Research article

Comparison of Inoculated Eri Cocoon and Eco-block for Pollutant Removal

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Abstract The most popular medium for microorganism immobilization is eco-block. The easiest microorganism to obtain in Japan is *Bacillus natto* and used for producing food for human consumption. While, eri Cocoon has proven to be an efficient immobilization medium for bacteria. *Lactobacillus spp.* is a beneficial bacteria easily obtained in every country in the world. The main objective was to compare the performance of inoculated eri cocoon and inoculated eco-block for glucose and NO_3-N consumption. As a secondary objective we compared, through the inoculation rates, the capacity of eri cocoon and eco-block to allocate *Lactobacillus* and *Bacillus natto* separately. The methodology of this study was divided into two stages. First, a comparison of eco-block and eri cocoon's ability to allocate microorganisms within its structures; and second, the comparison of consumption between inoculated eri cocoon and inoculated eco-block. The comparison of the amount of *Lactobacillus* and *Bacillus natto* to be allocated by the eco-block and the eri cocoon, we found that there are no significant differences between the amounts of both *Lactobacillus* and *Bacillus natto*which can allocate, however, a higher inoculation rate was found in eri cocoon medium. Addition to the consumption of glucose for both microorganisms showed a significant difference between *Lactobacillus* and *Bacillus natto*.*Lactobacillus*had a higher consumption rate. When comparing within both different bacteria, the result foundthat the eri cocoon performed better against eco-block. In comparison the use of inoculated eri cocoon showed better results in both consumption of glucose and NO_3-N of both *Bacillus natto* and *Lactobacillus*. However, when comparing *Bacillus natto* and *Lactobacillus*, the last one showed better consumptions when combined with eri cocoon and *Bacillus natto* showing better results when combined with eco-block. In conclusion, pending an economical study, eri cocoon inoculated with *Lactobacillus spp.* presents the best option as a water pollutant removal tool.

Keywords eco-block, eri cocoon, *Lactobacillus spp*, *Bacillus natto*, water pollutant removal, NO_3-N consumption.

INTRODUCTION

The necessity for an effective and economic water pollutant removal tool, recently, has become an interest in the developing world. Previous studies has given information about the use of effective microorganisms for this effect (Ongley, 2000; de Vries et. al., 2008; Pebbles, 2003). These

effective microorganisms require an immobilization medium to make them easier to handle and apply.

A known microorganism immobilization medium for water filtering is the eco-block. The eco-block is any inert material where effective microorganisms can be immobilized and used for water quality improvement. Park and Tia (2004), conducted an experiment where porous concrete and industrial by-products was used for water purification. Although it was not inoculated the experimenter calculated the amount of organisms attached to the block by the consumption of dissolved oxygen. Matsunaga et.al. (2006), presented data where concrete eco-block inoculated with *Bacillus natto* performed better than regular block for water quality improvement. The use of immobilized microorganisms in blocks is usually for bio-filtration systems (Cohen, 2001).

Although, most of the substrates used to immobilize effective microorganisms are non-biological porous materials. The eri cocoon has the structures to immobilize *Lactobacillus spp.* within its fibers (Mendoza Tovar et. al., 2013); it was also determined the best treatment for the eri cocoon to be used. A study with *Lactobacillus acidophilus* has proven capable of removing up to 60% of Arsenic (III) from water solution within 3 hours (Singh & Sarma, 2010). In another experiment, *Lactobacillus spp.* isolated from shrimp farm water samples were capable of simultaneous removal of pathogenic bacteria and nitrogen (Ma, et.al., 2009)

OBJECTIVE

The main objective of this study was to compare the performance of eri cocoon and eco-block as porous mediums in glucose and NO_3-N consumptions.

As a secondary objective, was to compare eri cocoon and eco-block based on the amount of bacteria that can be allocated through the inoculation rates from both *Lactobacillus* and *Bacillus natto*.

