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Cellulose coating and chelation of antibacterial compounds for the protection of flax yarns against natural soil degradation

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A B S T R A C T

Natural cellulosic fibres such as flax fibres present interesting mechanical properties as well as biodegradability, and by-products, such as short flax fibres, could be used to produce geotextiles to stabilise soils. Today, geotextiles are often made of coir fibres, which have a high lignin percentage leading to their slow degradation in soil. Fibres with a high cellulosic content, such as those of flax, exhibit lower resistance to soil degradation. This study investigates solutions to improve this parameter with a view to increasing their service life and therefore their credibility compared to coir fibres for geotextile applications.

For this purpose, a cellulose coating of yarns made of short flax fibres was performed and its stability under a water flow was assessed. The ability to form a cellulose sheath was estimated by chromaticity measurements of flax fibres after applying a dye specific to lignin. Infrared spectrometry analysis to monitor the level of protection against degradation by cellulolytic enzymes was also carried out. It appears that the cellulose coating provides an efficient physical protection, preventing access of these enzymes to their fibrous substrate. Then, the possibility of conferring antibacterial properties on the cellulose coating by chelating phytoalexin molecules such as gramine on it was assayed and proven to be effective against soil cellulolytic bacteria such as *Cellvibrio fulvus* and *Cellvibrio vulgaris*. This study therefore establishes that coating flax yarns with cellulose associated with antibacterial molecules could contribute to obtaining a longer service life in soil for geotextiles manufactured from flax fibres.

Keywords:
Antibacterial
Cellulose coating
Enzymatic degradation
Fibres
Flax

1. Introduction

Flax fibre is a natural fibre used in the textile industry or as a reinforcement for crude earth constructions and composite materials due to its high mechanical properties and relatively low cost [1–10]. Short flax fibres are a by-product of the textile industry and a renewable resource considered fully biodegradable making them an interesting candidate for manufacturing geotextiles. Nevertheless, although biodegradability is required for such products, it has to be quite slow for geotextile applications. Today, most geonets are manufactured from coir fibres. Coir and flax fibres are mainly composed of cellulose, hemicellulose, pectin and lignin but coir fibres have a higher lignin percentage [10,11]. Lignin is known for its resistance to degradation as lignin-degrading organisms are less widespread than cellulolytic ones. Consequently, when submitted to natural soil degradation by microbial populations [12], flax fibres are likely to show greater sensitivity than coir fibres. To overcome this higher degradability, flax fibres could be coated to increase their service life by providing physical protection. However, the coating must keep the environmental interest of flax fibre geotextiles and therefore also be biodegradable since a slowing down not an abolition of degradation is required. Cellulose seems a credible and interesting candidate because it has already been used for coating [13,14] and this compound, composed of β-1,4 glucosidic linked D-glucopyranose units, is a renewable, biodegradable and the most abundant biopolymer on earth [15]. On the other hand, flax fibre yarn is very flexible so any coating applied should be at least as flexible to remain in place. As flax fibre flexibility is due to

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its main component, cellulose, this seems to be one of the rare biodegradable candidates with the needed flexibility. Moreover, knowing the high sensitivity of cellulose to biodegradation, we have considered a combined protection approach: antimicrobial molecules could be associated with a cellulose coating in order to increase its resistance toward biological attacks, as already done on coir fibres using oil [16].

This work investigated the feasibility of a cellulose coating on flax fibre yarns and an antibacterial compound bound to the cellulose coating to assess the efficiency of such treatment against degradation processes, mimicking natural soil degradation. For this purpose, the ability of the cellulose coating to prevent enzymatic degradation using a cellulase cocktail from *Aspergillus niger* [17,18] was analysed. Polyquaternium-10, a polymeric quaternary ammonium salt of hydroxyethyl cellulose currently used in shampoo to coat hairs due to its ability to form a film easily, was used here as a positive control for coating ability and was assayed to provide a basis for comparison. Then, the possibility of associating antibacterial compounds with flax fibre yarns (previously coated or not with cellulose) was explored using antibacterial phytoalexins. This chemical protection was then evaluated against the soil-borne cellulolytic bacteria *Cellvibrio* [19], a microorganism already used in previous experiments [17]. Our aim was to investigate the possibility of using such natural products (with low environmental impact) to increase the potential service life of flax fibre-based geotextiles with (and without) a cellulose coating.

