Moringa oleifera functionalised sand – reuse with non-ionic surfactant dodecyl glucoside

Frances E. Williams, Andrew K. Lee, Sanaz Orandi, Sarah K. Sims and David M. Lewis

ABSTRACT

Moringa oleifera seeds are well known for their ability to cause flocculation in turbid water and facilitate bacterial inhibition. These effects are due to the cationic polypeptide $MO_{2.1}$, which affects the surface charge of suspended particles and causes lysis of bacterial cells. However, the attachment of bacteria to $MO_{2.1}$ prevents further bacterial attachment, reducing the effectiveness of the seeds. This research investigated the effect of surfactants on functionality and reuse of *Moringa* seeds to develop a sustainable water treatment technique. The seed extracts ($MO_{2.1}$) were used with a functionalised sand system, and the sands were exposed to commercially available (ionic and non-ionic) surfactants, dodecyl glucoside and sodium dodecyl sulfate. Artificially polluted water contaminated with *Escherichia coli* was used to evaluate the efficiency of the system. The non-ionic surfactant was found to be effective at separating *E. coli* from the functionalised sand without the detachment of the $MO_{2.1}$ and subsequent loss of the system efficiency. This was successfully repeated four times. The results demonstrated a sustainable, reusable technique to inhibit bacterial contamination in water.

Key words | dodecyl glucoside, *Escherichia coli*, functionalised sand, *Moringa oleifera*, sodium dodecyl sulfate, sustainable water treatment

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INTRODUCTION

Eleven percent of the world's population relies on untreated surface or ground water as a primary source of drinking water (WHO 2014). This water is often contaminated with pathogenic bacteria, and treatment for immediate use is commonly undertaken using tablets of sodium hypochlorite or calcium hypochlorite (Arnold & Colford 2007). This method is mainly used in regions which lack access to suitable water treatment infrastructure. The use of the tablets prevents recontamination, as the chlorine provides residual protection lasting from hours to days (Arnold & Colford 2007). High cost, an unpleasant taste and low availability of these chemicals have been identified as the main reasons which lead to their underuse or misuse (Islam *et al.* 2014).

The crushed seed kernels from *Moringa oleifera* are known to be an appropriate antibacterial agent for water treatment (Madsen *et al.* 1987; Jahn 1988). The seeds can be used directly, or as a crude water extract, to inhibit bacterial and viral organisms. They also cause coagulation of suspended particles in water (Ali *et al.* 2004; Kansal & Kumari 2014). However, the disadvantage of using the seeds in such a way is that the crushed seed particles quickly decompose and increase the amount of organic carbon in the water, promoting bacterial regrowth (Jerri *et al.* 2012).

The compound in the *M. oleifera* seed that causes the effects has been identified as a cationic polypeptide called $MO_{2.1}$ (Gassenschmidt *et al.* 1995; Suarez *et al.* 2005).

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This polypeptide is part of a dimer protein comprised of two polypeptide subunits linked by di-sulfide bonds (Ndabigengesere *et al.* 1995). The amino acid sequence of $MO_{2.1}$ has been documented (Gassenschmidt *et al.* 1995) and the molecular weight is 6,782 g/mol (http://www.uniprot.org/, accessed 28/09/2016).

$MO_{2.1}$ has been observed to adsorb to the surface of negatively charged solids, including silica nanoparticles, quartz sand, aluminum oxide and micro-emulsion prepared magnetic iron oxide nanoparticles (Kwaambwa *et al.* 2010; Jerri *et al.* 2012; Okoli *et al.* 2012; Hellsing *et al.* 2013). When the $MO_{2.1}$ is adsorbed to sand, this 'functionalised' sand (known as *f*-sand) displays the bactericidal and coagulant properties of the non-bonded polypeptide (Jerri *et al.* 2012). This simple method of purifying $MO_{2.1}$ has scope to be used in water treatment, as it negates the previously described disadvantages of the crude extract as well as reducing the costs associated with isolating the polypeptide by other methods. The *f*-sand can be used to attach and inhibit *Escherichia coli* in water (Jerri *et al.* 2012). In practice, the obstacle which currently prevents the use of *f*-sand to treat water is that the *E. coli* does not desorb once bonded to the *f*-sand, preventing its reuse (Jerri *et al.* 2012).

