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Structure and properties of fibres from sea-grass (*Zostera marina*)

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Abstract:

This paper presents results from a study of fibres extracted from Zostera marina eel-grass collected from the Baltic coast. This species of sea-grass is shown to contain small diameter (around 5 µm) fibres composed of ~57% cellulose, ~38% of non-cellulosic polysaccharides (mainly xylan) and ~5% of residual matter so-called Klason lignin. This composition is quite different to that of commonly used terrestrial fibres. Single fibre stiffness values up to 28 GPa were measured. This stiffness combined with a low density could provide an attractive reinforcement for composite materials, and may be particularly suitable for use in bio-degradable structures.

Keywords: Fibre, Sea-grass, IR spectroscopy, Mechanical behaviour, Tension, Xylan

INTRODUCTION

Natural fibres have been used in structural applications, particularly for ropes, for many years but they are currently attracting increasing interest for polymer reinforcement in applications seeking environmentally friendly materials. Sustainable development is becoming an issue in material selection and this is likely to increase in the future as fossil fuels become rarer. The use of biological resources is one option, and fibres which have been studied to date include flax, jute, sisal and hemp. Several recent papers have presented their mechanical properties, [1-4]. Compared to the E-glass fibres currently widely used all appear promising and a number of composite applications are now being considered, particularly for the automotive industry. All of these fibres are based on cellulose of terrestrial plant origin. Very little work has been reported on fibres from marine sources, but ocean resources could offer considerable potential for natural fibres if appropriate species could be identified. One possible source of fibres is sea-grass, and this paper presents a study of the structure and properties of fibres extracted from one species. Sea-grasses are flowering plants (angiosperm). They can be found in most of the oceans and are the only angiosperms which have adapted to the marine environment and permanent immersion in shallow water [5]. There are around sixty different species in the world and *Z. marina*, commonly known as eelgrass, is the most widely distributed species in the Northern hemisphere [6]. Sea-grasses are an invaluable element of the coastal environment and their depth of growth is used as an indicator of water quality [7]. The sea-grass is not a direct food source, at least when growing, due to a high cellulose content, low nitrogen levels and the presence of phenolic acids. A large proportion of sea grasses therefore degrade *in situ* at the end of their life cycle. Sea-grass beds shelter many marine life-forms including shrimps and small fish, providing protection and breeding

grounds. For these reasons the removal of living sea-grass is not allowed in Europe. However, in some coastal regions, and particularly along the Baltic coast, the proliferation of sea-grass washed ashore on beaches following storms is a costly nuisance requiring regular removal and disposal. Alternative uses of this resource are therefore of interest, and the present work was initiated to evaluate whether fibres observed in these materials could provide reinforcement for environmentally friendly structural composites. Previous studies have indicated a number of possible uses for eelgrass. For example, they have been studied as a possible food source, the grain of eelgrass (*Z. marina*) consumed by Seri Indians of Sonora Mexico is the only known case of a sea grain being used as human food [8,9]. Other studies have examined their use in anti-fouling compounds. Zosteric acid is a natural product made by the seagrass *Z. marina* and has been found to prevent biofouling at non-toxic concentrations [10] and a patent exists for this application [11]. Davies examined new sources of fibres for paper-making [12], and aquatic plants were among those studied (*poseideonia australis, potamogeton perfoliatius L., potamogeton tricarinatus A. Benn*). The posidonies have been studied since 1919 for this application [13].

Concerning bio-composites from marine sources few publications exist. Iannace et al. [14] studied the properties of a composite consisting of fibres from algae collected on Sardinian beaches in Italy in biopolymers such as polycaprolactone (PCL). Tensile properties were measured as a function of fibre content but values were quite low and the authors estimated the axial modulus of the fibre to be around 6 GPa. Injection moulding was used to prepare the specimens.

In the present work eelgrass fibres from the Baltic sea beaches were studied. First, the grass blades were examined to determine their global fibre content and structure. Then the mechanical properties of the fibres extracted from the grass blades were determined, and compared to those of other vegetal and synthetic fibres. The properties were found to differ

from those of fibres such as flax, so then more detailed analyses were performed on the fibres to understand the reasons for these differences. Recent studies on flax fibres have led to an improved understanding of their microstructure [4,15], and characterization at this scale is essential if the relations between growth conditions and variations in properties are to be understood.

MATERIALS

The materials tested were collected on the German Baltic coast by machines which clean the beaches regularly. They were identified as *Z. marina*. Other types of sea-grass and *Z. marina* samples from other sources have also been evaluated, but in this paper only the Baltic species is described as so far it appears to provide the most promising fibres from a mechanical point of view. This may be due to the severe climate to which these are exposed. Samples were collected at different periods during 2004 (January, March and June). The results described here are from tests performed on fibres extracted from samples collected in June. After collection on the beach, samples were sent in sealed bags to France. Some were then dried for 2 weeks, others were tested as-received. The plant stem is a yellow-brown colour, 2 to 5 mm in diameter. The branches have 3 to 8 leaves or blades which measure up to 1.2 metres long, and are 2 to 12 mm wide, Figure 1 [16].