2. Materials and methods

2.1. Biological material

Twisted yarns (90 turns/m) made of short flax fibres (1 cm long) produced from tows originating from various flax fibre cultivars were provided by Groupe Depestele (France).

Cellvibrio fulvus and *Cellvibrio vulgaris* strains were purchased from the BCCMTM/LMG bacteria collection, Gent University (Belgium). They were cultivated in a tryptic soy broth medium in the presence of flax fibre yarn (raw or previously treated as described below). To measure the antibacterial effect, growth was monitored by spectrophotometry at 630 nm after 12 h at 28 °C with shaking (300 rpm). The antibacterial compounds gramine or saponin were bound to the yarns using the metal chelation process described by Kennedy et al. (1974) [20]. This cellulose-metal chelation protocol uses iron III chloride as the chelating metal.

2.2. Chemicals and enzyme

Cellulose (C6288), *p*-nitrophenyl β-D-glucopyranoside (N7006), sodium carbonate (57795), sodium phosphate monobasic (58282), sodium phosphate dibasic (57907), iron III chloride (157740), gramine (G10806), saponin (47036) and *Aspergillus niger* cellulase (22178) were purchased from Sigma. Polyquaternium-10; JR-400 was purchased from Wenlin.

2.3. Coating of yarns with cellulose or Polyquaternium-10

1% w/v cellulose or Polyquaternium-10 solutions were prepared by suspending the powder in boiling distilled water. Flax yarns were then immersed and shaken in cellulose or Polyquaternium-10 suspension for 8 h at 25 °C. The impregnated flax fibre yarns were then dried in an oven at 37 °C for 7 days.

2.4. Enzymatic degradation and activity

Flax fibre yarn degradation using *Aspergillus niger* cellulase from Sigma was optimised in a previous work: Renouard et al. [17] demonstrated that optimal degradation occurred at a pH of around 7, an incubation temperature of 37 °C, an incubation duration of at least 8 h and a cellulase concentration of at least 5 U/ml. Here, the optimal conditions were used with 5 U/ml as the cellulase concentration and 16 h as the incubation duration.

To measure enzymatic activity, a protocol derived from Renouard et al. [17] was used. An enzymatic cocktail (50 µl of a 5 U/ml solution in 0.2 M pH 7 phosphate buffer) with *p*-nitrophenyl β-D-glucopyranoside (50 µl of a 0–10 mM solution in 0.2 M pH 7 phosphate buffer) was applied on cellulose or Polyquaternium-10 (100 µl of 1% w/v cellulose, Polyquaternium-10 solutions or water as the control) for 30 min at 25 °C and then stopped by applying 100 µl of 1 M sodium carbonate solution. The amount of *p*-nitrophenolate released by the enzyme activity was measured by spectrophotometry at 405 nm. The results were expressed in arbitrary units of absorbance per min (AU/min). As *p*-nitrophenyl β-D-glucopyranoside digestion induces glucose release, the glucose inhibition curve on *Aspergillus niger* cellulase from Sigma was established prior to the enzymatic assays and showed that no significant activity difference was detected up to 0.05% glucose. In our conditions (0.045% was the maximal glucose concentration), glucose inhibition did not have to be considered (Supplementary Fig. 1). The Lineweaver-Burk plot was used to estimate the Michaelis constant, Km, and the maximum reaction rate, Vmax. Using this method, the y axis-intercept is related to 1/Vmax and the slope to Km/Vmax.

2.5. Fibre coloration analysis

Yarns were placed for 5 min in a phloroglucinol solution (phloroglucinol 1% w/v in ethanol 95%) and then for 5 min in a 5 M HCl solution. This led to the pink coloration of the lignin promoted by the phloroglucinol solution. The fibres' final colour was determined using a Minolta Chroma CR-200 colorimeter. The results are presented on a chromacity diagram: this system, called a CIE diagram, can describe any colour through the use of only three elementary colours (red, green and blue).

