Fibers of the seagrass Posidonia oceanica as substrate for germination of lentils seed

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ABSTRACT

Environmental concern is increasing day by day. Industrial countries are aware of the problem wastes create and try to solve it by recycling and reusing different kind of wastes. Posidonia Oceanica is a Mediterranean endemic fiber which creates tons of wastes when collected from the sea line. This work demonstrates the feasibility to obtain substrates for plants from Posidonia Oceanica fibers. We analyse the fiber structure by SEM microscopy, the germination rate and the average germination time for lentils seeded on Posidonia substrates in comparison to the ones seeded on cotton. Posidonia fiber was studied with two different lengths of fibre and it was also studied the influence of desizing and bleaching the fiber. The values for gemination rate and germination time show similar or even better results than cotton and allow to conclude that Posidonia substrates are capable to be used for agriculture. These results offer a new application which valorise some wastes from Mediterranean coast by reusing the Posidonia fibers. Moreover, when Posidonia Oceanica fibers are draw together by means of a biopolymer, chitosan, results show the germination rate is higher than the ones without chitosan treatment and the average germination time is reduced in approximately one day. Thus, we can conclude that the germination process is faster and more effective.

KEY WORDS

Fiber, Posidonia Oceanica, germination, agriculture, nonwoven.

INTRODUCTION

Posidonia Oceanica is an endemic marine plant from the Mediterranean sea. It is easy to observe parts of the plant on the Mediterranean beaches. Posidonia Oceanica plays a critical role as it acts as a natural protection of the seabed thus preventing the advance of the erosive process [1]. Posidonia Oceanica fruit can be found on the beaches sand and it can be observed as a ball of brown fibers, as it can be seen in figure 1, which are also known as Neptune balls.

Figure 1. Neptune balls of Posidonia Oceanica algae.

These balls can be considered as a renewable resource. Considering it is part of a plant, it is made of lignocellulosic fibers. Many studies have been conducted regardin Posidonia Oceanica composition. B Ferrero et al.[2] analysed its composition and they reported Posidonia Oceanica is comprised of 70% of cellulose, 8% of hemicellulose, 12 % of inorganic components and around 10 % of lignin. This means that cellulose is the predominant component and there is a large amount of carbohydrate fraction around 90 %. Lignocellulosic-based fibres are the most widely used as biodegradable reinforcing elements for composite materials [3]. Other authors [4] found the same percentage of inorganic components but different amount of cellulose (40 %). This difference could be due to the hydrolysis method used or because of the origin where it was collected.

Every City´s Town Hall invests a high amount of money on removing this parts from the sand or beaches ground in order to keep them clean, specially in summer time. The problem is not only the money invested to keep the sand on beaches clean but to manage the tons of this product. A solution to the problem could be the use of the fibres for different purposes. Posidonia Oceanica have been studied as a low-cost adsorbent for removing dyes [5], ammonium [6] as a substrate for papermaking [4] as a starting material for cellulose derivatives [7] or as green composites [2]. It is important to valorise those fibres as Khiari et al did on their publication [3], and it can also be considered as a potential fuel for combustion [8]. However, those fibers offer a wide variety of different applications among which it could be agriculture, the one studied in this project and no publications could be found related to this purpose.

On the other hand, focusing the study on agricultural solutions and moving to seeds germination, it is important to notice that the growth of seeds is important for human subsistence. The seeds can be contaminated due to external issues such as transport, conditioning, storage, packaging, etc., and it can deal into germination problems. Mitra et al [9] conducted some studies on plasma treatment to demonstrate the importance of reducing the microbiota attached to the surface to improve the seed germination rate the speed of germination.

