

1 Supporting information

2 *Moringa oleifera* *f*-sand Filters for Sustainable Water

3 Purification

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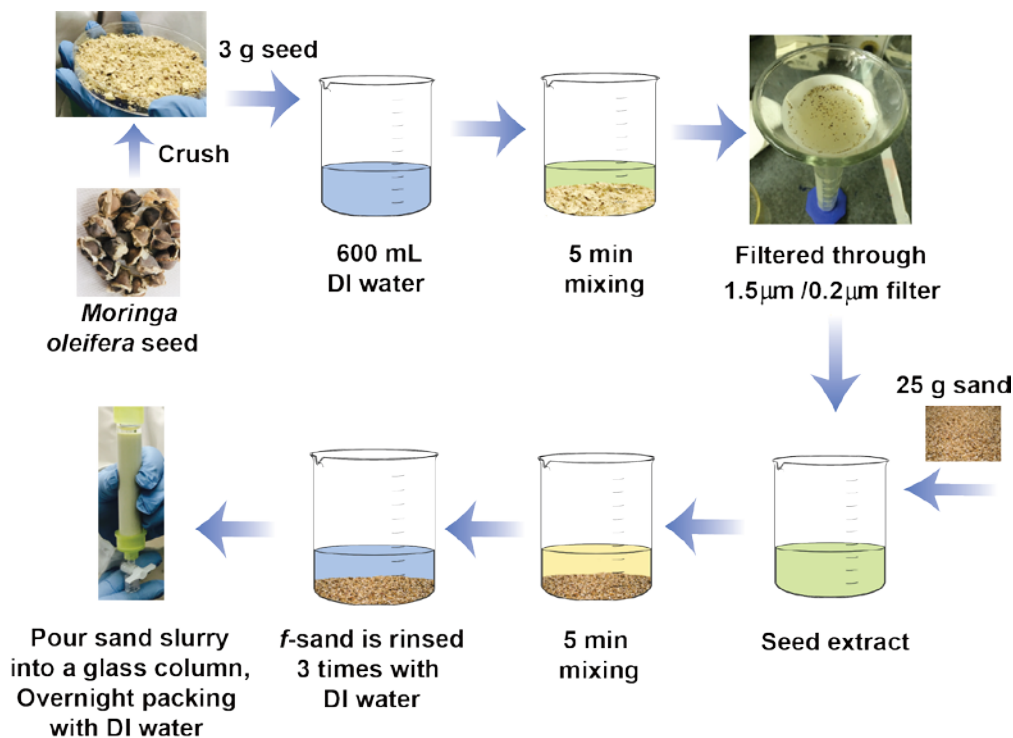
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15 *f*-sand preparation procedure for column tests

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17 *Procedure* This detailed description below provides an example of how an *f*-sand column at specified
18 conditions was prepared. Fully packing a sand column (1.6 cm I.D. and 10 cm L) required 25 g sand
19 with a size of 106 μm. 3 grams of ground seed was added into 600 ml water for 5 min, generating a
20 seed concentration of 0.005 g/ml, followed by filtration of the seed extract through a 1.5 μm glass
21 fiber filter and then a 0.2μm cellulose acetate filter. The filtered seed extract (600ml) was then mixed
22 with 25 g sand for 5 min. This generated a seed loading of 5.6 g/m². The supernatant was then
23 discarded and *f*-sand was used for packing after rinsing with DI water three times. To pack the
24 column, the *f*-sand slurry was quickly poured into the glass column and gently mixed in the column to
25 remove any trapped bubbles before packing overnight by gravity-fed DI water.

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28 **Figure S1.** Procedure of preparation of an *f*-sand column using *Moringa* seeds. Image was created
29 using Adobe Illustrator.

30
31 *Experimental details* For column experiments with different seed loadings, only seed amount was
32 changed while the volume seed extract volume and sand amount remained constant, as presented in
33 **Table S1**. For experiments with different collector sizes, we first determined roughly 26 g sand was
34 used to fill the glass column. Seed extract concentration (0.005 g/ml) and seed loading (5.6 g/m²) was
35 kept constant throughout different collector size experiments, while seed amount (g) and seed extract
36 volume (ml) was then determined as presented in **Table S2**.