2.6. ATR-FTIR analysis

Reflectance spectra were recorded using a Bruker V70 interferometer working under a dehydrated airflow in reflectivity mode, with an ATR (Attenuated Total Reflectance) accessory containing a gold crystal. The measurements were performed for wave numbers situated in the middle of the infrared range (between 600 cm^{-1} and 4500 cm^{-1}). The instrument resolution was about 4 cm^{-1} and measurements were averaged over 64 scans. As previously described [21], ATR-FTIR ratios were used to quantify crystalline cellulose (1375 cm^{-1}/1595 cm^{-1}), amorphous cellulose (897 cm^{-1}/1595 cm^{-1}), xylan (1089 cm^{-1}/1595 cm^{-1}), xyloglucan (1078 cm^{-1}/1595 cm^{-1}) and pectin (1610 cm^{-1}/1595 cm^{-1}) using the lignin-specific band (1595 cm^{-1}) as the reference since lignin is not degraded by cellulase treatment.

2.7. Statistical treatment of data

All data presented in this study are the mean and standard deviation of at least 3 independent replicates. A comparative statistical analysis of groups was carried out using Student's test. Statistical tests were considered to be significant at $p<0.05$, the *p*-value being the probability of finding the observed, or more extreme, results when the null hypothesis is true. Graphical and statistical processing was performed using Microsoft EXCEL 2010 software.

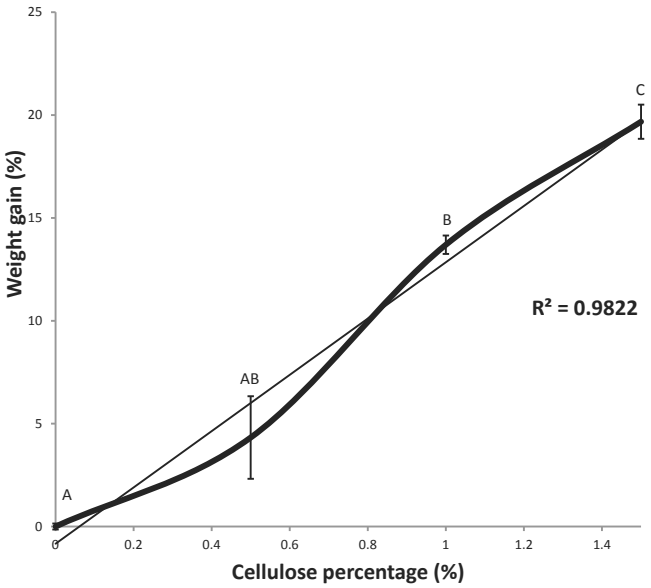


Fig. 1. Flax fibre yarn weight gain induced (by cellulose coating) evolution according to the cellulose percentage of the soaking solution completed with the correlation line (grey line) and associated correlation coefficient (R^2). Measurements associated with different letters differ significantly from each other at $p < 0.05$ using Student's test.

3. Results and discussion

3.1. Coating ability of cellulose

Based on the coating protocol of Hamzeh et al. (2013) [22], flax fibre yarns were coated using a 1% cellulose suspension.

3.1.1. Weight gain of flax fibre yarns after cellulose coating

The yarns exposed to the cellulose suspension showed a weight gain of 13.91%, demonstrating the coating ability of cellulose. According to Hubbe (2006) [23], water can be considered a solvent for segments of macromolecules such as hemicellulose and cellulose, and these segments tend to mix with each other. Thus, a random process of molecular motions would be expected to result in interpenetration and tangling. This mutual diffusion process at facing surfaces explains the cellulose coating ability on flax fibre.

3.1.2. Property of cellulose coating

The high positive correlation (0.982) between the weight gain and the cellulose percentage of the coating solution (Fig. 1) suggests cellulose stacking. The mutual diffusion process describe by Hubbe (2006) [23] can also explain it, considering the possibility of tangling between several cellulose fibres.

3.1.3. Cellulose coating stability

Coated flax fibre yarns were submitted to water movement during 14 days to test the stability of the cellulose coating (Fig. 2A). This information is crucial if this protective coating is to be used to increase the service life of geotextiles exposed to moisture and/or rain flow. No significant weight loss was observed after water agitation treatment on flax fibre yarns coated with cellulose (Fig. 2B). The interpenetration and tangling process due to the mutual diffusion at facing surfaces described by Hubbe (2006) [23] enables flax fibre yarn to be coated with cellulose and this coating presents a credible stability.