METHODS

- Microscopy

Optical microscopes and a scanning electron microscope (Jeol JSM 6460LV) have been used to examine samples. For the former a staining technique (carmin plus iodine green) was used

in one case to identify lignin regions (in green). For the latter a thin Au-Pd coating was applied to avoid charging.

- Tensile tests

Single fibres were extracted from fibre bundles as described below. They were then bonded to cardboard windows and placed in an MTS test machine with a 2 N load cell. The side supports were cut just before testing to allow stress-strain curves for single fibres to be determined, at a loading rate of 10 mm/minute. The longitudinal tensile Young's modulus of the fibres was measured using the standard method for single fibres [17], by taking into account the compliance of the loading system. The test machine compliance calibration was performed by running tests on glass fibres of different lengths. The load cell allows measurements in the range of 0 to 2 N with an accuracy of 0.01%. The accuracy of the displacement measurement is $\pm$ 1 μm.

- FTIR (Fourier Transform Infrared Spectroscopy)

Infrared microspectroscopy was used, which is a surface examination technique as in the reflection mode the penetration is only 1 to 2 microns. FTIR micro-spectroscopic experiments were performed using a *Protégé* 460 spectrometer coupled to a *Continuμm* microscope (*Nicolet*) operated in the reflection mode (not in attenuated reflectance, ATR). The microscope was fitted with a MCT detector and a computer controlled stage. The aperture of microscope was controlled by setting the adjustable mask so that light was collected from a narrow area which exactly overlaps two or three adjacent fibers. Each spectrum was acquired at a 4 cm^{-1} resolution and represented the average of ten accumulations of 128 scans each.

- Sugar analysis

After their isolation, fibres were poured in boiling ethanol for 15 min to inhibit cell wall enzymes and further treated in organic solvents (chloroform/methanol and acetone) to dissolve lipids and wax. Then two types of sugar extraction were performed on the cleaned fibres. In each case two independent samples were analysed.

(1) Fibres were extracted with boiling water in order to collect non cellulosic polysaccharides from the surface of the fibres.

(2) Fibres were incubated in 2 M trifluoroacetic acid (TFA) 2h at 110°C, washed with distilled water and filtered on Millipore 0.45 µm. The filtrates, containing non cellulosic polysaccharides (NCPs), were kept aside for further sugar analyses. The cellulose residues were dried, weighed and further incubated at room temperature for 1 h in concentrated sulphuric acid. After dilution to 1 M, the samples were cured for 2 h at 110°C and subsequently treated as for TFA.

The amount of uronic acids and total sugars were measured in the filtrates, using the colorimetric methods of Blumenkrantz and Asboe-Hansen [18] and Dubois et al [19]. Methanolysis of the samples and their sylilation were performed and sugar molar composition determined [20].

RESULTS AND DISCUSSION

Global structure of *Z. marina* sea-grass

Sea grass samples were first examined using an optical microscope. Figure 2 shows an example of a broken blade and fibres are clearly visible at the fractured end. Further optical and scanning electron microscopy was then used to examine several samples in order to establish where the fibres were located and the quantity present, Figure 3. It can be seen that the eelgrass can be considered as a composite, with fibres reinforcing a matrix. The damage

mechanisms are similar to those of industrial composites, including matrix cracks, fibre breakage and fibre/matrix interface debonding. After drying the matrix has a lower elongation to failure than the fibres.

The blades are organised in a closed cell structure, Figure 4. The fibres are present in bundles of 6 to 12 fibres mainly located near the outer surfaces of the blade on the faces of this structure. Sections through the blade treated with carmin green stain showed no green colour which indicates that there is very little lignin present. This will be discussed further below. SEM micrographs clearly reveal the presence of very fine fibres. These appear to be approximately circular, with diameters between 4 and 5 microns, Figure 5.

Mechanical behaviour of *Z. marina* fibres

In order to be able to test single fibres it is necessary to extract them from the grass blade. Initially various chemical digestion methods were examined in order to remove the 'matrix' and leave the fibres but an appropriate solvent which did not damage the fibres was not found. A mechanical extraction method was therefore developed, in which fibre samples were extracted in bundles from wet grass blades. By cutting carefully and separating the two halves of a grass blade it is possible to obtain bundles of fibres as shown in Figure 6. The two halves are then bonded to a cardboard window and under a microscope all the fibres except one are cut using a scalpel. The diameter and length of this fibre are measured, using an optical microscope linked to image analysis software (*Q-Win*). The remaining fibre is then mounted in the test machine described above and tested to failure.