Surfactants are a class of amphiphilic compounds able to wet surfaces and solubilise fatty material (Glover *et al.* 1999). Anionic surfactants, such as sodium dodecyl sulfate, are reported to be bactericidal; affecting the permeability of cell membranes and causing cell lysis at high surfactant concentrations (Glover *et al.* 1999; Van Hamme *et al.* 2006). Non-ionic surfactants are also bactericidal and are known to solubilise the bacterial lipid membrane (le Maire *et al.* 2000). When solubilised in water, surfactants self-assemble into compounds called micelles, and the minimum surfactant concentration required for micelle formation is called the critical micelle concentration (Privé 2007).

M. oleifera seed extracts have been observed to interact with sodium dodecyl sulfate and other anionic surfactants (Kwaambwa & Maikokera 2007; Maikokera & Kwaambwa 2007; Beltran-Heredia & Sanchez-Martin 2009). The crude seed extract has been reported to remove up to 80% of sodium dodecyl sulfate via flocculation (Beltran-Heredia & Sanchez-Martin 2009). Both $MO_{2.1}$ and the dimer protein are cationic and readily adsorb to the anionic sodium dodecyl sulfate (Beltran-Heredia & Sanchez-Martin 2009; Beltran-Heredia *et al.* 2012). This has been reported to neutralize the charge (Maikokera & Kwaambwa 2007). The process begins below the critical micelle concentration of $8.2–8.3 \times 10^{-3}$ M, at the critical aggregation concentration of 1×10^{-5} M, and can be used to remove sodium dodecyl sulfate from solution (Beltran-Heredia & Sanchez-Martin 2009).

Dodecyl glucoside is a non-ionic surfactant, and no studies investigating this surfactant and the *M. oleifera* crude or purified seed extracts were identified in the literature. Okoli *et al.* (2012) report that a 0.1 wt% solution of the non-ionic surfactant Tween 20 can be used to wash coagulated clay particles off *M. oleifera* protein functionalised magnetic nanoparticles. These particles can then be reused as coagulants with some loss of efficiency. Triton X, another non-ionic surfactant, does not interact with the *M. oleifera* coagulant protein (Kwaambwa & Maikokera 2007).

This study demonstrates the use of surfactants in regenerating *f*-sand for continued *E. coli* removal. All concentrations of dodecyl glucoside tested against the *f*-sand were above the critical micelle concentration of 1.9×10^{-4} M (Neugebauer 1990). This is a novel approach for the regeneration of *f*-sand and has the potential to be applicable in water treatment for developing countries.

MATERIAL AND METHODS

This investigation was conducted in two stages. The first stage was focused on testing two surfactants, dodecyl glucoside and sodium dodecyl sulfate, to examine their effect on attachment of the $MO_{2.1}$ to the sand surface. The second stage evaluated the efficiency of these surfactants at separating *E. coli* from the $MO_{2.1}$.

Preparation of *Moringa oleifera* functionalised sand

The functional sand (*f*-sand) was prepared using *M. oleifera* seeds. The preparation was based on the method described by Jerri *et al.* (2012) with some modifications. Dry, whole seeds were purchased from AustraHort, Australia. The seeds were dehusked and the kernels pulverised to a fine powder. A 0.05 g/mL crude solution was prepared by mixing 20 g of the seed powder with 400 mL of a 0.1 M

sodium chloride solution for 1 hour at 150 rpm. The solution was sieved and filtered to remove the seed particles.

100 g of commercially available, fine grained silica sand was thoroughly rinsed with deionised water, dried then autoclaved. The sand was combined with 400 mL of the filtered crude extract solution and mixed on a shaker plate at 150 rpm for 1 hour. After the *f*-sand settled, the crude extract supernatant was removed and the *f*-sand was rinsed with deionised water. The *f*-sand was dried at 50 °C.