Therefore, the aim of this study is to determine whether the fibres from Posidonia Oceanica (PO) can be used for agriculture purposes. In this study, we prepared some substrates from Posidonia fibers and some lentils seeds were germinated in order to determine the possibility to valorise the residues as agricultural substrate. The separation of fibres from Neptune balls was conducted manually, getting what we called long fibres, or mechanically milling, treatment which offered short fibres. We prepared some Posidonia fibers by washing off them with water. Some of the fibers were tested without any other treatment whereas some were tested after a bleaching treatment. Furthermore, all the treated fibers (rinsed, or bleached) were also studied once they had been compacted as a nonwoven with chitosan. Thus, different substrates were prepared and some lentils were seeded on them and compared with cotton substrates.

EXPERIMENTAL

Materials

Posidonia Oceanica balls were collected from Oliva and Denia beaches, both located in the Spanish Mediterranean Coast at the Comunidad Valenciana. Lentils were bought from the agricultural cooperative in Cocentaina, also at the Comunidad Valenciana. They were classified as ecological agriculture. Leophen RA, was used as a wetting agent and was supplied by Basf, NaOH, Sodium Silicate and H_2O_2 was supplied by Panreac. Chitosan medium molecular weight was supplied by Sigma Aldrich and acetic acid to solubilize chitosan by Panreac.

Preparation of Posidonia fibre

Posidonia Oceanica balls were subjected to mechanical or a hand mill process to scatter the Posidonia Oceanica fibres (PO) from the ball. After the milling process fibers were washed with distilled water in order to remove sand, vegetables and other wastes caught on the ball. Clean fibres were dried in an oven at 60º C for 4 hours and kept at room temperature.

Desizing and bleaching of Posidonia fibre

Washed fibers were treated with NaOH (8 g/L, 1:40 w/v) and Leophen RA (wetting agent; 1g/L, 1:40) solution at 90-100º C for 1 hour. Later on every fiber was washed with distilled water and dried at 60º C for 4 hours.

The bleaching was applied at 1:40 w/v ratio. The liquor bath composition was:

25 g/L H_2O_2

1   g/L NaOH

0.5 g/L Leophen RA (wetting agent)

1   g/L Sodium Silicate (Na_2O 8%, SiO_2 27%) (stabilizator)

The bath was heated till 100º C and kept for 1 hour. Later on every fiber was washed with distilled water and dried at 60º C for 4 hours.

Germination process

There are standard procedures which have been used to enhance the germination of dormant seeds [10](ISTA 1985). They are based on washing and chilling. Other type of seed treatments to improve plant growth and productivity could be the ones described by Taylor et al [11]. However, this study is not based on the germination efficiency or yield consequently, in our test we used commercial lentils seeds and they were only washed.

In order to prepare the substratum 5 grams of fibre were placed on a glass vessel and four lentils were distributed approximately equidistant (see figure 2). Afterwards, lentils were covered with 5 more grams of the same fiber. Five pans were prepared for the same kind of fiber what implies a total amount of 20 seeds. The study was conducted with 4 different substratum:

- Posidoinia Oceanica washed (PO washed)
- Posidonia Oceanica washed + desized (PO desized)
- Posidonia Oceanica washed + desized + bleached (PO bleached)
- Bleached Cotton fibers (Cotton)