The longitudinal tensile Young's modulus was measured taking into account the compliance of the system. An example of a stress-strain plot, after the machine compliance correction, is shown in Figure 7. Stress values are determined using the measured diameter and assuming a circular cross-section. The response is fairly linear to failure. Results from diameter

measurements and tensile tests on eight specimens are shown in Table 1. The diameters are all around 5 microns. This is similar to the diameters of carbon fibres used in composite reinforcement but much smaller than glass or most other natural fibres. Modulus values obtained range from 10 to 28 GPa. There is considerable scatter in values but this is not unusual for natural fibres. It is of interest to compare the mechanical property results obtained here with those reported for other vegetal and synthetic fibres, Table 2. The stiffness values are promising, higher than those of many industrial polymer fibres such as polyamide (around 5 GPa) or polyester (13 GPa). They are similar to Sisal and Jute but lower than those of flax fibres (58 GPa). The comparison with E-glass fibres is of particular interest as these are the most widely used reinforcements for industrial composites today. The stiffness values measured on these *Z. marina* fibres from the Baltic are lower than those of E-glass but the latter are relatively heavy. When the lower density of the sea grass fibres is taken into account, (in the range 1000-1500 kg/m^3, compared to 2700 kg/m^3 for glass fibres), specific stiffness appears more favourable. It should be emphasized that this was a preliminary screening project, to evaluate different sea grass species. While the results are already interesting, there has been no attempt to optimise the mechanical properties for the *Z. marina* fibres, the sea-grass samples were simply collected on the shore, the fibres were extracted and tested. Differences were noted between grasses collected at different times, and appropriate environmental conditions during growth might be expected to result in improved properties. These fibres also contain significant quantities of water. Moisture has a strong influence on natural fibre properties and drying processes must be investigated further. Nevertheless, given these potentially attractive mechanical properties for composite reinforcement, it was decided to examine the fibre composition in more detail, in order to see if there were differences compared to the more traditional natural fibres.

Composition of fibres in *Z. marina* sea-grass

Two methods have been used to study the composition of these fibres. First, IR analysis by micro-spectroscopy of the fibre surface was performed. Then analysis of sugars in elements of the fibre, after different solvent extractions, provided more detailed information.

IR Microspectroscopy of fibre surface

These analyses were performed on single intact fibres after removal from wet grass blades. An example of the spectra obtained is shown in Figure 8. The broad band around 1600-1610 cm^{-1} may be assigned either to lignins or pectins. In this case, it seems likely that the main component must be pectin since the spectral pattern in the 1600 cm^{-1} to 1735 cm^{-1} cm^{-1} (ester bonds) is characteristic of pectins compounds. This is also supported by the valley at 1510 cm^{-1} which corresponds to a major lignin absorption band. The bands observed in the 1034-1057 frequency domain mainly arise from cellulose. As a whole, the FTIR data suggest that the *Z. marina* fibers cell walls exhibit a high content in polysaccharides and pectins with a low lignin content.

For comparison, our data concerning *Z. marina* fibres were matched with those obtained in a previous report on different natural fibres [27]. Those authors have used two intensity ratios $R_1=I_{1595} / I_{1105}$ and $R_2=I_{1595}/I_{2900}$ to quantify the lignin/cellulose and lignin/organic matter ratios, respectively. As mentioned above, the 1595 cm^{-1} absorption band cannot be unambiguously assigned to lignin alone since pectins also absorb in this frequency domain. Figure 9 is based on both the data published by Garside & Wyeth [27] and the data measured in the present study, for which the R1 and R2 intensity ratios have been determined in exactly the same way including the baseline correction protocol used by those authors.

The present data were obtained in reflection rather in ATR mode but similar R1 and R2 values are observed for flax fibres (flax and µ-flax) whatever the infrared acquisition mode.

The *Z. marina* fibre is quite different to the other fibres, showing a much higher R2 ratio. As

discussed above, this high R2 ratio cannot be due to a high lignin content but, rather from a relatively low amount of organic compounds, at least in the fibre's cell wall.

Sugar composition of non cellulosic polysaccharides.

Two experiments, (1) extractions with boiling water and (2) hydrolysis with TFA, were run in order to approach the sugar composition of the non cellulosic polysaccharides (NCPs) of Zostera fibres (Table 3). The first extract essentially originated from the primary cell-wall (CW-I), at the surface of the fibres, while TFA hydrolysed the NCPs both from the surface and from the heart of the fibre (secondary wall, CW II).

The results from the sugar analyses are given in Table 4 and show:

1- The main NCPs at the surface of the fibre consisted of xyloglucan and to a lesser extent of pectins (20%), half homogalacturonan (HG) and half rhamnogalacturonan-I (RG-I). ATR IR spectroscopy showed clearly the presence of pectins (1605 cm^{-1}) in the outer cell walls (data not shown). Globally, the polysaccharide composition is similar to that of the primary wall of aerial plants when the cells had been grown in the dark [28], which might be related to the growth of *Z. marina* in deep and dark water.

2- The TFA extract represented about 40 % of the mass of the fibres and consisted of two thirds hemicelluloses and one third pectins. The sugar composition indicates essentially the presence of xylans , i.e. hemicelluloses typical of the secondary wall in lignocellulosic fibres [29]. Homogalacturonan is the main pectin identified in the secondary wall. Incidentally, it may be noted that the amount of pectins in secondary was larger than in primary wall. This last point might be related to the significant amount of pectins identified in cellulosic fibres present in the stems of flax [5], Table 5.