3.2. Protective ability of cellulose and Polyquaternium-10

3.2.1. Cellulose and Polyquaternium-10 coatings protect the yarn from cellulase action

To mimic natural degradation conditions, an *Aspergillus niger*

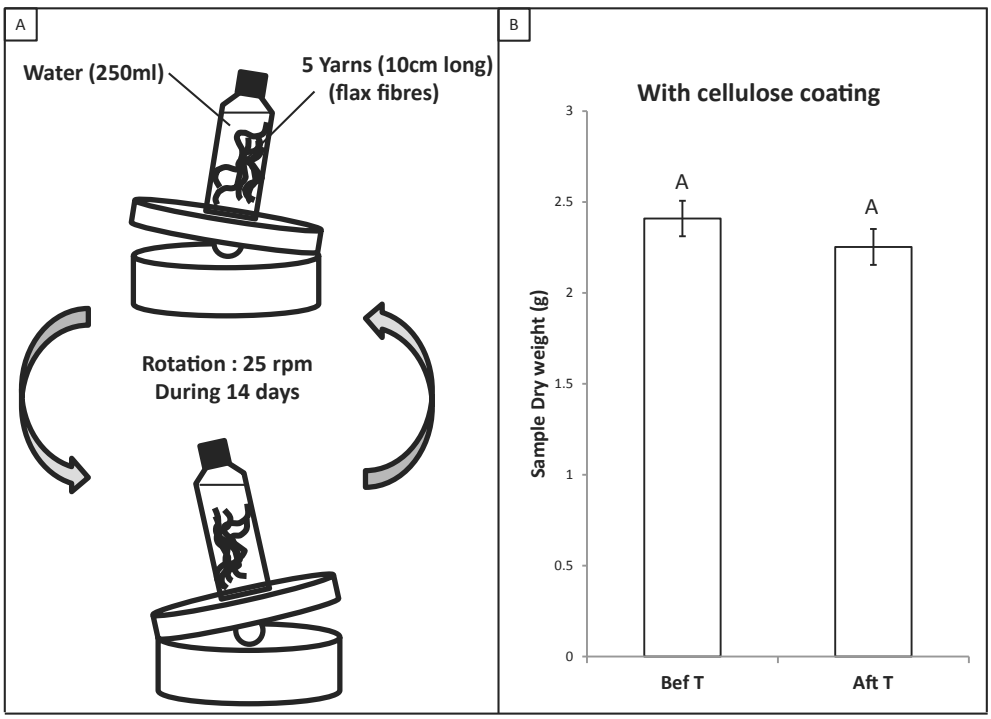


Fig. 2. A. Flax fibre yarn agitation experiment: diagram of the coating stability test conducted on 10-cm-long flax fibre yarn using a bottle containing 250 ml of water with stirring at 25 rpm at 25 °C for 14 days. B. Dry weight before (Bef T) and after water agitation treatment (Aft T) for flax fibre yarns with 1% cellulose coating. Measurements associated with different letters differ significantly from each other at $p < 0.05$ using Student's test.

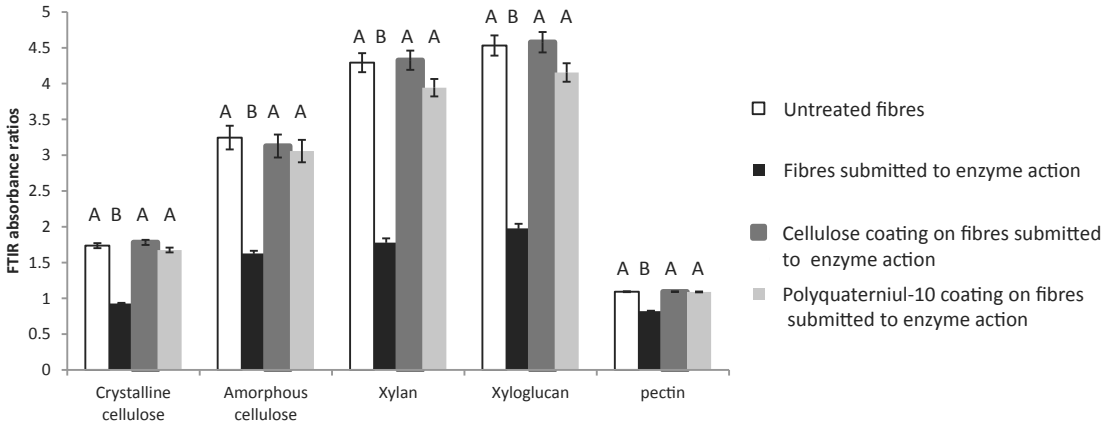


Fig. 3. ATR-FTIR data. Each bar is the relative absorbance normalised using the lignin band as the reference). Measurements associated with different letters differ significantly from each other at $p < 0.05$ using Student's test.