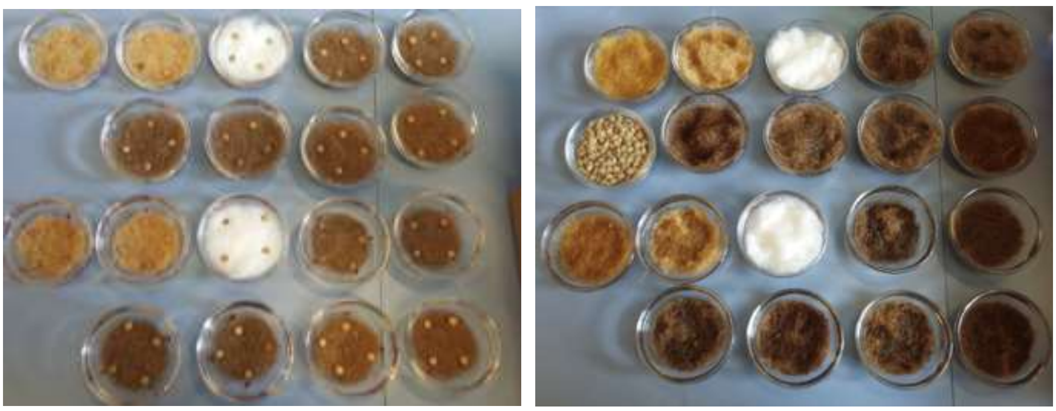


Figure 2. Lentils on the fiber in the glass vessel before covering them. a) Distribution of lentils seeds. b) Lentil seeds covered by fiber.

Germinated seeds will be counted at the same time every day. When the root reaches 10 mm, the seed will be considered germinated [12]. When germination is complete, the germination rate was calculated according to the equation (1).

$$\textit{Germination rate (\%)} = \textit{(total number of seeds germinated / total number of seeds)} \times 100 \quad (1)$$

The average germination time will be calculated according to the following formula [13]. In the formula, f is the number of seeds germinated on the census day; X indicates the number of days counted

$$\textit{Average germination time (days)} = \Sigma\,(fx) \,/\, \Sigma f \quad (2)$$

In order to ensure wetness of fibers to induce the germination process, 5 grams of water were sprayed on the upper fibers every morning for 7 days, on the 8^{th} day the germination was evaluated. Samples were kept at the laboratory at room temperature (20-23º C).

Scanning Electron Microscopy

Every sample was observed by means of electron microscopy (Scanning Electron Microscopy, SEM) with a FESEM (ULTRA 55, ZEISS). Each sample is placed on a surface and covered with a layer of gold in order to transform them into conductive by using the Sputter Coater EMITECH mod. SC7620 (Quorum Technologies Ltd., EastSussex, UK). The samples were analysed with the appropriate magnification and with an acceleration voltage of 10 KV.

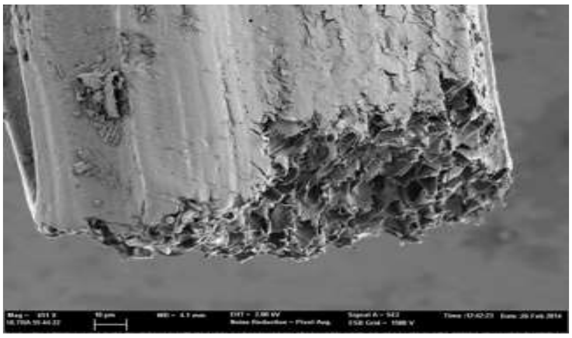
Cohesion treatment

Once the fibers had been scattered from the Neptuno ball, they were tested without any cohesive treatment however, considering the possibility to develop industrial products, if they happen to be joined together with a fixed shape they could be sold in the shape of flowerpots, etc. This consolidation has been given by a biopolymer used as a binder. In this case we used chitosan a biopolymer from the shells of shrimp and other crustaceans. We prepared a 5 g/L of chitosan which was solved by magnetic stirring for 24 hours at room temperature with 3g/L of acetic acid.

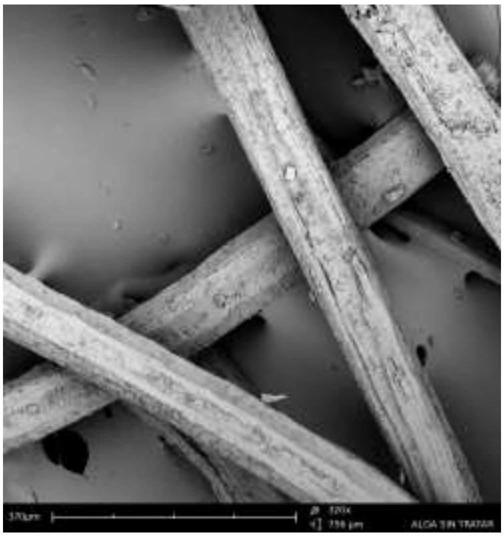
To consolidate the fibres 5 gr of PO were placed on a glass vessel and 10 mL of chitosan solution were sprayed. Later on, the glass vessel with the fibres and chitosan solution were dried in an oven at 150º C for 10 minutes. Once it was cooled we could observe the fibres were binded and the structure was consolidated and easy to manipulate. Another sample with the same kind of fibres (washed, desized, bleached or even cotton) was prepared to cover the seeds and conduct the germination test.