3- The cellulosic residue (~57 %) obtained after the TFA treatment was shown to contain mainly cellulose. What was designated as Klason lignin (KL) residue after sulphuric acid treatment, which also contained oxidized sugars, minerals etc. represented less than 5% of the total mass of the fibre. This means that the zostera fibres were hypolignified as was observed on the stained sections in figure 4.

Overall, the biochemical data indicate a particular family of fibres, that are neither cellulosic nor lignocelluosic (Table 5). We propose to designate them as xylo-cellulosic fibres.

CONCLUSIONS

This paper shows results which indicate that fibres in sea grass samples collected on Baltic coast beaches possess interesting mechanical properties. The structure of these fibres differs from that of flax fibres. First, their dimensions are much smaller, around 5 microns, compared to around 18 microns for flax. Preliminary analysis of the internal structure of these fibres indicates that they are composed of ~57% cellulose, ~38 % of non cellulosic polysaccharides (mainly xylan) and ~5 % of residual matter so-called Klason lignin. The designation 'xylo-cellulosic fibres' is proposed. The mechanical properties of these fibres are very promising. In their natural state tensile modulus values up to 28 GPa and strengths up to 770 MPa were measured. These values are comparable to those of other terrestrial fibres (sisal and jute) currently being considered for polymer reinforcement in environmentally friendly composites. Proliferation of sea grass washed ashore on beaches requires costly removal and disposal. If fibres can be extracted from sea-grass this may provide an economic outlet for what is currently a costly nuisance, but more detailed studies of the four stages of production, extraction, purification and transformation must first be examined, so that a balanced

comparison with other natural fibres can be made. Further work is underway to understand the relation of structure to growth conditions.

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Tables

Test	Diameter μm	Strength MPa	Modulus GPa
1	4.6	497	20.3
2	5.0	565	24.2
3	4.2	481	9.9
4	4.5	596	16.5
5	5.0	454	10.5
6	4.6	776	28.4
7	4.0	726	24.5
8	4.6	491	23.9
Mean (Std. Dev.)	4.6 (0.3)	573 (120)	19.8 (6.8)

Table 1. Results from tensile tests on single *Z. marina* fibres.

Fibres	E (GPa)	Failure strain (%)	σ_u (MPa)	D_f (μm)	Reference
Z. marina	19.8 ± 6.8	3.4 ± 0.3	573 ± 120	4.6 ± 0.3	Present work
Flax	58 ± 15	3.3 ± 0,8	1339 ±486	17.8 ± 0.5	[4]
Hemp	35	1.6	389	10 - 50	[21]
Jute	26.5	1.5-1.8	393-773	25 - 200	[22,23]
Sisal	9 - 21	3 - 7	350 - 700	7 - 47	[2,24]
Polyester	13	13	1260	20 - 30	[25]
E glass	76	1.8-3.0	1400 - 3500	8-14	[26]

Table 2. Comparison of properties of *Z. marina* fibres with those of other natural fibres.

Fibre	R1	R2	Reference
Cotton	0.39	0.09	[27]
Flax	0.70	0.33	[27]
μ-Flax	0.98	0.43	Our data
Hemp	0.61	0.12	[27]
Jute	0.96	0.24	[27]
Ramie	0.10	0.02	[27]
Sisal	1.37	0.51	[27]
μ-*Zostera*	0.75	1.24	Our data

Table 3. Published and measured R1 and R2 ratios from IR spectroscopy for different natural fibres.

Sugar	Boiling water	TFA
Arabinose	1.4 ± 0.4	3.3 ± 0.7
Galactose	5.5 ± 0.5	5.1 ± 0.5
Rhamnose	1.3 ± 0.3	3.8 ± 0.3
Galacturonic Acid	10.9 ± 0.9	23.8 ± 0.8
Glucuronic Acid	3.2 ± 0.4	6.4 ± 0.5
Glucose	47.0 ± 3.0	6.5 ± 0.7
Fucose	1.0 ± 0.3	0.7 ± 0.1
Mannose	0.1 ± 0.1	0.3 ± 0.1
Xylose	30.0 ± 3.0	50.0 ± 0.1
Homogalacturonan	9.5 ± 1.5	20 ± 2
Rhamnogalacturonan-I	9.5 ± 1.5	16 ± 2
Hemicelluloses	81 ± 3	64 ± 4

Fibre samples were treated either with boiling water or with TFA (see methods). Sugar amounts are molar percentages relative to the total amount of sugar analysed. Pectins (HG and RG-I) and hemicelluloses (HC) were calculated as follows: HG = GalA- Rha; RG-I= (2x Rha) + Ara +Gal , HC = Fuc + GlcA + Glc +Man +Xyl. HC in TFA consisted of a xylan (xyl/Glc~8) rather than a xyloglucan (xyl/Glc <1) as observed in boiling water i.e. in CW-I

Table 4: Sugar composition of different extracts of zostera fibre.

