

Moringa oleifera f-sand Filters for Sustainable Water Purification

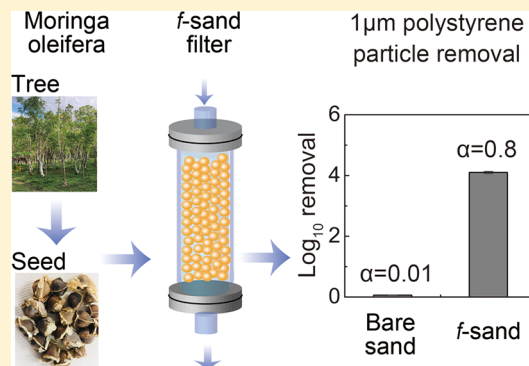
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Supporting Information

ABSTRACT: The purpose of this work is to determine parameters for the design of a *Moringa* seed sand filter for water purification. *Moringa oleifera* seeds containing cationic antimicrobial proteins have been used as natural coagulants for the removal of turbidity; however, a low removal efficiency and high residual organic levels limit their applications. In this work, *Moringa* seed extracts were used to reverse the charge of sand (*f*-sand) to 10 mV at a seed dosage of 5.6 g of seeds/m² of sand. This *f*-sand filter demonstrated ~4 log removal of 1 μ m polystyrene particles and >8 log removal of *Escherichia coli* compared to <0.1 log removal for bare sand. Enhanced removal for particles and *E. coli* was dominated by attractive electrostatic interactions. Clean bed filtration modeling predicts a sticking coefficient (α) of 0.8 for *f*-sand compared to a value of 0.01 for bare sand. This α was further validated under a wide range of filtration conditions. Preliminary scale-up analyses suggest a point-of-use *f*-sand filter that requires a very small amount of seeds annually. The outcome of this work presents the scientific basis for the design of a water purification solution for developing regions, requiring only locally available resources and no use of synthetic chemicals or electricity.



INTRODUCTION

The World Health Organization (WHO) has reported that 2.2 million deaths were caused by waterborne diseases in 2016.¹ The employment of point-of-use technologies for household level water purification reduces diarrheal disease by 30–40%;^{2,6} proposed techniques include boiling, chlorination, biosand filters, and ceramic filters.^{3–5} Nevertheless, their application in developing areas remains limited because of the inaccessibility of available raw materials. Therefore, it is critical to provide water purification technologies that are affordable, effective, and derived from locally produced materials to provide safe water in the developing world.

Moringa oleifera grows widely in many equatorial regions of the world where public health is threatened by unsafe drinking water.^{7–9} The seed contains cationic and antimicrobial proteins (MOCP), which comprise 1.2% of the total protein and are readily soluble in water.¹⁴ The antimicrobial properties are caused by the presence of a helix–loop–helix motif that causes fusion of inner and outer cell membranes.¹⁴ Because of these unique properties, MOCP has been used as coagulants/flocculants for turbidity.^{10–19} However, its application as a flocculant is challenged by the fouling of treated water over time as a result of the residual organic matter released from seeds.^{10,20} Our previous work showed that MOCP can be adsorbed onto sand via electrostatic attraction creating functionalized sand (*f*-sand): the antimicrobial and flocculating

capability of *f*-sand remains comparable to that of the original seeds, while the residual organic matter is eliminated.²¹ The application of *f*-sand to point-of-use water purification is still limited for the following reasons: (1) limited removal (<1 log removal) of pathogens at dilute concentrations (commonly occurring in surface water) by *f*-sand suspensions due to mass transport limitations, (2) a lack of optimization of seed dosage and preparation procedures, and (3) no existing design parameters for scale-up.