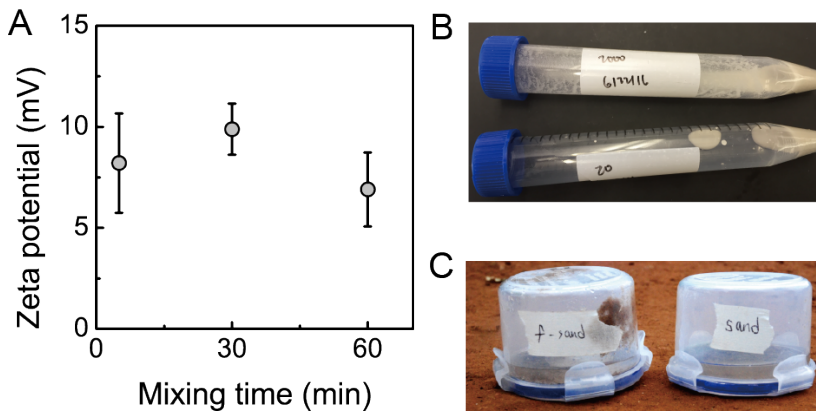
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38 **Table S1.** Details of *f*-sand column preparation for column experiments at different seed loadings.

Seed/surface area (g/m ²)	Seed concentration (g/ml)	Seed (g)	Seed extract (ml)	Sand (g/ml)
1.12	0.001	0.6	600	0.042
5.59	0.005	3	600	
11.17	0.01	6	600	

40
41 **Table S2.** Details of *f*-sand column preparation for column experiments at collector sizes.

Collector size, specific surface area	Seed concentration (g/ml)	Seed (g)	Seed extract (ml)	Sand (g/ml)
106 μm, 0.021 m ² /g	0.005	3	600	0.0042
256 μm, 0.0091 m ² /g	0.005	1.3	256	0.098
512 μm, 0.0045 m ² /g	0.005	0.6	128	0.196

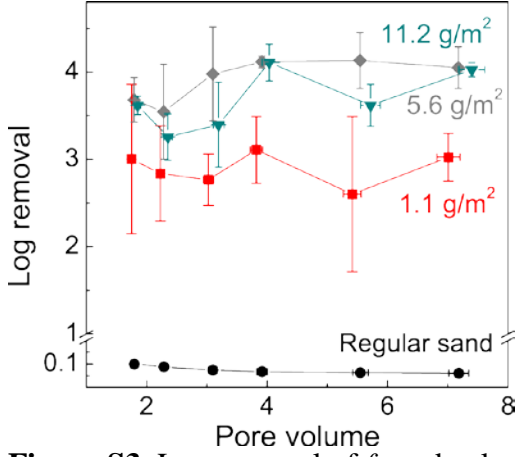
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Figure S2. (A) Five minute mixing time for glass beads and 0.005 g/ml and 5.6 g/m² moringa serum is sufficient to yield charge reversal of sand, resulting in the surface potential of 8.2±2.4 mV. Mixing time up to 30 min only led to an increase in zeta potential to 9.8±1.3 mV. 3µm SiO₂ particles (original surface potential of -42 mV) are used as substitute for sand in order to eliminate settling challenges during zeta potential measurements of 106 µm glass beads. (B) A simple “stick test” can quickly be used to determine the effectiveness of charge reversal of sand by *Moringa* seed protein. *f*-sand coated with 2.3 g/m² moringa seed stuck on the side of the plastic tube (upper image) due to positive surface potential, compared to *f*-sand coated with 0.02 g/m² moringa seed (lower image) showing no sticking effect. (C) Stick test was performed in Kigali, Rwanda using locally available sand and moringa seeds (left:*f*-sand, right:regular sand). This test can be done with plastic or glass containers in the field to quickly determine the optimal seed dosage given various sizes of sand material. Both photos (B and C) were taken by one of the authors (Emma Clement).