Fibre	**Pectins**	**Hemi-cellulose**	**Cellulose**	**KL**	**Reference**
Cotton	6		83	11	[30]
Flax	6 ± 3	7 ± 3	82 ± 5	5 ± 2	[5]
Hemp	2.5	5.5	78,3		[31]
Jute	0.2	12	64.4		[31]
Ramie	1.9	13.1	68.6		[31]
Sisal	0.8	12	65.8		[31]
Zostera	10 ± 2	28 ± 5	57 ± 3	5 ± 1	Present work

Table 5: Cell-wall composition of various fibres, including *Z. marina*.

List of figures

Figure 1.

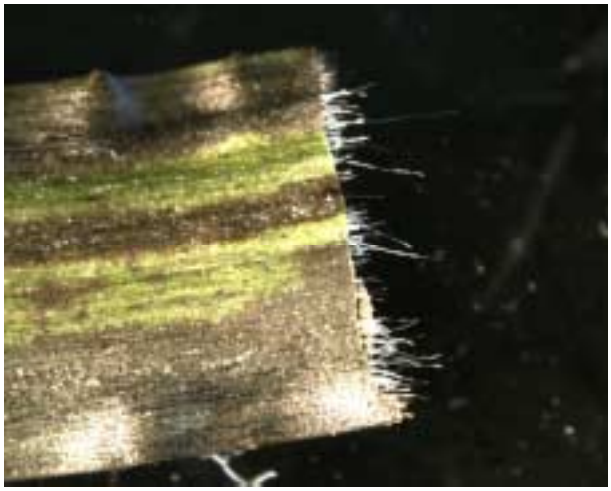


Figure 2.

