Research article

Utilization of Inoculated Eri Cocoon for Water Pollutant Removal

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Abstract The eri cocoon may have the potential to immobilize effective microorganisms although it needs to be tested in order to be used as a method to remove pollutants from water. *Lactobacillus acidophilus* has proven to be capable of removing up to 60% of Arsenic (III) from water within 3 hours. The main objective of this study was to quantify the amount of glucose absorbed by inoculated eri cocoon as an indicator of the capacity for pollutant removal. *Lactobacillus* spp. was incubated at 37 ^{o}C for 48 hours. By microscopic observation, structures were described. The rate of inoculation was calculated as colony forming units (cfu) in the eri cocoon divided by cfu of the inoculation solution times one hundred. An absorption trial was set up. The absorption rate was calculated as final glucose concentration minus initial divided by time. The untreated (UT) eri cocoon presented nano-tubes where bacteria can easily penetrate. By soaking eri cocoons in distilled water (WS), cavern-like structures appeared within the silk fibers. When the eri cocoon was autoclaved (AC), the cavern structures were more frequent. The twisted yarn (TY) showed almost no difference with the fibers in the UT eri cocoon. The sample counts gave no statistical differences between UT and AC. Eri cocoon has the natural structures to accommodate microorganisms within its fibers. In the inoculation rate there was no difference between UT and AC treatments. The consumption of glucose showed no difference between UT, AC and WS; but there was a significant difference between the first three and TY. TY had the lowest glucose consumption.

Keywords pollutants, eri cocoon, lactobacillus, glucose, absorption

INTRODUCTION

Sericulture is defined as the breeding and raising of silk worms for the production of silk. There are different species of silk worm used for this purpose. This study will be focused on the eri silk worm (*Samia cynthia ricini*). The eri culture, as is called the rearing of eri silk worm, takes place in different areas of Asia and India.

In the North-East region of India, eri farmers adopt various traditional indigenous practices for rearing (Sarmah et. al., 2010). Eri culture has different uses such as a poverty alleviation strategy (Ramalakshmi, 2009) and the use of the eri pupa as a food for human consumption (Sarmah, 2011). But the main purpose of eri culture is for the production of silk.

Some studies on the structure of the silk fiber have been conducted. However, these studies have focused on the protein structure rather than the physical structure (Asakura and Nakazawa, 2004). In another study the eri cocoon was analyzed in an aqueous solution and dry-frizzed to obtain a fibroin powder to be used for its thermal properties and protein structure study (Yaowalak et. al., 2009). Asakura and Nakazawa (2004) concluded that the silk fiber has a spiral protein structure focusing in poly-Alanine chain and Glycerine areas with a Carbon and Nitrogen terminals.

Structural studies of the filaments have been done by Akai and Nagashima (2001, 2002) on different silk moths. Porous and compact filament formations are caused or regulated by continued lysosome releases which are affected by the progress of silk gland degradation (Akai and Nagashima, 2001). These porous structures and shell holes, as described by Akai and Nagashima (2001, 2002), can supposedly be present in other Saturniids as well and used as a niche for effective microorganisms.

The use of different methods for water pollutant removal has been considered one of the important actions taken to protect the environment. There have been some experiments to immobilize microorganisms and used for bioremediation (Krumme et. al., 1994; Arango, 2004; Hosseini et. al., 2007; Singh and Sarma, 2010). Most of the substrates used to immobilize effective microorganisms are non-biological porous materials. The eri cocoon has the potential to immobilize effective microorganisms and from there be used as a method to remove pollutants from water. A study with *Lactobacillus acidophilus* has proven that this bacteria is capable of removing, at a concentration of 2 mg dry wt/ml biomass, up to 60% of Arsenic (III) from a 1000 ppb water solution at pH 7 within 3 hours (Singh and Sarma, 2010). In another experiment, *Lactobacillus* spp. isolated from shrimp farm water samples was capable of simultaneous removal of pathogenic bacteria and nitrogen (Ma et al., 2009).

OBJECTIVE

The main objective of this study was to quantify the amount of glucose absorbed by inoculated eri cocoon as an indicator of the capacity for pollutant removal. Secondary objectives were: to describe the structures that eri cocoon present in order to be a niche for microorganisms; to observe the effects of different treatments on the physical structures of the cocoon fiber; and to determine the ability of eri cocoon to allocate *Lactobacillus* spp. within its structure.

METHODOLOGY

The experiment was done in a two stages stile. Stage one the description and inoculation, and stage two the glucose consumption. When analyzing the inoculation the treatments was cocoon treatments only and when analyzing the glucose consumption the statistical differences were calculated based on cocoon treatments and glucose concentration levels.