METHODOLOGY

The methodology of this study was divided into two stages; the first was the comparison between two mediums, eri cocoon and eco-block, to allocate microorganism, and second was the comparison of consumption of glucose and NO_3-N between inoculated eri cocoonand eco-block (Fig. 1).

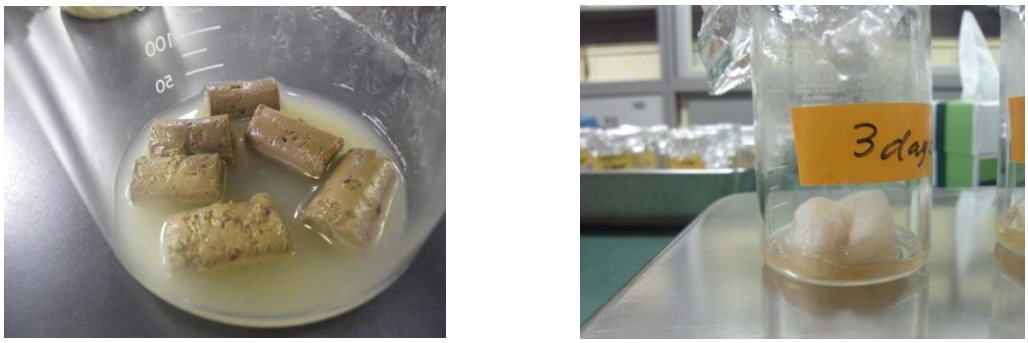
eri cocoon **eco-block**

Fig. 1 Immobilization mediums

Both eco-block and eri cocoon were inoculated with *Lactobacillus spp.* and *Bacillus natto* and cultivated for 3 days before making a dilution method to count the colony forming units of each one. Both microorganisms had the same management for both immobilization mediums.

The inoculation solution was added directly in order to cover half of the sample and allowing the other half to absorb by capillarity. The inoculated samples were incubated for 72 hours at 37 ^{o}C

(Fig. 2). Five count repetitions were made to get an average colony forming units (cfu) per milligram of both mediums in order to observe the difference.