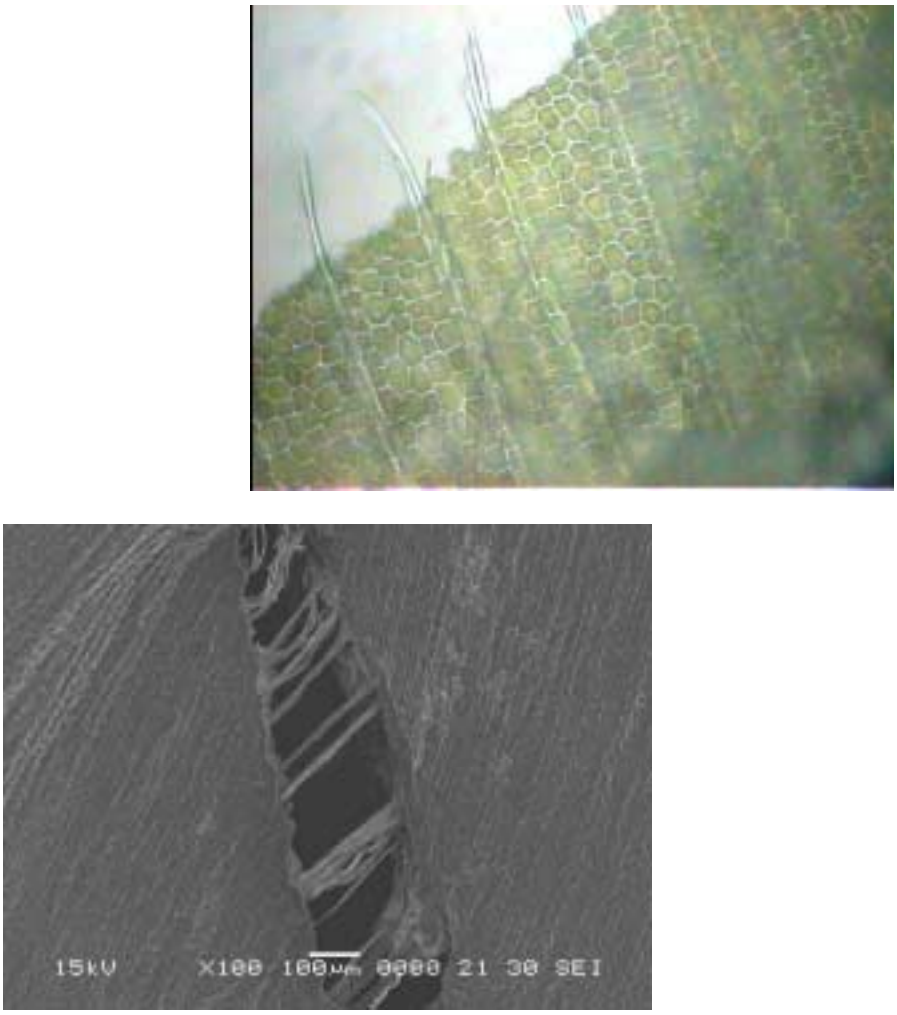
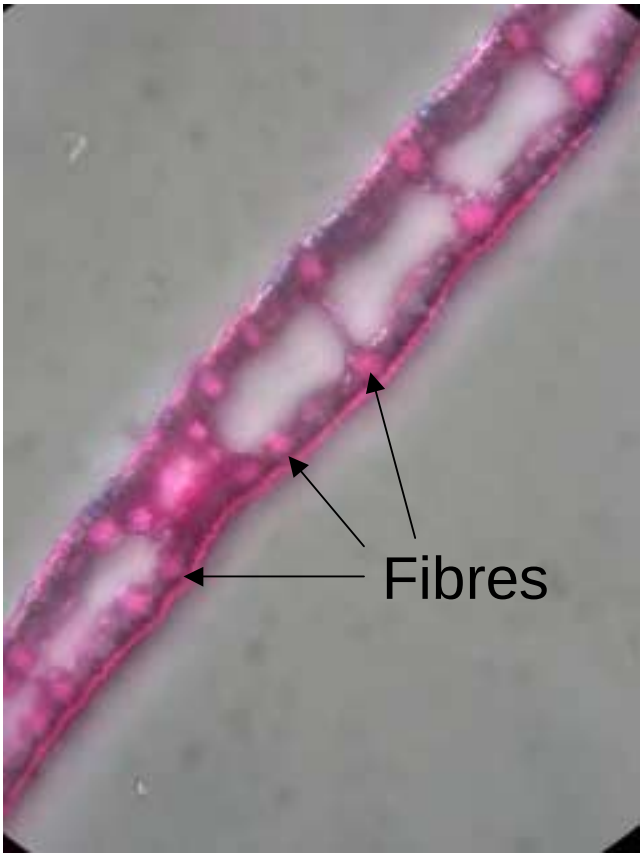
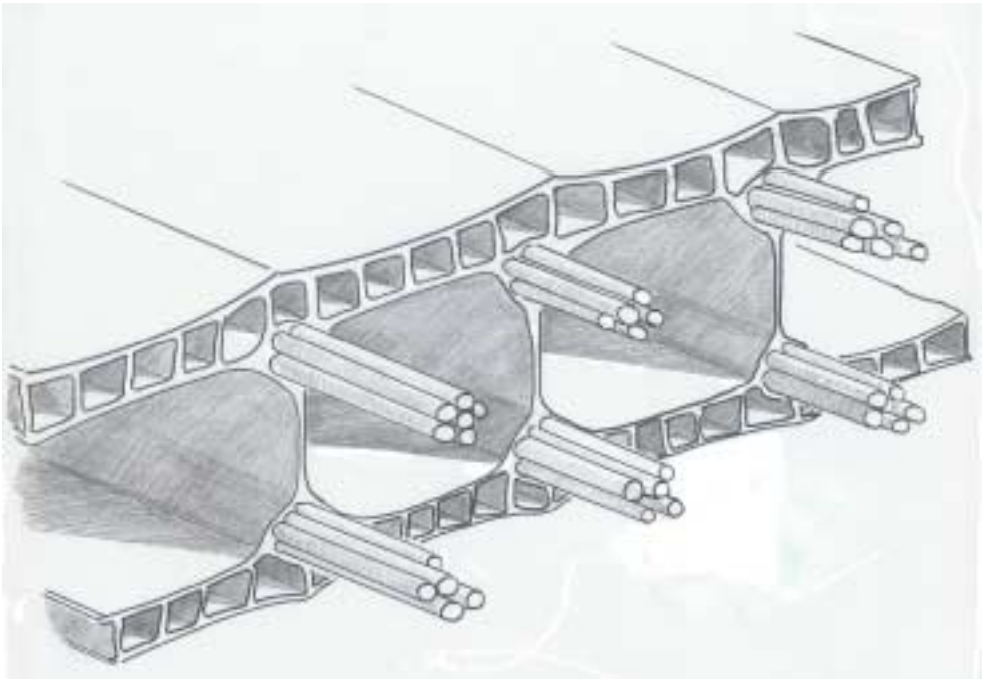


Figure 3.





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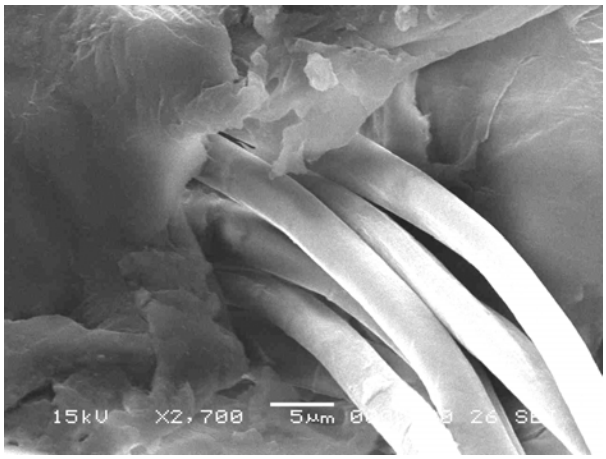