cellulase was used [17] [18]. ATR-FTIR enables quantitative analysis of the flax fibre composition with or without enzymatic treatment by using ratios with lignin as a reference [21]. To achieve a direct fibre analysis, the coatings were removed before spectrometry measurements by shaking the yarns in boiling water. FTIR analysis showed that the enzymes applied to the flax yarns did not have any effect on the flax fibre main components: cellulose (crystalline or amorphous), hemicellulose (xylan, xyloglucan, etc.) and pectin when the yarns were coated with cellulose or Polyquaternium-10. On the contrary, raw yarns (which were not coated) underwent degradation, revealed by the drop in the intensity of the specific band ratios of their components (Fig. 3, Supplementary Fig. 2) [17]. To sum up, a cellulose coating confers a protection on flax fibre yarns against enzymatic attacks.

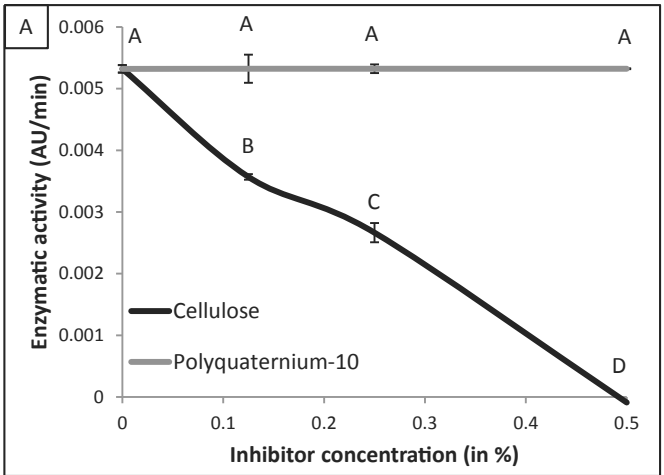
3.2.2. Characterisation of cellulose protection against enzymatic degradation

In an attempt to understand how the protection provided by the coating works, further experiments were performed to test two hypotheses. The first was that the coating products can affect the ability of the enzyme to degrade its cellulose substrate. In this case, the enzymatic parameter would undergo a change revealing the kind of enzymatic activity inhibition. The results are shown in Fig. 4 (Supplementary Fig. 3): Polyquaternium-10 had no impact on enzymatic activity, but cellulose inhibited the cellulase activity as expected logically since cellulose is the natural substrate of cellulase. Enzymatic parameter measurements indicated that cellulose induced only an increase in the Michaelis constant (Km) without modification of the maximum reaction rate (Vmax). In other words, there was competition between nitrophenyl β-D-glucopyranoside, the substrate provided in the experiment to measure the cellulase activity, and cellulose itself. This seems logical as cellulose acts as a lure but does not inhibit the enzyme's intrinsic ability to act on its substrate. Thus, neither cellulose nor Polyquaternium-10 will change the capacity of cellulase to attack the fibre by classic enzymatic degradation.

Consequently, the protection provided to flax fibre yarns by the coating could result from the formation of a sheath that covers them completely and prevents access of cellulase to the fibres. Polyquaternium-10, which can form a film, does not change the enzyme activity and therefore the ability to attack the cellulose. It is used here as a positive control to observe what may happen when a physical barrier, such as a coating that hinders the access of enzyme to its substrate, is applied.