Physical description: Microscopic images were taken from different treatments of eri cocoon. From the images, physical description and differences were observed to determine the space that microorganisms could inhabit. The treatments were untreated cocoons (UT); the pupa was removed and only the silk cocoon was utilized. 1 week soaked in water (WS); the cocoon was soaked in distilled water for seven days without stirring at 25 ^{o}C. Autoclaved (AC); the eri cocoon were immersed in destilled water and autoclaved at a temperature of 110 ^{o}C and at a pressure of 152 kPa. Twisted yarn (TY); an already processed silk yarn was used.

Inoculation: *Lactobacillus* spp. was incubated and inoculated to calculate the ability of the treatments to allocate bacteria by the cocoon. The inoculation solution was added directly to the treatments by 6 ml per sample in order to cover half of the sample and allowing the other half to absorb by capillarity. The inoculated eri cocoon was incubated for 72 hours at 37 ^{o}C. Five count repetitions were made to get an average of colony forming units (cfu) per milligram of eri cocoon in order to observe the differences between treatments.

Glucose consumption: Two levels of glucose solutions were used: a high concentration of 15 g of glucose dissolved in 100 ml of distilled water; and 5 g of glucose dissolved in 100 ml of distilled

water, which equals 15% glucose solution and 5% glucose solution respectively. The inoculated eri cocoon treatments were placed in 60 ml of glucose solution and sampled at 3, 24, 48, 72, 120, 192, 288, 360, 408, 480 hours. The four treatments had four repetitions and two repetitions of control. The control treatment was distilled water instead of the glucose solution. The glucose consumption was recorded and analyzed by the SPSS 15 for windows system in a two factorial analysis. The treatments resulted in UT15%, UT5%, WS15%, WS5%, AC15%, AC5%, TY15%, and TY5%.

RESULTS AND DISCUSSION

Physical description

The UT presented entrances and nanotubes where bacteria can easily penetrate (Fig. 1a). After using a filter to observe the spaces within fibers the nanotubes were easily observed (Fig. 2). After treating the cocoon by soaking it for seven days cavern like structures began to appear within the silk fibers (Fig. 1b). Although no entrances where observed, these cavern-like structures present more suitable and larger spaces than those on the normal nanotubes present on the UT. When the eri cocoon was autoclaved the cavern-like structures were more frequent and entrances to them were observed. These cavern-like structures presented more space within the fiber and also were longer that those present on the WS treatment (Fig. 1c). The twisted yarn showed almost no difference to the fibers in the untreated, the only difference was the order that they aligned with each other making a lower exposure area (Fig. 1d).

Inoculation

In Fig. 3, the inoculation data are shown. The inoculation solution showed an average of 1.15 x 10^{16} colony forming units (cfu) per milligram of cocoon. UT and AC were not statistically different and had the higher inoculation count in cfu per milligram of cocoon. WS and TY had the lowest inoculation and were statistically different from AC and UT.

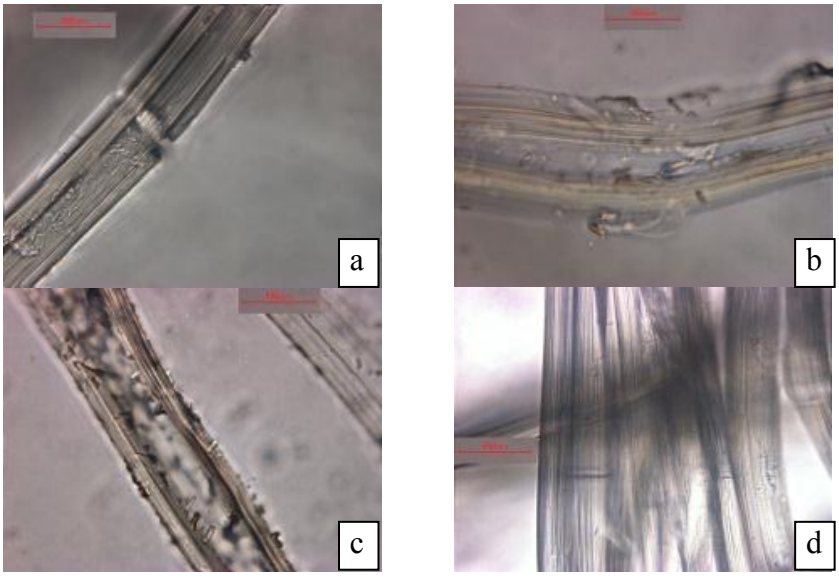

Fig. 1 Fig. 1 Microscopic cross-sectional views of eri-cocoon
(*a untreated, b 1week soaked in water, c autoclaved, d twisted yarn)*

