



## Moringa-Functionalized Rice Husk Ash for Potential Use in Water Disinfection

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**Abstract:** Moringa oleifera, a tree indigenous in tropical and sub-tropical areas, has been widely used in water purification in many low-income regions such as Africa, India, and Asia. Its seeds are known to contain proteins that possess coagulating and antimicrobial properties but its use in water treatment is compromised by the co-release of soluble organics in the seeds. These organics can serve as food for pathogen regrowth, causing problems with the storage of treated water. The current study investigated the potential for selective functionalization of the Moringa protein onto rice husk ash (RHA). RHA was soaked in different concentrations of Moringa solution until equilibrium sorption was achieved. The dissolved protein in solution was measured using an optical density meter at 280 nm. Moringa sorption on the RHA surface was also examined under a confocal laser scanning microscope (CLSM) by tagging the protein with green fluorescent dye. The potential leaching of bound Moringa was investigated by taking TOC (total organic carbon) measurements in water before and after addition of the functionalized RHA. Results show a strong potential for eliminating undesired organics in treated water through Moringa protein sorption onto RHA. Further, the Moringa protein binds strongly onto the RHA surface, and that the sorption process is irreversible. The antimicrobial property of the Moringa-functionalized RHA was also tested using different *E. coli* in water ( $10^3$ - $10^5$  CFU/mL). The functionalized RHA exhibited bactericidal property, and high *E. coli* removals were observed (%). *E. coli* removal in the control samples (bare RHA) was also observed but further CLSM analysis of tagged *E. coli* showed that the *E. coli* was inactivated in the functionalized RHA but not on the bare RHA. In summary, these results indicate a potential solution for the storage problem of water treated with Moringa, and that Moringa functionalization can effectively enhance the capability of typical adsorbents such as rice husk ash which inherently cannot inactivate bacteria.

**Key Words:** Moringa; rice husk ash; water disinfection

## 1. INTRODUCTION

Populations around the world, especially poor communities located in tropical and sub-tropical regions, depend on untreated sources of drinking water such as surface water and ground water. Even with the achievement of the global water target under the UN Millennium Development Goals, large populations worldwide are still affected by common water borne diseases such as diarrhea. Approximately 1.7 billion reported cases of diarrhea, which accounts for 760,000 child deaths each year, (WHO, 2013) prove that safe access to drinking water is still significantly low. The number of people affected emphasizes the immediate need for a cost-effective water treatment technology.

A potential technology that could potentially increase access to safe drinking water is MO-based water treatment using MO seeds. MO seeds are known to exhibit coagulation and flocculation properties and these have been extensively studied by various literature. It has been reported that the MO seed is able to remove turbidity (Ndabigengesere et al., 1995) and demonstrate bactericidal activity (Suarez et al., 2005). MO seeds have also been shown to improve water quality by reducing water hardness (Muyibi and Evison, 1995) and removing toxic metal ions such as arsenic in drinking water (Kumari et al., 2006). Furthermore, high removal of anionic dyes observed indicates its versatility in various wastewater applications (Beltrán-Heredia and Sánchez Martín, 2008). Collectively, these facts support the use of MO seeds as an alternative water treatment technology.

The capability of MO to purify water is due to a coagulating/flocculating protein found in its seeds (Gassenschmidt et al., 1995). While this protein is essential in MO water treatment, the seed also contains soluble organics that are introduced into the treated water (Okuda et al., 2001; Ndabigengesere and Narasiah, 1998). This causes a problem where additional treatments steps are necessary MO seed application. These organics need to be removed to improve overall water quality, prevent regrowth of bacteria, and prolong water shelf-life. Therefore, elimination of these post treatment steps is the main challenge in using MO seeds to produce potable water.

This research investigates the immobilization of the MO protein (MOP) onto RHA. This method is seen as a means to eliminate consequences associated with the use of MO seeds.

Once MOP is extracted from the seed, it adheres to the surface of the bare RHA, and the functionalized RHA is subsequently used in water disinfection. Saturation level of MOP on bare RHA was investigated since literature have not yet established these parameter. Batch Escherichia coliform (E. coli) experiments were performed to confirm whether the antimicrobial properties of the immobilized MOP were retained.

## 2. METHODOLOGY

RHA was investigated as a potential adsorbent for MOP immobilization. RHA was kindly provided by Central Luzon State University and whole MO seeds were purchased from Bureau of Plant Industries. The particle size of the MO seeds were reduced using mortar and pestle and were only crushed and powdered prior to experiments to minimize ageing effects. Milli-Q deionized water was used in all batch experiments.

### *2.1 Batch MOP Adsorption Experiments*

RHA was functionalized using a modified method described in literature (Jerri et al., 2012). MOP saturation levels were investigated by preparing aqueous MOP solutions of different concentrations ranging from 0.25 to 1.7 mg/ml. Heavy matter was allowed to settle naturally for an hour and MOP supernatant was carefully collected afterwards. Bare RHA was incubated with this supernatant at different MOP concentrations until equilibrium was reached. Initial and final concentrations of MOP were read at 280 nm.

