MATEC Web of Conferences **268**, 01005 (2019)
RSCE 2018
https://doi.org/10.1051/matecconf/201926801005

Moringa-functionalized rice husk ash adsorbent for the removal of amoxicillin in aqueous solution

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Abstract. Lack of stringent policies requiring water treatment facilities to combat occurrence of residual antibiotics in effluents critically impairs the resiliency of low-income communities to drug-resistant pathogens. In an attempt to mitigate the effects of residual drugs in aqueous media, we investigate the extent to which rice husk ash (RHA) functionalized by Moringaoleifera protein (MOP) sequestrates amoxicillin in solution.A semi-factorial design was implemented to evaluate the influence of initial amoxicillin concentration, initial MOP functionalized RHA dosage, and contact time on the removal on amoxicillin in water. Results of our experiments have shown that MOP functionalization enhanced RHA by doubling its rate to sequestrate amoxicillin molecules in solution. This strongly indicated that MOP adhered on the surface of RHA significantly improved its capacity to remove amoxicillin contamination in aqueous solution. Statistical analysis employed further supported our results by implying a significant difference between the performance of MOP-functionalized and bare RHA. In conclusion, our results strongly suggest that MOP functionalization can be a potential practical solution to alleviate the vulnerability of communities to emerging antibiotic pollution.

1 Introduction

Apprehensions to the occurrence of pharmaceuticals as emerging pollutants have grown significantly due to their toxicity and persistence in the environment [**1-2**]. Currently, about 4000 pharmaceuticals are known to be actively consumed by humans and animals [**3-4**]. After consumption, these pharmaceuticals are being introduced into diverse environmental systems through wastewater treatment plant and sewage effluents [**5-6**]. The prevalence of these compounds in environmental matrices then changes the behavior of natural flora and fauna in the environment [**7-8**]. As a consequence, the natural metabolism of organisms is disrupted and this induces propagation of antibiotic-resistant microorganisms [**9-10**]. This therefore increases the risks of developing superbugs in the environment.

Developing countries (i.e. Philippines) are seen to be most vulnerable to this emerging problem. As published literature suggests, advance oxidation processes, such as photocatalysis, are the primary treatment methods that efficiently removes antibiotic residuals in aqueous solutions [**11-12**]. These processes however provide numerous technical barriers which often impede their full implementation and operation [**13**]. As a result, these countries may not yet be ready to mitigate this growing environmental concern. There is then a need to explore an alternative treatment method that may be suitable to the conditions and technical capacity of these developing countries.

Rice husk ash (RHA) is known to be a low-cost adsorbent common to agricultural and developing countries. Its capacity to remove various contaminants in solution has already been demonstrated in literature [**14**]. For example, RHA has been proven to effectively sequestrate dyes in aqueous media [**15**]. In a different study, RHA was shown to achieve a high removal of heavy metals in solution [**16**]. RHA has also been used for the removal of selected organic pollutants in water [**14,17**]. Moreover, related studies have also explored modification of the RHA surface to enhance the adsorptive capabilities of RHA as nanoporous adsorbents in the field of biomedicine [**18**]. Collectively, these evidences establish the potential of RHA as an effective adsorbent suitable to various environmental applications.

RHA has also been demonstrated to be an effective excipient in the pharmaceutical industry [**19**]. Due to its high silica content and good stability as a porous medium, RHA has been shown to have high affinity for pharmaceuticals [**20-21**]. However, despite this fact, the utilization of RHA in removing residual pharmaceuticals in water has been very limited. To address this gap, we extend the application of RHA to the removal of amoxicillin residuals in solution. In the present work, we test the extent to which functionalization of RHA by Moringaoleifera proteins (MOP) can effectively enhance the adsorptive capacity of bare RHA. We have previously presented that moringa-functionalization can be an effective way to enhance the adsorptive capability of RHA to various contaminants in water [**22-25**]. We also apply this methodology to assess the extent of amoxicillin removal using the moringa-functionalized RHA.

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2 Materials and methods

2.1 Materials and reagents

RHA used in this study was kindly provided by a local power plant in Camarines Sur, Philippines. The RHA was sieved to achieve a uniform particle size (30-50 mesh size) and the sieved particles were vigorously washed with distilled water to remove excess carbonaceous materials. Whole moringa seeds used in this study were purchased from local suppliers in Pangasinan, Philippines. Dry seeds that exhibited no signs of discoloration, softening, and extreme dessication were carefully selected to ensure the freshness of the seeds [**26-27**]. The shell and wings of the moringa seeds were removed and the seed kernels collected were powdered using a domestic blender. Amoxicillin Trihydrate (AR grade) was used to prepare solutions that would simulate the behavior of amoxicillin in aqueous medium.