In this study, we determined critical parameters for the design of this *f*-sand filter (Figure 1). *Moringa* seed extract (simply crushed seed), rather than purified protein, was used, while seed dosage and filter preparation time were minimized for ease of field applications. The filter performance was tested using model polystyrene particles and *Escherichia coli* solutions. We applied classic clean bed filtration (CBF) models to determine critical parameters for scale-up design, which were validated under a wide range of filtration conditions. A simple saturation model was used to estimate the filter longevity. The findings enable the rapid and effective design of point-of-use

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Figure 1. *Moringa* seed extract-functionalized sand filter (*f*-sand) for enhanced pathogen and colloidal removal. Cationic and antimicrobial proteins in *Moringa* seeds can be readily dissolved and adsorbed onto a sand surface, reversing the charge of the sand particles. These *f*-sand particles can then be packed into a filter with improved hydrodynamics enabling superior log removal of pathogens. This enhanced sand filter is based on locally available materials and provides an attractive water sanitation option for resource-poor settings.

filters when different local materials are available during field applications at various scales.

MATERIALS AND METHODS

***f*-Sand Preparation.** Whole unshelled Nicaraguan *Moringa* seeds were ground with a coffee grinder and mixed with deionized (DI) water for 5 min followed by filtration through a 1.5 μm glass fiber filter (Whatman) and a 0.22 μm cellulose acetate filter (Millipore). The seed extract was mixed with unwashed glass beads (Sigma-Aldrich, St. Louis, MO) for 5 min; the supernatant was discarded, and the glass beads were rinsed with DI water three times to remove organic residues. The glass bead slurry (*f*-sand) was then used to pack glass columns. Glass beads with sizes of ≤ 106 , 212–300, and 425–600 μm were used for model development and validation. The specific preparation procedure and details are presented in Figure S1 and Tables S1 and S2. To study the surface charge of *f*-sand, 3 μm silica oxide particles [5% (w/v), Sigma-Aldrich] were used as a substitute. Measurements were conducted in 1 mM NaCl using a Zetasizer Nano instrument (Malvern Instruments).

Model Contaminants. Green-yellow fluorescent Fluospheres polystyrene particles with a diameter of 1 μm (Life Technologies) were used as model particulate contaminants. The particle concentration was analyzed using a FlowSight Imaging Flow Cytometer (Millipore). *E. coli* strain TG1 containing plasmids that express red fluorescent protein (pCA24N-rfp-lasR) were used as model pathogens at an influent concentration of 10^8 colony-forming units (CFU)/mL suspended in 10-fold diluted phosphate-buffered saline (PBS) buffer (0.016 M). Culture medium chemicals were removed from the cell suspension by rinsing pellets three times with PBS buffer. Culturing details were described in a previous study.²² A conventional plate counting method was used to quantify CFU. Confocal laser fluorescence microscopy (CLM) was utilized to visualize the attachment of *E. coli* to the sand surface after filtration. Images (20 \times objective lens) were taken at a laser excitation of 561 nm and emission of 595 ± 50 nm using a Nikon Inverted Eclipse Ti2-E System with a Nikon A1R⁺ confocal detector system.

Column Experiments. Particle and *E. coli* filtration tests and breakthrough experiments were performed with glass columns (Bio-Rad, Hercules, CA) either 10 or 5 cm in length (*L*) and either 1.5 or 1 cm in inner diameter (I.D.). The porosity of the packed sand column was gravimetrically determined to be 0.37. The *f*-sand slurry was quickly poured into the glass column and gently mixed by rotation along the length of the column to remove any trapped bubbles. The columns were then packed with DI water overnight and equilibrated with the background electrolyte for 20 pore volumes before switching to appropriate influent solutions prepared in the same background electrolyte. Sterilized water and PBS buffers (10-fold diluted) were used for *E. coli* removal experiments, while sterilized vials were used to collect effluent samples. A constant flow rate was achieved using a peristaltic pump (Cole-Parmer) with the feed solution entering from the top of the column.