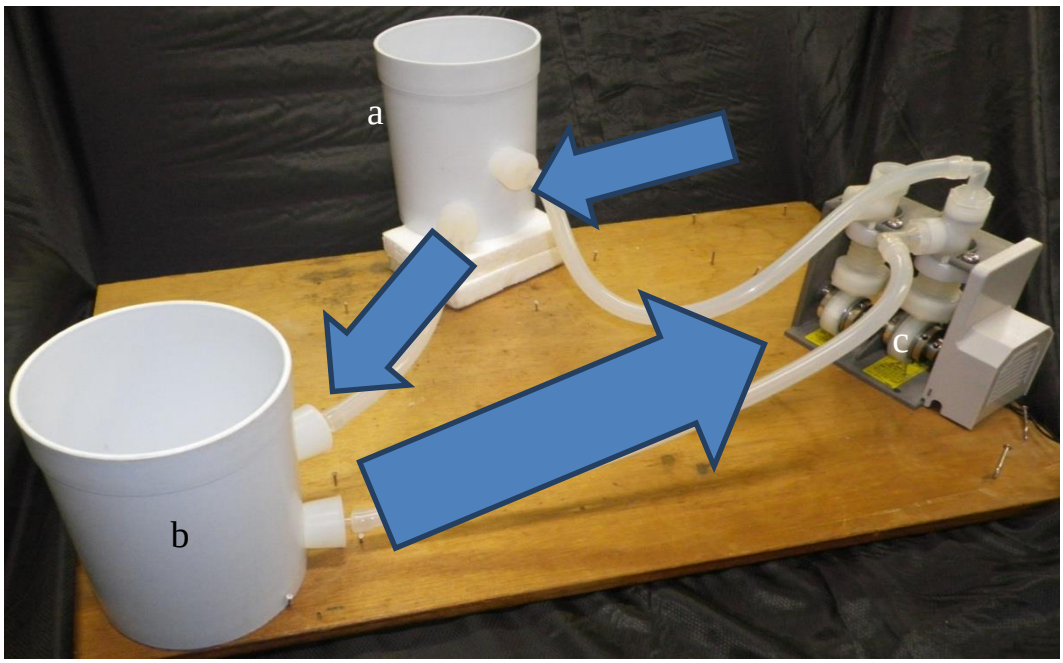


Inoculation of eco-block **Inoculation of eri cocoon**

Fig. 2 Inoculation method

Simultaneous consumption experiments for both inoculated immobilization mediums by *Lactobacillus* and *Bacillus natto* were carried out in a water recirculation system to simulate water movement in a reservoir (Fig. 3). Glucose at two level of concentration; low concentration was of 5% glucose solution and high concentration of 15% glucose solution. NO_3-N was determined at 10 mg/lat 0 hours before data collection. The data was collected at 3, 12, 24 and 48 hours and analyzed within 24 hours of the sample taking.

TheSPSS Statistics Version 19 was used to analyze the significant differences and comparisons among different microorganism, mediums, and consumptions.



a. *Medium application reservoir* ***b.*** *Solution application reservoir* ***c.*** *Water pump* ⇒ *Water flow*

Fig. 3 Experiment water recirculation system

RESULTS AND DISCUSSION

Within the inoculation rates (cfu/g), there was a significant difference between the eri cocoon and eco-block (Fig. 4) placing the eri cocoon as the best immobilization medium between them. Nevertheless, the low mass of ericocoon is an advantage over the eco-block when comparing them

by the same unit of mass of inoculated medium. It is also suspected that the clay material of which the eco-block consists may have had an effect on the immobilization ability or inoculation rate.

Even though there was no significant difference between bacteria allocation within each medium, *Bacillus natto* presents a higher rate in both mediums, hinting that *Bacillus natto* may be more effective in the immobilization process.The document by Matsunaga et.al. (2006) presented no inoculation rate of *Bacillus natto* in the concrete block used in his experiment; however, observing the dissolved oxygen in the document, it can be inferred that during the experiment they multiply after application.