To investigate this second hypothesis, i.e. the ability of a cellulose coating to form a complete physical protection for flax fibre yarns, phloroglucinol coloration (pink coloration) of the accessible lignin of the flax fibres was performed. The chromaticity diagram shows that uncoated flax fibre yarns reveal pink coloration whereas yarns coated with cellulose or Polyquaternium-10 do not (Fig. 5). Consequently, it can be deduced that cellulose, as well as Polyquaternium-10, forms a protective sheath around flax fibre yarns thus establishing a physical barrier that hinders the access of phloroglucinol to the lignin of the flax fibres. One can suppose that the coating will also prevent the access of the enzymes to the fibres. Consequently, it can be concluded that, similarly to chitosan used in a previous work [17], cellulose, as well as Polyquaternium-10, can

A



B

	Equation curves	R^2	Vmax	Km
Control	y = 147.63x + 48.14	0.999	0.021	3.067
Cellulose 1%	y = 164.33x + 49.888	0.998	0.020	3.294
Polyquat. 1%	y = 149.12x + 49.285	0.998	0.020	3.025

Fig. 4. A. *Aspergillus niger* enzymatic activity evolution according to the percentage of cellulose or Polyquaternium-10. B. Kinetic parameter modification due to cellulose and Polyquaternium-10; calculated according to Supplementary Fig. 2. R^2: correlation coefficient; Vmax: maximum initial reaction rate, Km: Michaelis constant. Measurements associated with different letters differ significantly from each other at $p < 0.05$ using Student's test.

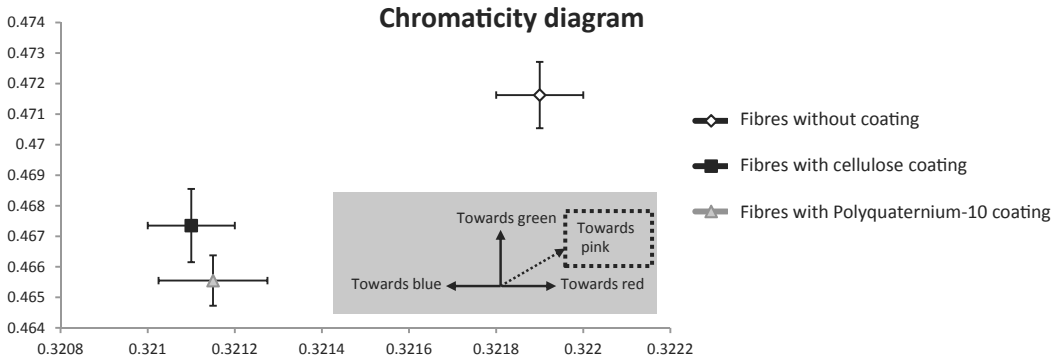


Fig. 5. Coloration of fibres with phloroglucinol presented on a chromaticity diagram.

form a sheath that completely covers the flax fibre yarns and efficiently protects them against enzymatic attacks.

3.3. Antibacterial chelating possibility

To prevent coir fibre geotextile degradation, Sumi et al. (2016) [16] applied antifungal lipids on them, which reduced the microbial activity. For our flax fibres, a process specifically adapted to cellulose was used on coated and uncoated flax yarns to bind phytoalexins, which are biodegradable natural compounds produced by plants to fight their pathogens. Kennedy et al. (1974) [20] developed a protocol to chelate antibacterial compounds on cellulose using iron III as the mediator to convert cellulose into a more reactive form: the cellulose-metal form. Metal ions can then be replaced by electron-donating groups of antibacterial molecules, leading to antibacterial compound chelation on cellulose. This protocol was tested here to chelate the saponin and gramine phytoalexins, which possess antibacterial activity [24] [25] [26], on cellulosic flax fibre yarns. The inhibitory effect of phytoalexins on *Cellvibrio fulvus* and *Cellvibrio vulgaris,* which are soil bacteria that produce enzymes causing flax fibre degradation [17] [19], was then tested on cellulose-coated and raw flax yarns.

To estimate the antibacterial activity conferred by the chelated phytoalexins on cellulose coating or flax fibre yarns, bacteria were quantified compared to the corresponding control without phytoalexins using the absorbance at 630 nm, which is directly related to the bacterial amount. The antibacterial assay principle is that antibacterial activity will slow down bacterial growth so to investigate bacterial growth retardation, a difference in bacterial amount is measured. Therefore, the experiment was carried out after 12 h, corresponding to the exponential phase of the two *Cellvibrio* strains (Supplementary Fig. 4), during which the most sensitive difference in bacterial amount can be measured to provide better evidence of the difference in bacterial growth.