2.2 Preparation of moringa-functionalized rice husk ash

Functionalization of RHA was carried out according to a method described in our previous work [**24**]. Powdered moringa seeds were mixed with distilled water and the resulting mixture was gently shaken for one hour at room temperature. Insoluble matter was allowed to settle for another hour and the supernatant solution collected was filtered to remove floating particles. This filtered solution was used as a source of active MO proteins. MO protein saturation on the surface of RHA was achieved by soaking RHA in known concentrations of MO solutions (400-1700 mg/L). Sorption equilibrium was observed by reading initial and final equilibrium concentrations of MO proteins in solution at 280 nm [**25**].

2.3 Preparation of amoxicillin solution

Amoxicillin stock solution with a concentration of 1000 ppm was prepared by mixing 1.14 g of amoxicillin trihydrate with distilled water. Amoxicillin solutions with lower concentrations (100-900 ppm) were prepared by serial dilution. Desired concentrations achieved were read by performing a full wavelength scan using a Perkin Elmer Lambda 45 UV-Vis spectrophotometer. The highest absorbance recorded was attained at a wavelength of 229 nm.

2.4 Experimental design

A time-series experiment was employed using the functionalized RHA to determine at what point amoxicillin removal in solution reaches equilibrium. Functionalized RHA was soaked in amoxicillin solution of known concentration and this suspension was gently agitated for a total of 90 minutes. Sampling was carefully done at an equal interval of 10 minutes. Once equilibrium was established, a semi-factorial design was implemented to investigate factors (see **Table 1**) influencing the magnitude of amoxicillin removal using functionalized RHA. Effects of these factors to the response were assessed through analysis of variance. Control experiments were also employed. All experimental runs were performed in duplicates.

Table 1. Factors influencing amoxicillin removal.

Factor	Unit	Factor Level		
		Low	Medium	High
Adsorbent Dosage	g	0.5	1.0	1.5
Initial Amoxicillin Concentration	ppm	100	200	300
Contact Time	min	30	60	90

3 Results and discussion

3.1 Effect of contact time

The highest percent amoxicillin removal attained at a moringa-functionalized RHA dosage of 0.5 g was 86%. The amoxicillin molecules in solution were found to be destabilized and sequestrated at a fast rate when RHA was functionalized by MOP. As shown in **Fig. 1**, equilibrium was already achieved at 10 min. In a separate experiment, we found that bare RHA was able to reach equilibrium at 20 min. The rate of removal of amoxicillin molecules were observed to have doubled when MOP was allowed to immobilized on the surface of RHA. Although the presented evidence is not sufficient for a concrete explanation, we hypothesize that a similar sequestration mechanism described in literature is displayed in these results [**22-25, 28**], have revealed that the chemical structure of MOP suggests that the tail of this protein does not display any coagulation capabilities. This tail may have been the part of the MOP that attached to the surface of RHA and allowed MOP to be immobilized. As a result, the active component of the MOP was allowed to freely move and display its coagulation capacity which enhanced the adsorptive capability of RHA.

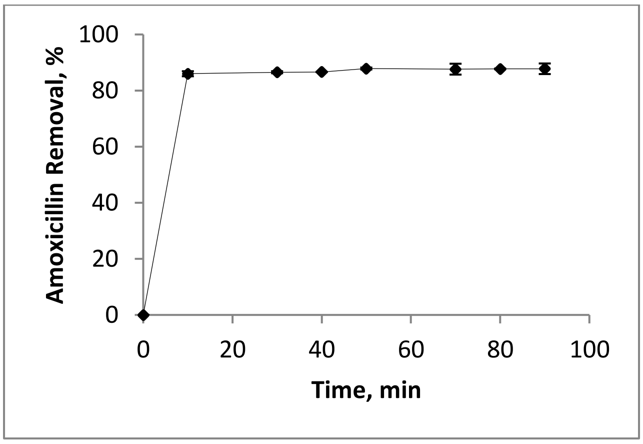

Fig. 1. Effect of Contact Time on Amoxicillin Removal; moringa-functionalized RHA dosage 0.5 g; solution volume 20 mL; temperature 25°C; mixing speed 150 rpm; initial amoxicillin concentration 100 ppm.

3.2 Effect of adsorbent dosage

The highest percent amoxicillin observed was attained at an adsorbent dosage of 1.5 g. As presented in **Fig. 2**, it was noted that the percent amoxicillin removal also increased as adsorbent dosage increased. This observed results indicated that higher adsorbent dosages were found to be favorable for amoxicillin removal in solution using bare and moringa-functionalized RHA. Essentially, increasing the adsorbent dosage also increased the surface where amoxicillin molecules can interact with either bare or moringa-functionalized RHA. The existing driving force for the interaction of the adsorbent and adsorbate molecules was also increased with a wider platform introduced. This would then result to a positive effect on the amount of amoxicillin molecules removed in solution.