### *2.2 Batch Disinfection Experiments*

Bacterial contamination was simulated using deionized water using Escherichia coli (ATCC® 11775™). This was grown in liquid medium for 12 hours at 37°C to an approximate concentration of 10<sup>8</sup> CFU/ml. The concentrated bacterial solution was diluted using phosphate buffer solution to achieve a final concentration of 10<sup>3</sup>, 10<sup>4</sup>, and 10<sup>5</sup> CFU/ml. Previous coliform analysis of water samples from a

local municipality in the Philippines was used as basis for coliform concentration. 1 g of functionalized RHA (in duplicates) was suspended in the bacterial solution and this was gently shaken until equilibrium. As a control, batch disinfection experiments were also conducted using bare RHA.

### 2.3 TOC Experiments

MOP aqueous extracts were prepared at concentrations ranging from 0.25 to 1.7 mg/ml. 30 ml of these extracts were subjected for TOC analysis. The MO functionalized RHA was suspended in 30 mL deionized water and was gently shaken until equilibrium was achieved. The TOC of the MOP aqueous extract was compared to the TOC of the supernatant of the functionalized RHA suspension.

### 2.4 CLSM Experiments

Functionalization of RHA was evaluated by tagging the adhered MOP with negatively charged microfluorescent particles. The functionalized RHA was washed vigorously prior to imaging to assess the retention of MOP on the surface of RHA. Bacterial staining experiments were also conducted to assess the degree of disinfection on the surface of functionalized RHA and as well as bare RHA. The retention of MOP bactericidal activity was indicated by the viability of *E. coli* adhered on the surface of RHA.

## 3. RESULTS AND DISCUSSION

### 3.1 MOP Functionalization of RHA

The highest MOP adsorption capacity was found to be 32 mg/g RHA. The adherence of MOPE onto the surface of RHA was evaluated by tagging the proteins with green fluorescent dye. Results of CLSM imaging confirmed the presence of MOP on the RHA surface. The adsorption of MOP onto the RHA surface was due to difference in electrostatic forces between the protein and adsorbent. The

binding of MOPE onto the active sites of RHA is defined by dense and thick layers of proteins that are indicative of cooperative adherence of each protein molecule (Kwaambwa et al., 2010).

### 3.2 *E. coli* Removal of Functionalized RHA

Figure 1 shows the results of batch disinfection experiments. High *E. coli* removal was achieved for  $10^3$  CFU/ml *E. coli* solution but removal was observed to decrease as functionalized RHA was subjected to higher concentrations of *E. coli*. This indicated that the functionalized RHA was reaching saturation. Higher initial *E. coli* concentrations is expected to further decrease the *E. coli* removal due to the decrease in MOP levels on the RHA surface as bacterial membranes continue to interact with MOP. The viability of adhered bacteria on the functionalized RHA surface was also assessed. Results of imaging showed that *E. coli* cells were disrupted and destroyed as it was adsorbed onto the functionalized RHA surface. These results indicate that the immobilized MOP still retained its antimicrobial properties. In addition, although *E. coli* removal was also observed for bare RHA, imaging results revealed that *E. coli* cells remained viable on the bare RHA surface. This implied that regrowth of bacteria is still highly probable even after bacterial sorption using bare adsorbents and poses risks such as bacterial recontamination if it is not managed effectively.

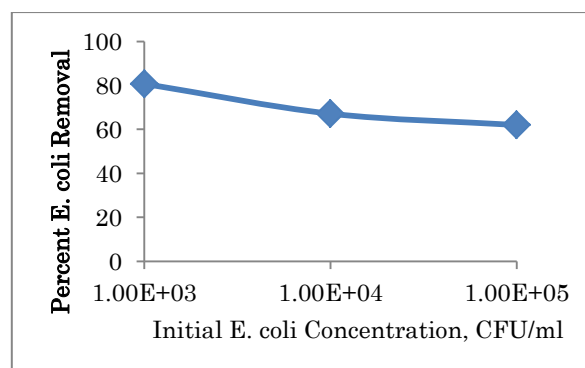


Figure 1. *E. coli* Removal of Functionalized RHA at Different Initial *E. coli* Concentrations



### 3.3 TOC Comparison

Results showed that 99.67% TOC removal was achieved by immobilizing MOP onto the RHA surface. This inferred that adsorption of MOP onto a matrix is an effective solution of eliminating the consequences of unwanted release of residual organics into the treated water.

## 4. CONCLUSION

Results of this study have demonstrated that immobilization of MOP onto RHA could eliminate the problematic applications of MO seed water purification. The protein is selectively adsorbed onto the sorbent due to its high diffusion coefficient and electrostatic charge (Jerri et al., 2012). These findings suggest a promising solution to the bacterial regrowth and water storage life problem associated with the application of MO seed in water treatment.

## 5. ACKNOWLEDGMENTS

This work was partially funded by the Grand Challenges Canada (Grant number S6 0505-01-10).

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