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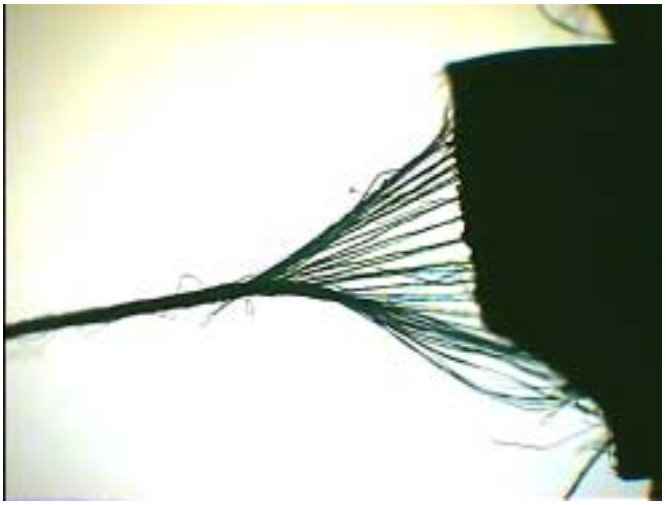

Figure 6.

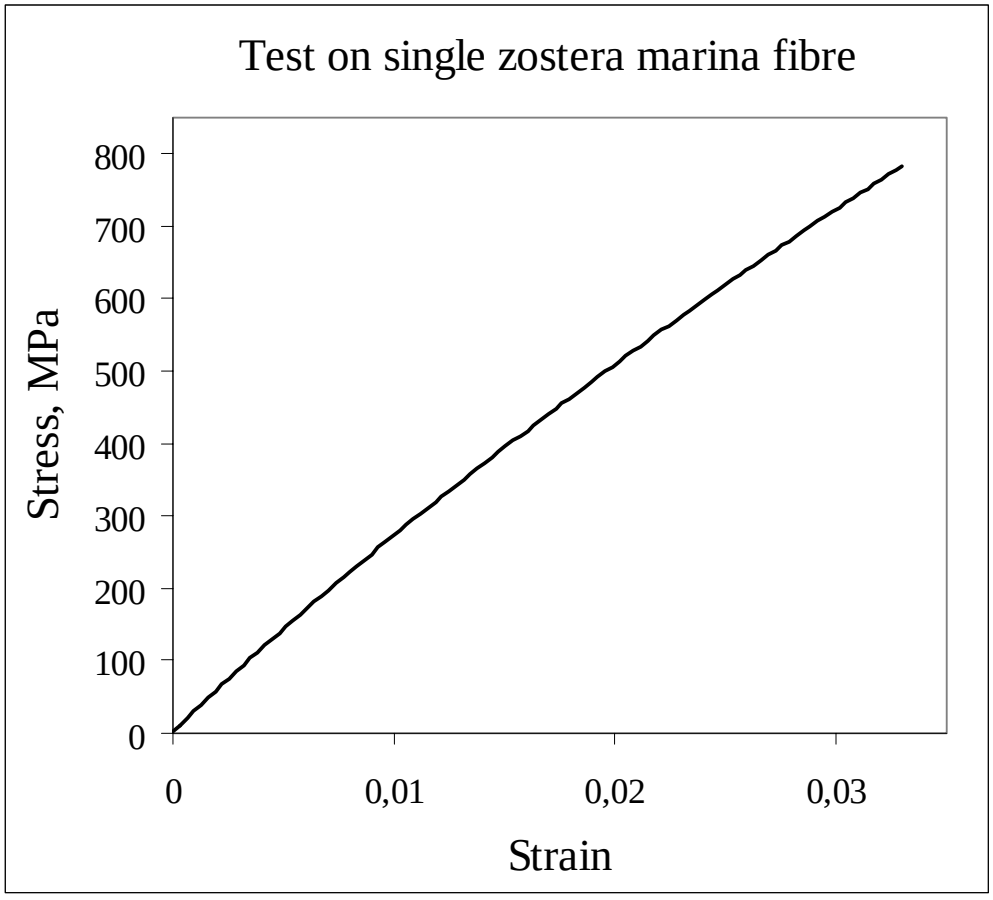
Test on single zostera marina fibre
Stress, MPa
800
700
600
500
400
300
200
100
0
0
0,01
0,02
0,03
Strain

Figure 7.