Experimental log removal (pC^*) for particles was calculated using eq 1:

$$\log_{10} \text{removal}_{\text{exp}} = -\log_{10} \left(\frac{N}{N_0} \right) \quad (1)$$

where N and N_0 are the effluent and influent particle concentrations, respectively. To validate α at various ionic strengths, collector sizes, and flow rates, filtration experiments were conducted with 1 μm particles (10^6 mL^{-1}) as the influent. The default conditions were 1 mM NaCl, a flow rate of 1.6 mL/min, and collector diameter of 106 μm . Breakthrough experiments were conducted with 1 μm particles (10^7 mL^{-1}) as the influent at a flow rate of 0.7 mL/min with the same collector size.

Model Calculations. Classic clean bed filtration models^{23–29} are widely applied to describe the colloidal/particle deposition and transport in saturated porous media. The extent of deposition can be estimated from the collector efficiency (η_0), defined as the probability of a particle striking a collector given the column specifics and hydrodynamic conditions. Equation 2 demonstrates the correlation between log removal and η_0 :

$$\ln \left(\frac{N}{N_0} \right) = -\frac{3}{2} \frac{(1 - \varepsilon) L \alpha \eta_0}{d_c} \quad (2)$$

where d_c is the collector diameter, ε is the column porosity, and L is the column length. The model developed by Tufenkji and Elimelech (TE model) was used to calculate the theoretical η_0 because of the superior agreement of the predicted values with experimental data.²⁶ The sticking coefficient, α , describes the probability of a particle sticking to a collector upon collision and has a theoretical range from 0 to 1. The values of α for *f*-sand and bare sand columns were determined using eq 2 and $\log \text{removal}_{\text{exp}}$ under various column and flow conditions. Predicted log removal values ($\log \text{removal}_{\text{pred}}$) were then calculated using SI-eq 1 with a standard deviation calculated using SI-eq 2. A saturation equation (SI-eq 5) was used to calculate the fraction (f) of sand area occupied by particles at breakthrough. Details are discussed in the Supporting Information.

RESULTS AND DISCUSSION

Minimal Seed Dosage and Time for Achieving Charge Reversal of *f*-sand. We optimized the seed mass per total

surface area of sand (grams per square meter) by evaluating ζ potentials of 3 μm silica oxide particles in 1 mM NaCl at seed concentrations ranging over 4 orders of magnitude (0.02–230 g of seeds/ m^2 , 2×10^{-5} to 0.2 g/mL). As shown in Figure 2A, the ζ potential of sand reverses from -42 to 10 mV (1 mM NaCl) at a seed dosage of 1–10 g/ m^2 . These concentrations