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Figure S3. Log removal of *f*-sand column coated with various amounts of *Moringa* seed over 2-8 pore volumes. Removal values at each pore volume are an average of triplicate experiments. Experimental conditions: 1 μm polystyrene particle at a concentration of $10^6/\text{ml}$, 1mM NaCl, 1.6 ml/min with 106 μm glass beads.

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Calculation of predicted log removal

$$\text{Log removal}_{\text{pred}} = -\log_{10}\left[e^{\frac{-3(1-\varepsilon)L\eta_0\alpha}{2d_c}}\right] \quad \text{Equation (1)}$$

$$\sigma_{\text{log removal}} = -\log_{10}(e) \left(\frac{-3(1-\varepsilon)L\eta_0}{2d_c}\right) \sigma_{\alpha} \quad \text{Equation (2)}$$

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Interaction energy calculation

74 The electrostatic interaction energies were calculated using equation (4) developed by Hogg *et al.*¹:

$$\Phi_{\text{EDL}} = \pi\epsilon_0\epsilon_r a_p \left\{ 2\psi_p\psi_c \ln\left[\frac{1+e^{-kh}}{1-e^{-kh}}\right] + (\psi_p^2 + \psi_c^2) \ln[1 - e^{-2kh}] \right\} \quad \text{Equation (3)}$$

77 Where ϵ_0 is the dielectric permittivity in vacuum, ϵ_r is the relative dielectric permittivity in water, a_p is the particle radius, ψ_p and ψ_c are the surface potential of particle and glass beads (collector) experimentally determined as zeta potential, k is the inverse of debye length and h is the separation distance between particle and collector. The van der Waals interaction energies were calculated using equation (5)

$$\Phi_{\text{VDW}} = -\frac{Aa_p}{6h} \left[1 + \frac{14h}{\lambda} \right]^{-1} \quad \text{Equation (4)}$$

83 Where A is the Hamaker constant and a value of $1 \times 10^{-20} \text{J}$ was used for the polystyrene-water-quartz system². λ is the characteristic wavelength of the dielectric (100 nm^3).

85

Saturation model equation

87 **Eqn 5** was used to calculate the maximum fraction (f) of sand area that is occupied by particles at breakthrough, which is defined as the fractional coverage when N/N_0 exceeds 0.1.

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89 $f = \frac{A_s N_s}{J_p V_b A_p}$ Equation (5)

90 where A_s is the surface area of one sand particle, N_s is the total number of sand particles in the
 91 column, J_p is the flux of influent particles, V_b is the volume filtered at breakthrough and A_p is the
 92 cross-section area of an influent particle.

93 **Interaction area calculation of sphere and rod-shape bacteria**

94 The interaction area between spherical polymer particle with a diameter ($2r$) of $1 \mu\text{m}$ is
 95 estimated using equation⁴:

96 $A = 2\pi r \kappa^{-1}$ Equation (6)

97 The interaction area between rod-shape bacteria with a diameter of $1.2 \mu\text{m}$ and a length (L) of $3.7 \mu\text{m}$
 98 is estimated using this Eqn:⁴

99 $A = 2L\sqrt{r\kappa^{-1}}$ Equation (7)

100 Where κ^{-1} (nm) is the Debye length⁴ in 1 mM NaCl. The calculation was under the assumption of the
 101 distance between particle or bacteria and sand surface is equal to the Debye length. The sand surface
 102 is considered a flat surface given the significant size difference between particle and sand.

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104 **Considerations for selecting collector size and flow rate for lab scale column experiments**

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106 **Collector size** We justify our choice of collector size in our study according to a previous
 107 scale-down analysis of granular activated carbon that suggests a proper scaling between the small and
 108 large column empty bed contact time (EBCT) can be determined from the ratio of adsorbent particle
 109 sizes:⁵

110 $\frac{EBCT_{sc}}{EBCT_{lc}} = \left[\frac{d_{sc}}{d_{lc}}\right]^2$ Equation (8)