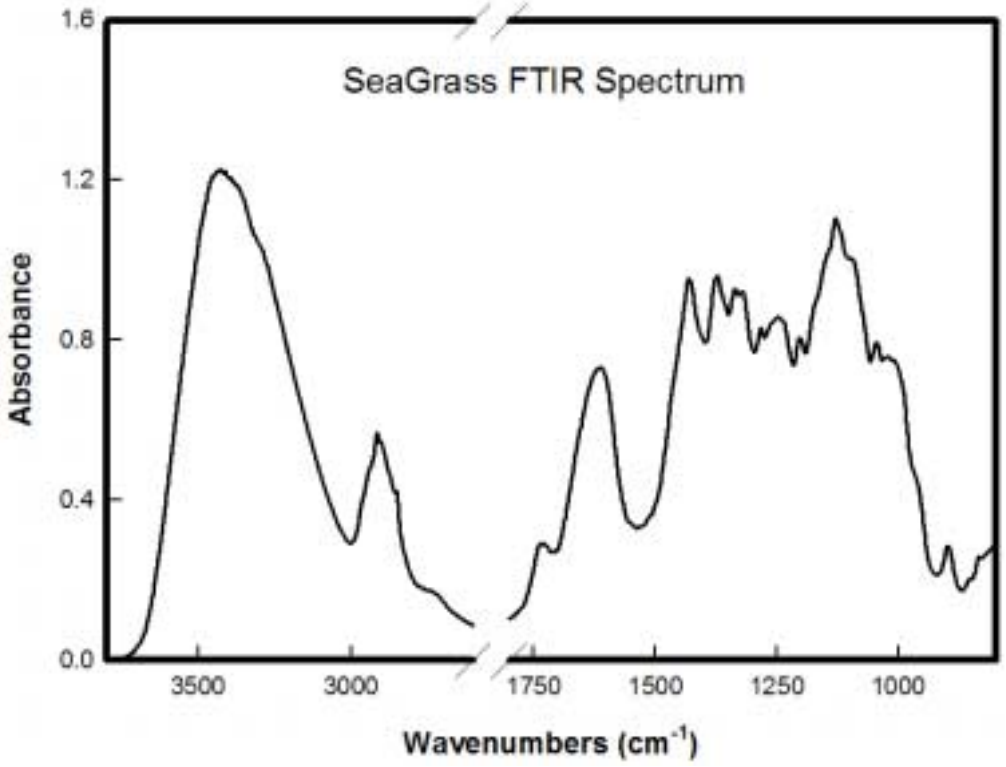
SeaGrass FTIR Spectrum
Absorbance
1.6
1.2
0.8
0.4
0.0
3500
3000
1750
1500
1250
1000
Wavenumbers (cm-1)

Figure 8.

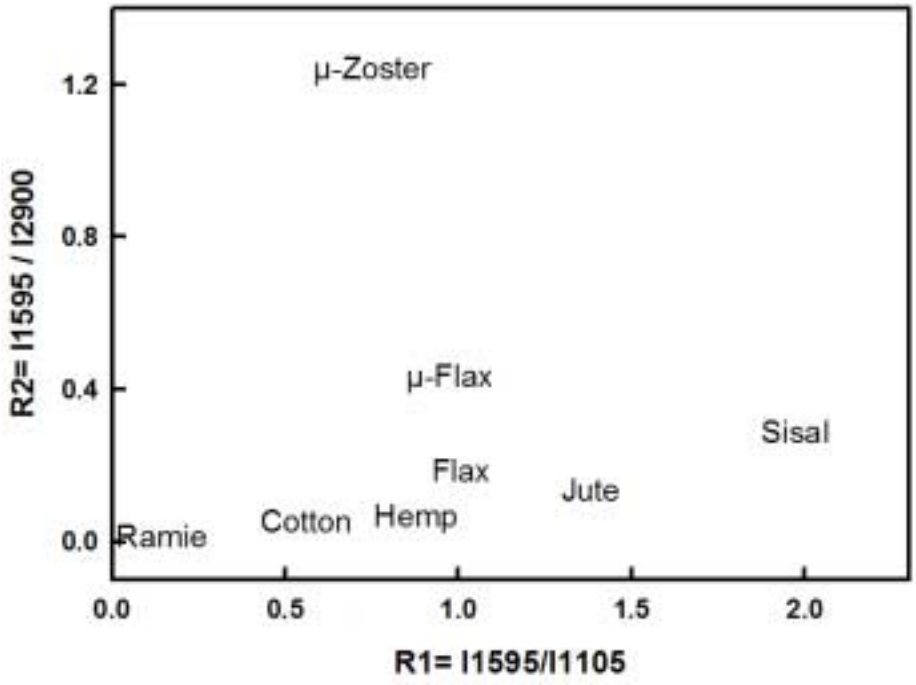
μ-Zoster
μ-Flax
Sisal
Flax
Jute
Cotton
Hemp
Ramie
1.2
0.8
0.4
0.0
0.0
0.5
1.0
1.5
2.0
R2= I1595 / I2900
R1= I1595/I1105

Figure 9.

Author reply to reviewers' comments.

Reviewer 1.

We have included all the corrections suggested by the reviewer in the revised version.

Reviewer 2.

The study is continuing. The assumption that fibres are circular is certainly a strong assumption. Figure 5 gives an indication of the form of the fibres, which does appear to be approximately circular, but in this preliminary study we did not manage to obtain a convincing photo of a single fibre cross-section. The ductile nature of these very small diameter fibres makes that quite difficult, but we are continuing to examine the statistics of fibre dimensions.

We have done some work on the influence of drying, but in this preliminary study samples were tested without controlling this parameter. However, we certainly appreciate that this is a very important parameter, and as stated on page 8 this is something which requires detailed further study.