For both *Cellvibrio* strains, the same results were observed (Fig. 6). When applied on flax fibre yarns without prior cellulose coating, no antibacterial activity was conferred by the phytoalexin treatment (Fig. 6). On the contrary, with prior cellulose coating on flax fibre yarns, gramine provided a significant inhibition of bacterial growth for both *Cellvibrio* strains compared to the control samples (Fig. 6). As expected, treatment with saponin did not lead to bacterial growth inhibition since saponin only acts against Gram-positive bacteria [26] and *Cellvibrio* bacteria are Gram-negative. This clearly shows that it is possible to chelate antibacterial compounds to the cellulose coating of the fibre but not directly on the fibre. These results also demonstrate that the antibacterial activity observed is due only to the specific activity of the chelated molecule.

To understand why there is no antibacterial activity without prior cellulose coating, the phytoalexins that remained unbound after treating the flax fibre yarns were quantified: this showed that without prior cellulose coating, there was no phytoalexin chelation (Supplementary Fig. 5). One can hypothesise that lignin prevents the access of the phytoalexin compound to the flax fibre cellulose

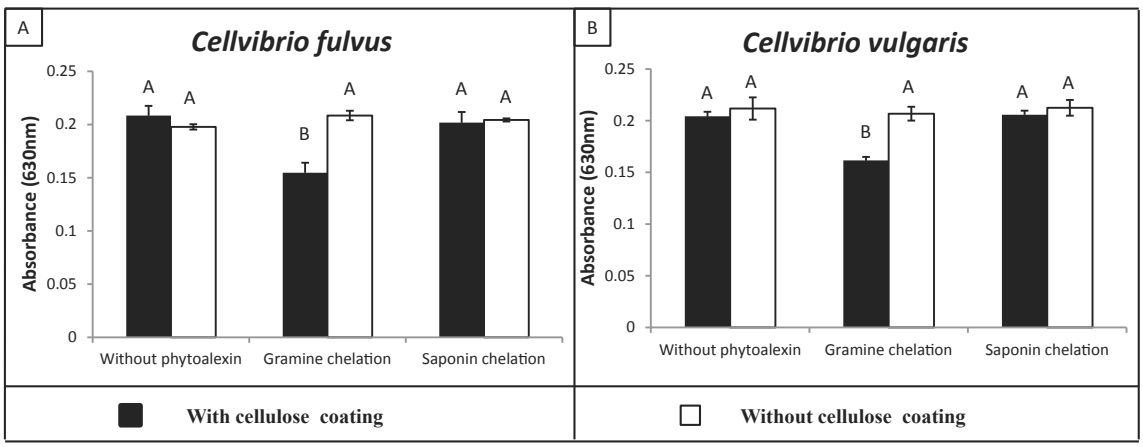


Fig. 6. A & B. *Cellvibrio fulvus* (A) and *Cellvibrio vulgaris* (B) growth after 12 h at 28 °C with an antibacterial component (gramine, saponin or control) bound on yarn with (black columns) or without (white columns) prior cellulose coating. Bacterial cell growth was estimated by the absorbance at 630 nm. Measurements associated with different letters differ significantly from each other at $p < 0.05$ using Student's test.

due to its hydrophobicity. Another possibility is a repulsion of iron III cations (needed for chelation of antibacterial molecules) by cations such as calcium, which are part of the flax fibre composition [27].

To summarise this section, the observations show that antibacterial chelation is only effective if a cellulose coating is previously applied to the flax yarns. The bacterial growth inhibition by a natural compound such as gramine could contribute to slowing down the natural degradation of the cellulose-based yarns while preserving the biodegradable aspect, since phytoalexins themselves can be degraded as they are natural plant compounds.

4. Conclusions

In this work, it was established that flax fibre yarns can be coated in cellulose by dipping. This coating was also shown to be resistant to a water flow. The cellulose coating provides a complete physical protection to flax fibre yarns, which hinders their degradation by the cellulase of one telluric microorganism. Moreover, the presence of this coating offers the possibility of binding natural antibacterial compounds such as gramine or saponin since the binding of an antibacterial phytoalexin active against Gram-negative bacteria was effective at preventing the development of cellulolytic bacteria such as *Cellvibrio fulvus* and *Cellvibrio vulgaris* cultivated in the presence of flax fibre yarns treated in this way. These treatments, only carried out using natural compounds, could therefore be used to increase the service life of flax fibre-based geonets.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.polymdegradstab.2017.02.006.

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