111 where $EBCT_{sc}$ and $EBCT_{lc}$ are EBCT of small and large columns, which can be calculated from
 112 column volume divided by superficial velocity. d_{sc} and d_{lc} are adsorbent (collector in our case)
 113 particle size. The equation is under the assumption that the porosity, bulk densities and capacities are
 114 identical in the two scales and that intraparticle diffusivities do not change with particle size. We
 115 considered a slow sand filter and a rapid sand filter at the typical full scale, and used **Eqn 8** to perform
 116 scale-down analysis to calculate collector size given the column dimension and flow rate used in our
 117 study. Media diameter, filter length and flow rate used the calculated collector size is presented in
 118 **Table S1**. The scale-down collector size ranges from 0.03-0.075 mm considering a slow sand filter

119 and ranges from 0.7-1.6 mm considering a rapid sand filter. We chose to use 0.1-0.6 mm collector size
 120 for our study.

121 **Table S3.** Scale-down collector sizes were calculated from the design parameters of full scale slow
 122 sand filter and rapid sand filters.

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	Full scale slow sand filter (low, high)	Small column scale down from slow sand filter	Full scale rapid sand filter (low, high)	Small column scale down from rapid sand filter
Filter Length (cm)	90, 150	10	60, 180	10
Flow rate (m/h)	0.05, 0.2	0.48	5, 15	0.48
Collector size (mm)	0.3-0.45	0.03, 0.075 (low, high)	0.5, 1.2	0.7, 1.6 (low, high)

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125 **Flow rate** Head loss calculations show that the collector size and flow rate used in this study
 126 were reasonable for a small-scale filter and comparable with the large scale sand filters. Head loss or
 127 the minimal head required is calculated using the equations below⁶. For a flow at Darcy flow regime
 128 at $Re < 1$, H_L (m) is calculated based on Poiseuille's law:

129
$$\frac{H_L}{L} = \frac{K_k \mu S^2 v}{\rho_w g \epsilon^3}$$
 Equation (9)

130 where K_k is Kozeny coefficient (unitless) which is an empirical coefficient and assumed to be about 5
 131 for spherical media⁷. S is the specific surface area ($\frac{6(1-\epsilon)}{d}$, m^{-1}), ϵ is column porosity, v is superficial
 132 velocity (m/s), μ and ρ_w is viscosity and density of water.

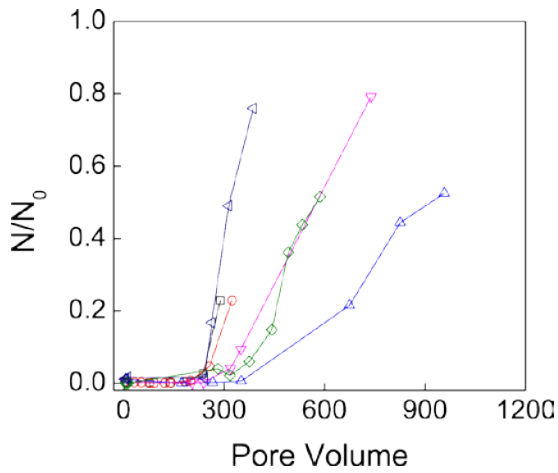
133 **Table S4.** Superficial velocity (v) and head loss of the filtration experiments with various collector
 134 sizes and flow rates. The values suggest the flow rates of filtration experiments were similar and
 135 higher than a slow sand filter, and were at Darcy flow regime that generated a head loss lower than a
 136 typical slow sand filter.

Collector size (μm)	Flow rate (ml/min)	v (m/h)	Head loss (m)
106	1.6	0.48	0.13
	3.2	0.95	0.25
	7	2.09	0.55
106	1.6	0.48	0.13
256			0.02
512			0.005

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 141 **Figure S4.** Breakthrough curves of 3-day column filtration experiments using a column with a
 142 dimension of 5 cm L and 1 cm inner diameter run at 0.7ml/min with 10^7 /ml $1\ \mu\text{m}$ polystyrene
 143 particles. Six repeated runs were presented. This data was used to calculate the fractional coverage (f)
 144 at breakthrough.
 145