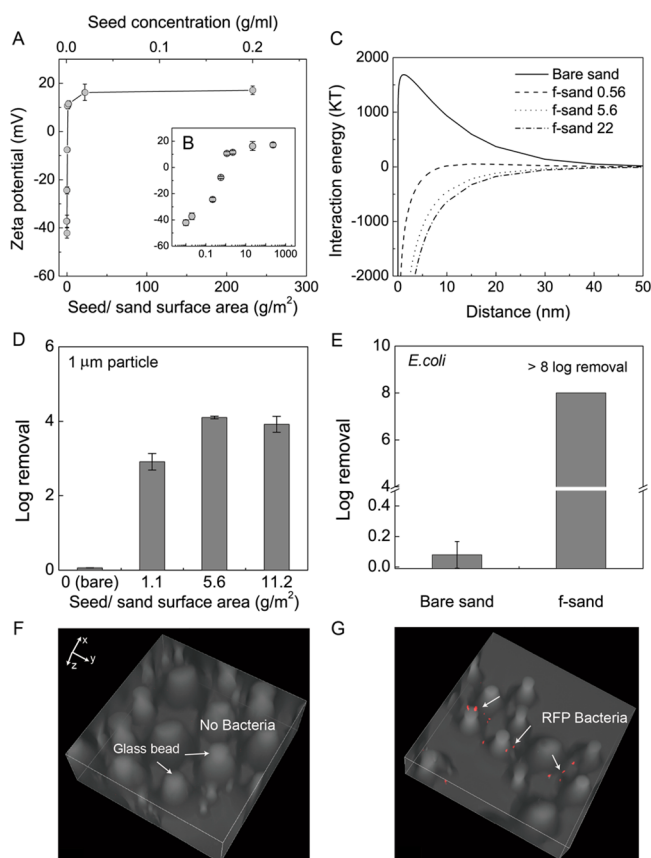


Figure 2. Increasing seed dosage induces charge reversal of *f*-sand, while the packed *f*-sand filter achieves ~ 4 log₁₀ removal of 1 μm polystyrene particle and >8 log₁₀ removal of *E. coli*. (A) ζ potential measurements of *f*-sand indicate charge reversal from -42 to 16 mV with increased seed loading. The bottom x -axis represents the seed per sand surface area, while the top x -axis shows the corresponding seed concentration. Silica particles 3 μm in size were used as a surrogate for sand and were mixed with *Moringa* serum for 5 min before the measurements (triplicates using 1 mM NaCl as the background electrolyte). (B) Identical ζ potential data from panel A plotted on a logarithmic scale. (C) DLVO theory-based interaction energy calculations suggest strong attractive forces between negatively charged polystyrene particles and the surface of *f*-sand with various seed dosages, in comparison to a large energy barrier seen in bare sand. Values were calculated considering a particle size of 1 μm in 1 mM NaCl and a Hamaker constant of 1×10^{-20} J. (D) 3 log to 4 log removal of 1 μm particles (10^6 mL⁻¹) by an *f*-sand filter with various seed loadings (grams of seeds per square meter of sand surface area) compared to <0.06 log removal by regular glass beads (bare sand). Filtration experimental conditions: 106 μm glass beads, 1 mM NaCl, 0.13 mm/s. (E) *f*-sand filters achieved >8 log removal of *E. coli* compared to 0.05 log removal by bare sand. (F and G) CLSM images. (F) Bare sand shows no bacteria attached after filtration for 8 pore volumes (2.8 bed volumes) of *E. coli* (10^8 CFU/mL), compared to (G) red fluorescent *E. coli* attached to *f*-sand. Light gray contours are glass beads. Bare sand image: x -axis, 186.16 μm ; y -axis, 186.16 μm ; z -axis, 60.80 μm . *f*-Sand image: x -axis, 269.84 μm ; y -axis, 269.84 μm ; z -axis, 60.80 μm .

are comparable to those previously identified.¹⁴ We also show (Figure S2A) that a 5 min mixing time for sand and seed extract is sufficient to enable charge reversal by MOCF (compared to 2–5 h using the previously described method²¹). In addition, a simple “stick test” was developed to enable field practitioners to determine if charge reversal of the sand was successful. Positively charged *f*-sand stuck on the side of the negatively charged plastic containers (Figure S2B,C) compared to regular sand showing no sticking effect.

***f*-sand Filters Achieved 3–4 Log₁₀ Removal of 1 μm Polystyrene Particles and >8 Log Removal of *E. coli*.** The *f*-sand filter achieved 3–4 log removal of 1 μm particles, compared to 0.060 log removal by the bare sand filter (Figure 2C). Polystyrene particles of 1 μm size were chosen because their size and charge represent many target microbial contaminants (such as coliform bacteria) and the collector efficiency is typically the lowest at this size as predicted by CBF models.²⁶ As shown in Figure S3, removal was consistent from 2 to 10 pore volumes (from 0.7 to 3.5 bed volumes); values were averaged and are presented for each specific seed loading in Figure 2D. Increasing the seed dosage from 1.1 to 5.6 g/ m^2 resulted in an increase in log removal from 2.9 ± 0.2 to 3.9 ± 0.4 (compared to 0.060 ± 0.008 log removal by bare sand filters). Further increases in seed loading did not increase surface charge or particle removal; thus, we chose a 5.6 g/ m^2 seed loading for all subsequent filtration experiments.