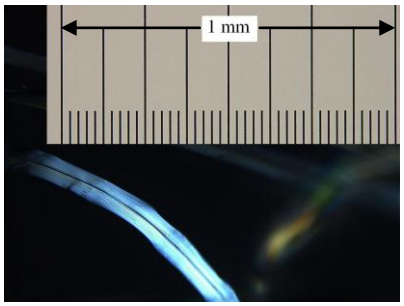

Fig. 2 Negative light filter fiber picture

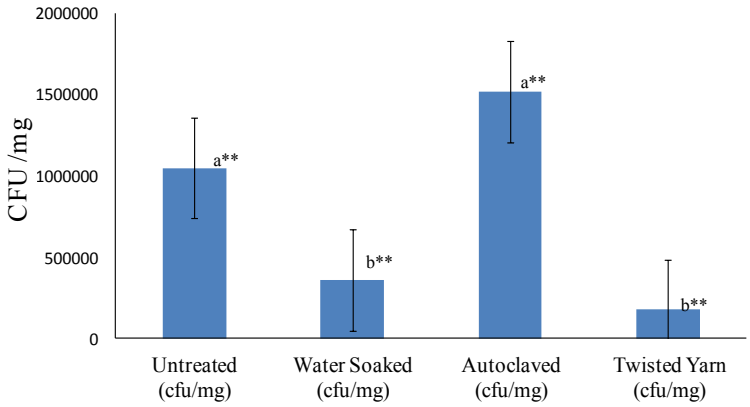

Fig. 3 Average colony forming units per milligram of eri cocoon

The alignment in TY and the treatment that it received beforehand may be the cause of such low cfu/mg count. It is also possible that if the TY was soaked in water before the inoculation this might have given a similar result to WS. WS and TY were not statisticalyl different; nevertheless, they both have the capacity to allocate a certain amount of *Lactobacillus* spp. within their structures.

Glucose consumption

The glucose consumption was taken and corrected against the control samples. There was a statistical difference in glucose consumption between UT15%, AC15%, WS15% and TY15%, UT5%, AC5%, WS5%, TY5%; TY15% was statistically different from UT5%, AC5%, WS5%, TY5%. There was no statistical difference between UT15%, AC15%, and WS15%. Within the UT5%, AC5%, WS5%, TY5% there was no statistical difference. Fig.4 shows the net consumption of glucose per treatment over time.

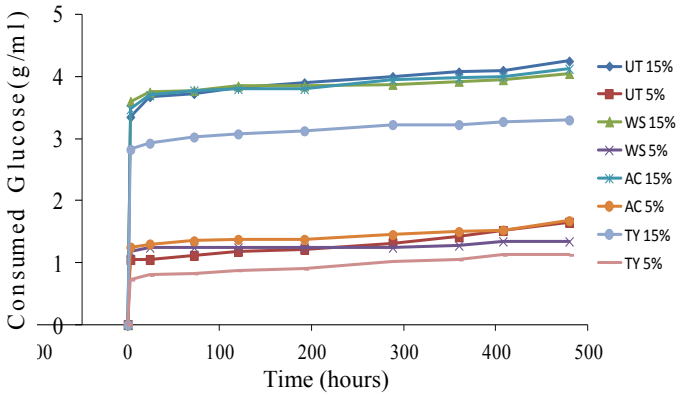

Fig. 4 Net glucose consumption by *Lactobacillus* spp. inoculated eri cocoon

CONCLUSIONS AND RECOMMENDATIONS

Eri cocoon has the natural structures to accommodate microorganisms within its fibers. Application of treatments such as soaking the eri cocoon in distilled water, or using an autoclave can improve the structures. Creation of yarn appeared to reduce the ability of eri cocoon fiber to accommodate bacteria. Eri cocoon inoculated with *Lactobacillus* spp. is able to absorb up to 29% of glucose out of a glucose solution at 15g/100ml or 15% glucose solution within 450 hours; and up to 26% within the first 3 hours.

Comparing physical structures, as the amount of space observable by microscopic imaging, and inoculation rate, the AC treatment presents the best structures and the highest rate of colony formation. Regarding inoculation rate there was no statistical difference between UT and AC which infers that the cheapest and best option for inoculation is the UT.

When comparing glucose consumption, the treatments AC15%, UT15%, and WS15% present the highest consumption. Nevertheless, using the inoculation rates as a reference point, the most effective treatments are AC15% and UT15%. Even though AC presents all the advantages for a filtering system, the use of UT is recommended to make a nitrogen absorption trial replacing glucose in the solution. The combination of UT and TY may give a better result when used together to make a net for water pollutant removal.

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