146 **Scale up analysis** Two different scales were considered for scale up: 5-person household scale
 147 for point-of-use, and 1000-person community scale. Filter flow rate was first determined based on the
 148 amount of people served for each scale assuming 2L per day of drinking water. In addition we
 149 specified a sand diameter of 0.5 mm. The specific diameter and length of the column was then
 150 determined in order to meet two requirements: 1) reasonable head required and 2) 4 log removal of
 151 $1\ \mu\text{m}$ particles at a concentration of 10^4 /ml concentration. The amount of sand was then calculated
 152 based on the porosity (0.37) while the mass of seeds was calculated using $5.6\ \text{g seed}/\text{m}^2$ sand area.
 153 The lifetime of the filter based on saturation was then calculated using 4% maximum fractional
 154 coverage (f). Finally the head loss was calculated using an non-linear Forchheimer flow equation
 155 when $\text{Re} > 1$:

$$156 \quad \frac{H_L}{L} = \frac{K_V(1-\varepsilon)^2 v \mu L}{\rho_W g d \varepsilon^3} + \frac{K_I(1-\varepsilon) v^2 L}{\rho_W d \varepsilon^3} \quad \text{Equation (10)}$$

157 where K_V is the head loss coefficient due to viscous forces and K_I is the head loss coefficient due to
 158 inertial forces. We used typical values for sand, $K_V=110$ and $K_I=2^6$, for our calculations.

159 Log removal increases with column diameter due to decreased superficial velocity and
 160 increased collector efficiency. Log removal also increases with column length given the CBF model
 161 (**Eqn 2** in manuscript). Yet column diameter and length are disproportional to each other due to given
 162 a fixed column volume. Analysis shows that, in order to meet both requirements, column volume has
 163 to be scaled large enough to generate the dimensions shown in **Table S3**.

164 The minimal volume was found to reach breakthrough in 140 years given our fraction of
 165 coverage of 4%. Therefore, in reality, the longevity of the column will not depend on the column
 166 capacity but rather depend on the duration and stability of adsorbed protein over extended period of
 167 time.

168 Analyses also suggest that the column filtration rate will be operated at low Re number <1,
 169 and with very small head required (<0.1 m). It was also found that the column with a larger media size
 170 such as 0.8 mm would result in a column (community scale) dimension providing too small of
 171 required head (0.008 m with a dimension of 1 m diameter and 1.6 m long) or too large of a scale (1.6
 172 m head loss but a dimension of 0.2 m diameter and 12.5 m long) that is unrealistic for implementation
 173 in the field.

174
 175 **Table S5.** Scale up specifics of *f*-sand filter. Two different scales were considered: 5 people
 176 household scale for point-of-use, and 1000 people community scale. Assumptions: 2 L/ day/person,
 177 porosity 0.39. 1 μm particle at 10^4 /ml concentration, sand size 0.5 mm, fraction of coverage: 4%,
 178 filter will not reach breakthrough in 140 years, although filters are assumed to be replaced every three
 179 months based on protein stability and effectiveness with sand being reused. Unit price of locally
 180 sourced sand is \$0.018/ kg⁸.
 181

Design parameters		Point of use (5 people)	Community based (1000 people)	Typical slow sand filter	Rapid sand filter
Treatment capacity	Daily output (L)	10	2000	NA	NA
	Yearly output (L)	3650	730,000	NA	NA
	Flow rate (L/d)	10	2000	NA	NA
	Filtration rate (m/h)	0.21	0.22	0.05-0.2	5-15
Column specifics	Filter volume (m ³ , porosity 0.39)	0.002	0.41	NA	NA
	Media diameter (mm)		0.50	0.3-0.45	0.5-1.2
	Filter diameter (m)	0.05	0.70	NA	NA
	Filter length (m)	1.04	1.06	0.9-1.5	0.6-1.8
	Log removal	4.1	4.2		
	Minimal head (m)	0.03	0.03	0.9-1.5	1.8-3
Sand and seed consumption	Total sand (kg)	2.1	423	NA	NA
	Cost of sand/ person/ year (\$, Unit price: 0.018\$/kg)		0.03	NA	NA
	Total seed (kg)	0.054	42.91	NA	NA
	Seed kg /person/ year		0.043	NA	NA

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