We further determined that the *f*-sand filters removed >8 log red fluorescent *E. coli*, as no bacteria were detected in the effluent (1 mL sample volume at pore volumes of 4, 6, and 8) when the influent concentration was 10^8 CFU/mL. Bare sand achieved only 0.05 log removal of *E. coli* under the same flow conditions (Figure 2E). CLSM images (Figure 2G) of *f*-sand after filtration show bacteria (red dots) attached to the *f*-sand surface, while little to no bacterial accumulation was observed on bare sand (Figure 2F). Previous BacLight stain tests have also demonstrated the loss of viability of the bacteria attached to the *f*-sand surface,²¹ likely because of the membrane fusion by the MOCF.¹⁴

To confirm the role of electrostatic attractive forces in the polystyrene particle removal in *f*-sand filters, the classic Derjaguin–Landau–Verwey–Overbeek (DLVO) theory (S1eqs 3 and 4) was used to demonstrate an attractive interaction energy between *f*-sand (modeled as a flat plate) and a polystyrene particle versus a repulsive energy barrier between bare sand and the same particle (Figure 2C). This strongly suggests a favorable deposition condition for negatively charged particles and bacteria on *f*-sand and thus a high value for α .

The Sticking Coefficient (α) Was Determined To Be 0.8 for the *f*-sand Filter Compared to 0.01 for the Bare Sand Filter. Our experimental data confirmed this trend: we calculated an α of 0.83 ± 0.08 for the removal of the 1 μm polystyrene particle by *f*-sand compared to an α of 0.011 ± 0.001 for bare sand. We validated the α value of *f*-sand with a series of filtration experiments at a range of ionic strengths, collector sizes, and flow rates, typical for water treatment processes. As shown in Figure 3, the predicted log removals agree well with experimentally measured log removals at various ionic strengths (1–10 mM) that are typical in natural waters. The model also agrees with experimental data at different collector sizes (106–520 μm); these collector sizes were selected after considering proper scaling between the filter diameter and the collector size from full scale sand filters based on a previous scale-down analysis of granular activated carbon

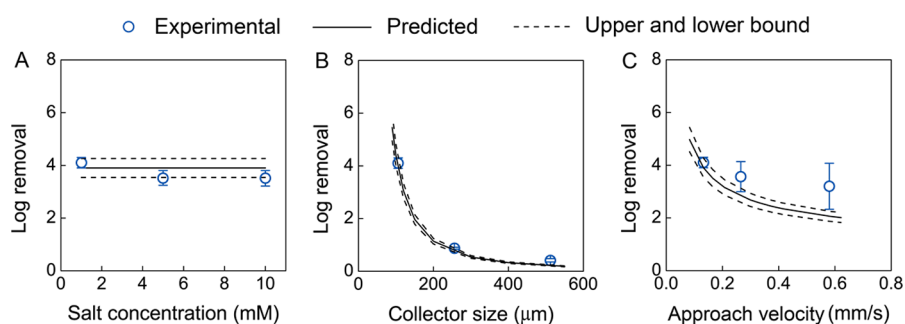


Figure 3. Experimental and predicted \log_{10} removals (10^6 mL^{-1}) of $1 \mu\text{m}$ polystyrene particles by *f*-sand agree well at various (A) NaCl concentrations (1–10 mM), (B) collector sizes (100–520 μm), and (C) approach velocities (0.13–0.58 mm/s, 0.48–2.08 m/h). The predicted removal was calculated using SI-eq 1 and a sticking coefficient of 0.83 ± 0.08 determined from experimental data. The upper and lower bounds were generated using the standard deviation calculated using SI-eq 2. If not specified, the filtration experiments were conducted in 1 mM NaCl with an approach velocity of 0.132 mm/s (1.6 mL/min, 0.48 m/h) with glass beads with a collector size of 106 μm at room temperature. All the *f*-sand has a seed loading of 5.6 g/m², prepared with 0.005 g/mL seed extract.