RESULTS AND CONCLUSIONS

Fibers were obtained by opening or disgregating the fibres from the neptune balls by hand or mechanically milling. During this process, it was clearly observed a solid residue basically comprised of sand. Posidonia Oceanica fibers were observed by SEM and it could be clearly observed (figure 3a) the fibers were comprised of a series of thin, hollow microfibres covered by a thin layer which keeps them all stikced together and compact. Moreover, it is appreciated that some particles still remain on the fiber surface once it has been washed off with water (figure 3b).



a)



b)

c)

Fig. 3 FSEM image of Posidonia fibers. a) Without treatment; b) after milling and washing c) with desizing treatment.

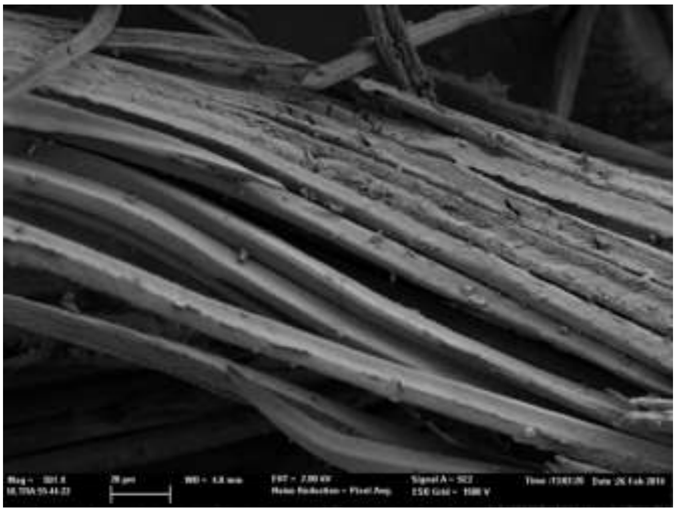


Fig. 4 FSEM image of Posidonia fibers after desizing and bleaching process

Once the desizing treatment has been applied onto the fibres, figure 3c evidences the treatment is directly affecting the coating which keeps the microfibrils joined together and compact, having applied the bleaching process on the fibres, it is clearly observed the coating surface can be practically dissolved, and only small particles from the original coating remain on the microfibres surface as it can be appreciated in figure 4.

Figure 5 reflects the ability of lentil seeds to germinate on PO fiber. It can be clearly shown how the lentil shoot appears among the brown posidonea fibers.

Fig. 5 Lentil seeds shooting among posidonia fibre. a) washed PO fibre. b) bleached PO fibre.

Regarding the germination process, table 1 shows results from the different samples tested. Stablishing comparisons between Cotton and PO it is evident that seeds on cotton germinate later (6,88) than the ones on PO (5,72-5,24). This increase on germination demonstrated the ability of PO to be used as a substratum. Moreover, it seems to be more effective than other substratums such as cotton fibers which have been used traditionally for thos experiments. This behaviour can be attributed, not only to the water retention by the cellulose, but to the water retention on the inner part of the fibrils which allows to keep more moisture on the PO fibers than on the cotton ones.