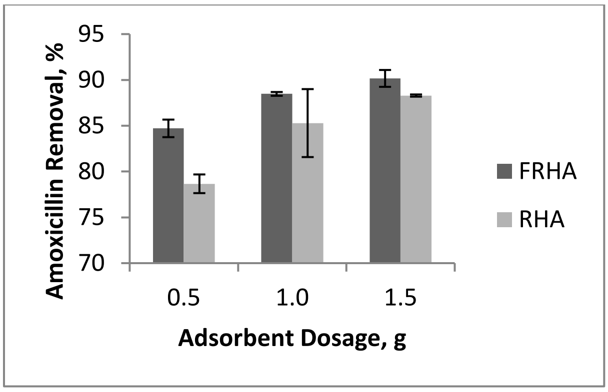

Fig. 2. Effect of Adsorbent Dosage on Amoxicillin Removal; contact time 30 min; solution volume 20 mL; temperature 25°C; mixing speed 150 rpm; initial amoxicillin concentration 100 ppm.

3.3 Effect of initial amoxicillin concentration

The highest percent amoxicillin removal displayed by both moringa-functionalized and bare RHA was observed at the lowest initial amoxicillin concentration (100 ppm). As shown in **Fig. 3**, the lowering of percent amoxicillin removal as initial amoxicillin concentration was increased showed that each adsorbent tested was already nearing saturation points. Since each adsorbent has only a limited number of pores for adsorbent and adsorbate interactions, the increase in amoxicillin molecules in solution can no longer be accommodated by the fixed amount of adsorbent introduced in solution. This then showed that an increase in adsorbent dosage would also be needed to increase the removal of amoxicillin molecules in solution. Hence, higher initial amoxicillin concentrations would require higher dosages of each adsorbent introduced in solution.

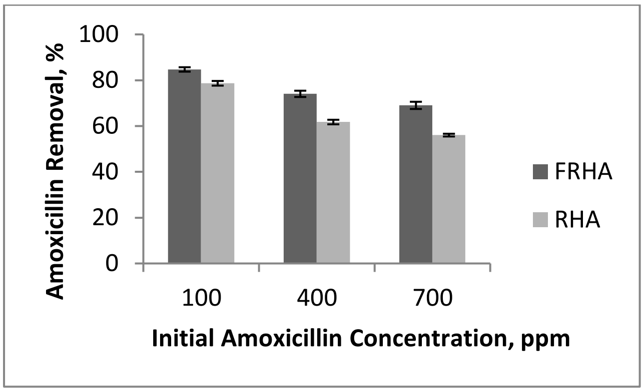

Fig. 3. Effect of Initial Amoxicillin Concentration; adsorbent dosage 0.5 g; contact time 30 min; temperature 25°C; mixing speed 150 rpm; initial amoxicillin concentration 100 ppm.

3.4 Statistical analysis

To assess whether functionalizing RHA with MOP enhanced percent amoxicillin removal of RHA, a one-way analysis of variance (ANOVA) was implemented. As shown in **Table 2**, moringa-functionalization of RHA did not have a significant effect (at a reference P-value of 0.05) on amoxicillin removal when the adsorbent dosage and initial amoxicillin concentration was varied. Although moringa-functionalized RHA gave higher percent amoxicillin removals than its bare counterpart, results of ANOVA strongly suggest that the performance of these adsorbents was relatively equal to each other. However, a p-value of 0.0051 for the performance of both adsorbents in terms of contact time highly indicated a significant difference between the performances of each adsorbent. As previously presented, functionalization with MOP allowed amoxicillin molecules to be sequestrated rapidly by RHA. On a large-scale operation perspective, a lower equilibrium time would allow lower costs in operation and manpower needed to remove amoxicillin molecules in effluent wastewater. This makes functionalization with MOP a viable alternative on enhancing shortening equilibrium adsorption time of commonly used adsorbents in the industry. Overall, evidences of higher percent removals and faster adsorption rates show that the adsorptive properties of RHA were successfully enhanced by MOP.

Table 2. Statistical Analysis of Factor Effects

Factor	P-Value	Remarks
Contact Time	0.0051	Strongly Significant
Adsorbent Dosage	0.32	Not Significant
Initial Amoxicillin Concentration	0.26	Not Significant

4 Conclusion

Utilization of RHA in removing pharmaceuticals in aqueous media has been very limited. In the present work, we have demonstrated that RHA can also be used as a viable alternative to capture residual drugs (i.e. amoxicillin) in solution. Moreover, we have presented evidences that MOP functionalization was seen as a potential approach to further enhance the potential of RHA as a low-cost adsorbent for these emerging pollutants. Collectively, this study contributed to research by extending the application of RHA as an adsorbent to remove pharmaceutical drugs in water.

We would like to thank Bicol University College of Science for allowing us to use their UV-Vis spectrophotometer and Bicol University College of Engineering – Chemical Engineering Department for granting us permission to use their facilities.

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