filters (details in Table S3).³⁰ The experimental log removals at various approach velocities (0.13–0.58 mm/s) were also within the range of predicted values, although the variation in average values is large compared to those under other conditions. These approach velocities are similar to or higher than that of a typical slow sand filter, with head losses between 0.1 and 0.6 m (calculation presented in Table S4), which is appropriate for gravity-driven household applications. This agreement strongly suggests that the determined sticking coefficient is robust and the *f*-sand surface retains its ability to attract and retain particles under a range of conditions. Natural organic matter (NOM) present in surface waters might interact with protein and sand surfaces; future work will further validate the sticking coefficient under various NOM types and concentrations.

The experimentally determined 8 log removal of *E. coli* is higher than the predicted value of 4.7. This predicted value is based on an equivalent diameter of 1.7 μm (for *E. coli* that are 1.2 μm in diameter and 3.7 μm in length)³¹ and a Hamaker constant of 6.5×10^{-21} J. The lack of agreement is likely due to the larger interaction area between flexible rod-shaped bacteria and that of rigid $1 \mu\text{m}$ polystyrene spherical particles (area calculation presented in the Supporting Information). The length of the bacteria can also enhance the collector efficiency by improved interception; the predicted log removal of a 3.7 μm sphere is calculated to be 10.49. We propose that the α of *f*-sand determined here be a conservative design parameter with the a safety factor, when considering the design of field filters for bacterial removal.

Filter Longevity. To estimate the capacity of the filter, we determined a fraction (*f*) of the sand surface area covered by polystyrene particle contaminants (projected area) at an experimentally determined breakthrough point. The breakthrough point occurred when N/N_0 increased above 0.1. The two pore volume values right before and after breakthrough were averaged and used to estimate the *f* value (using SI-eq 5). Six repeated runs (Figure S4) indicate a breakthrough pore volume of 350 ± 116 , which corresponds to 400–600 mL, generating an *f* value of $4.1 \pm 2\%$. This value can be used for preliminary scale-up design and requires further validation with breakthrough experiments at different column scales.

Environmental Implications. The outcome of this study provides the scientific basis for a synthetic chemical-free method employing *Moringa* seed extract to enable sustainable drinking water treatment in the developing world. Using the α and *f* experimentally determined here, preliminary scale-up

analyses were conducted considering a point-of-use household scale (five people) filter. We required that the filter provide 10 L/day and >4 log removal of $1 \mu\text{m}$ particles given a heavily contaminated source water ($10^4/100 \text{ mL}$) with an appropriate head loss. The analyses (Table S5) indicate that an *f*-sand filter, 5 cm in diameter and 1 m in length (and 3 cm head loss), would require 0.21 kg of seed/year (a 2-year-old *Moringa* tree produces 480 kg of seeds/year⁹). Similar analyses were conducted for a community-based scale filter (1000 people) (Supporting Information). In addition, our calculation shows that the filter longevity was not limited by the sand surface area capacity.

To fully implement the proposed filter in the field, we propose to further validate the values of α , *f*, and longevity in more complex solution environments, such as various concentrations and types of natural organic matter, colloids, and pathogens. Filter regeneration should also be considered for further work. For the treatment of highly turbid source waters, we propose a hybrid column with a regular sand column on top of an *f*-sand column. In addition, testing in the field is required before comprehensive environmental implications can be assessed.

■ ASSOCIATED CONTENT

● Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.estlett.7b00490.

A detailed description of the *f*-sand filter preparation procedure clean bed filtration model; calculation of interaction energy, head loss, and scale-up; and additional data for the longevity test (PDF)

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Notes

The authors declare no competing financial interest.

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