Among PO samples it seems the value is around 5. the germination time is slightly higher when the Posidonia fiber has only been washed or desized (5,72) in comparison to bleached PO (100 %). It seems that the chemical treatment on the fibre improves the germination process and allows the seeds to germinate faster. This can be due to the fact that the chemical treatment damages the coating that keeps sticked the microfibres. When the coating has been practically solved at all (PO bleached) the Average germination time (5,24) is lower than when the PO has been desized and the coating is partially damaged (5,54). Table 1. Germination values for PO, once the coating has been removed, are the highest (100%) this can be due to the fact that the fibre hosts more water and the seeds can germinate faster. The coating of the fibre protects the water to go inside, once it has been removed, it is easier for water to reach the cellulose in the fiber, and moisture is kept there.

Table 1: Germination values for PO short fibre.

	PO Washed	PO Desized	PO Bleached	Cotton
Germination rate (%)	83,33	83,33	100	58,33
Average germination time	5,72	5,54	5,24	6,88

Considering Neptuno balls had been mechanically treated to disgregate the fiber from the ball, the length was measured and it could be observed that they were considerably shorter (2-5 mm) than the original fiber (6-15 mm). In order to compare whether the length has an influence, the same test was conducted with long fibres from Neptune balls, the ones manually separated. Table 2 shows the results for the length influence. Apparently, it seems that long fiber allows the seeds to germinate slightly faster than when the seed were grown on the short fiber. This fact can be attributed to the interfibrillar space. The longer the fibre the higher interfibrillar space, and consequently more water can be kept on the substratum.

Table 2: Germination values for PO long fibre.

	PO washed	PO Desized	PO Bleached	Cotton
Germination rate	91,66	91,66	100	58,33
Average germination time	4,83	4,36	3,94	6,88

Despite PO could present some level of salinity, both table 1 and table 2 show no difference between the washed fiber or the desized one, however this salinity influence should be studied in further studies and its effect on the plant gorwth. Perhaps, salinity is not influencing the results as watter is washing it out from the fibres when it as sprayed. However, further tests will be conducted to demonstrate it. Once it has been demonstrated that PO is suitable to have some seeds grown between the fibers, a biopolymer has been used to give some cohesion to the fibres and simulate a flowerpot.

As it was described on the experimental part, chitosan was the selected biopolymer and table 3 shows the results from the germination test.

Table 3: Germination values for PO long fibre treated with chitosan.

	PO washed	PO Desized	PO Bleached	Cotton
Germination rate	100	100	100	75
Average germination time	3,82	3,39	2,84	5,56

Chitosa is known by its antimicrobial effect. Results from table 3 evidence that the chitosan treatment implies an increase in germination rate and a reduction on germination time. This can be attributed to the fact that chitosan treatment is characterised by the implementation of antibacterial properties. This effect was demonstrated by Mitra et al [9] who stated the importance of reducing the microbiota on the seed surface. Thus, it seems that the germination process is encouraged due to the presence of chitosan.

CONCLUSIONS

This study shows that there are substantial differences on the behaviour of the lentils depending on the fiber length. The lentils seeded on Posidonia substrates germinated faster than the ones in cotton demonstrating the capability of PO fiber to be used for agriculture and a possibility to revalorise the PO fiber.

However, some differences could be observed when the PO fibre had been treated. Desizing is affecting the topping shell the fibre shows on its surface, and bleaching removes it all. When the topping is altered or even removed more lentils seeds geminated and they did it faster. Probably, this fact is due to the characteristic structure of the fibres as it has been demonstrated the presence of a hollow inner, which enables to retain more moisture on the substrate.

In order to revalorise the PO fibers chitosan, a biopolymer, was used to confer cohesion to the fibres. The process allowed to obtain a nonwoven of PO binded chemically by chitosan. Regarding the germination process, we could observe the antibacterial properties, the chitosan confer when fibers are treated, improves both the germination rate and the germination time. Consequently, we can confirm this polymer used as a binder could be considered an appropriated one to be used as binder.

To sum up, we can conclude that PO fiber can be revalorised if they are considered as a substratum for different seeds, at least for lentils. Further research must be done on different seeds and with other binders in order to design the flowerpot or the plant pan.

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