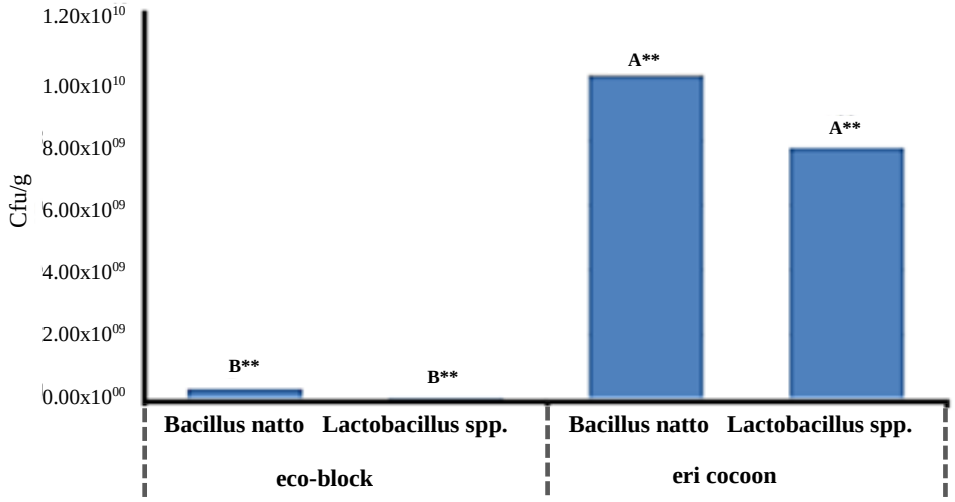


Fig. 4 Comparison of eri cocoon and eco-block by inoculation rate

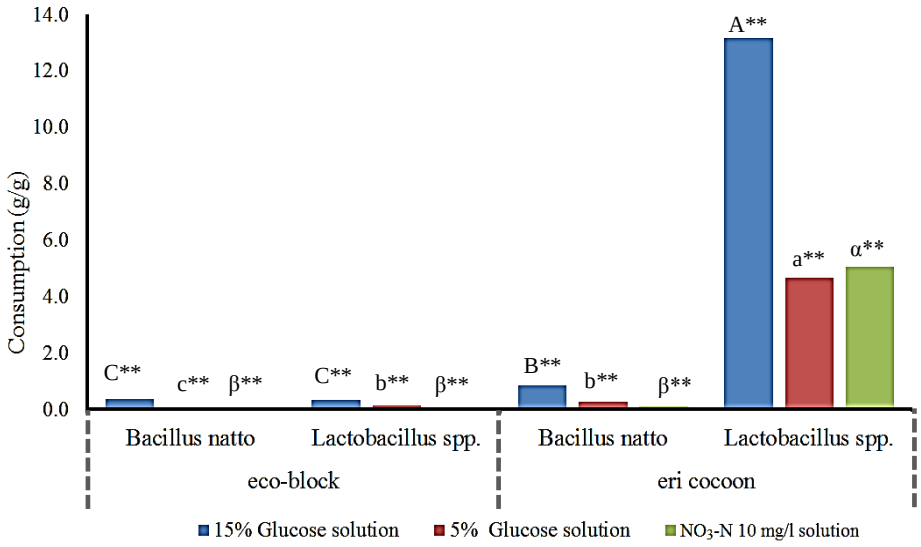


Fig. 5 Consumption comparison of inoculated eri cocoon and eco-Block

Fig. 5 shows the consumptions of both glucose and NO_3-N by both inoculated mediums. It is observed that the consumption of eri cocoon inoculated with *Lactobacillus* has a significant difference when compared with the other combinations. The differences in consumptions can be assigned to the *Lacto bacillus* consumption due to the lack of significant difference between inoculation rates within both mediums

The consumption of NO_3-N of eco-block in this experiment is better than the results by Matsunaga et.al. (2006) where after 24 hours of application there was an increase of NO_3-N.

During our experiments the consumption was observed to stabilize after 24 hours in the case of eco-block with both *Bacillus natto* and *Lactobacillus spp.* but there was no release of NO_3-N observed.

Overall in the consumptions ericocoon behaves better than eco-block having higher consumptions even if, when inoculated with *Bacillus natto,* does not present statistical difference. It is also observed that the consumption of glucose at low concentration presented a significantdifference between *Lactobacillus* and *Bacillus natto* inoculated in eco-block placing *Lactobacillus* in a higher consumption.

CONCLUSIONS AND RECOMMENDATIONS

As there was no significant difference between both microorganisms within the immobilization mediums while, a significant difference between mediums was found, the best immobilization medium by inoculation rate is eri cocoon. Due to the fact that it can allocate statistically the same amount of microorganisms in a smaller mass and volume than the eco-block. This sturdiness and lightness of the eri cocoon as an immobilization medium would make it easier to manage and to transport for any field application.

The eri cocoon inoculated with *Lactobacillus spp.* has the highest consumptions of all of the solutions. This infers that the best option to remove pollutant or create an economic pollutant removal tool is the eri cocoon inoculated with *Lactobacillus spp.Lactobacillus spp.* proved that is more effective at consuming nutrients from a water flow than *Bacillus natto*, nevertheless *Bacillus natto* may have with a different immobilization medium such as concrete have a better consumption results.

It is recommended that the tool may be utilized as a bio-string contactor with inoculated eri silk yarn; the combination of untreated eri cocoon and eri silk twisted yarn may give a better result when used together in water flow. The application could be by a spillway that ensures the contact of the bio-string contactor and the water for enough time for it to consume the pollutants in the water.

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