Mass determination of $MO_{2.1}$

The mass of $MO_{2.1}$ per gram of *f*-sand was determined by eluting the polypeptide on 1 g of *f*-sand using 5 mL of a 0.6 M NaCl solution. A baseline for the absorbance at 280 nm was set against the absorbance at 300 nm. A Shimadzu 1601 UV-visible spectrophotometer capable of scanning from 190 nm to 1,000 nm was used to detect the absorbance throughout this study. The Beer-Lambert law, $Abs = \varepsilon lc$, was applied using a path-length (l) of 1 cm and an extinction coefficient (ε) of 1,520 (Aitken & Learmonth 1996). The mass of $MO_{2.1}$ was determined in mg per gram *f*-sand using a molecular weight of 6,781.6 g/mol, based upon the reported amino acid sequence of $MO_{2.1}$ (http://www.uniprot.org/, accessed 28/09/2016).

Preparation of surfactants

Two surfactants were examined in this study: anionic sodium dodecyl sulfate (sodium lauryl sulfate, CAS 151-21-3) and non-ionic dodecyl glucoside (lauryl glucoside, CAS 11061-47-9) at concentrations ranging between 0.0005 M and 0.1 M. The concentrations of dodecyl glucoside were determined by UV-spectrophotometry. The absorbance was measured at 223 nm and was initially determined by serially diluting the supplied surfactant from a 49.74% w/w solution. A commercially available dishwashing detergent (Earth choice dishwashing concentrate, Natures Organics, Australia) was purchased from a local Australian supermarket. The detergent contained 10%–30% dodecyl glucoside as coco glucoside and <10% sodium dodecyl sulfate, and was tested at 1% and 2% v/v dilutions. The amount of dodecyl glucoside in the 1% solution was equivalent to 0.01 M dodecyl glucoside solution as confirmed by UV-visible spectroscopy.

Effect of surfactants on protein adsorption

The effect of the surfactants sodium dodecyl sulfate and dodecyl glucoside on the attachment of $MO_{2.1}$ to the sand was measured by exposing 1 g of *f*-sand to 5 mL surfactant at known concentrations. This was mixed for 1 hour and rinsed with deionised water. The samples were mixed between one and four times to determine the effect of multiple washes on the *f*-sand. The amount of $MO_{2.1}$ was measured as described above and the experiment was repeated in triplicate. The concentrations of sodium dodecyl sulfate examined were 0.01, 0.001 and 0.0005 M, and the concentrations of dodecyl glucoside were 0.1, 0.01 and 0.001 M.

Synthetic water preparation

Synthetic water with a known bacterial concentration was prepared to simulate non-potable water. *Escherichia coli* (ATCC® 25922™) was sourced from the School of Animal and Veterinary Sciences, The University of Adelaide and maintained on Bacteriological agar (Oxoid). Overnight bacterial suspensions were grown in nutrient broth (Oxoid). The suspension was centrifuged for 5 minutes at 3,000 g and the pellet was washed in a sterile 0.85% NaCl solution. This was centrifuged, and the pellet was re-suspended in sterile Milli-Q water diluted to an absorbance of 0.1 ± 0.005 at 600 nm. This was equivalent to a bacterial suspension containing 7×10^7 CFU/mL of stationary phase *E. coli* as confirmed by cultivatable counts. Fresh synthetic water was prepared for each set of experiments.

Functional sand column tests

Stage two evaluated the efficiency of the surfactants at separating *E. coli* from the $MO_{2.1}$. To do this, 50 g of dry *f*-sand was poured into a vertical glass column of 1.5 cm diameter. The *f*-sand was rinsed with Milli-Q water until the absorbance of the outgoing solution was less than 0.001 at 280 and 600 nm. The column was then subjected to a set of solutions in the following order: synthetic water, $2 \times$ Milli-Q water, surfactant, $4 \times$ Milli-Q water. Each set was repeated four times, so that the *f*-sand had been exposed to four discrete synthetic water treatments.

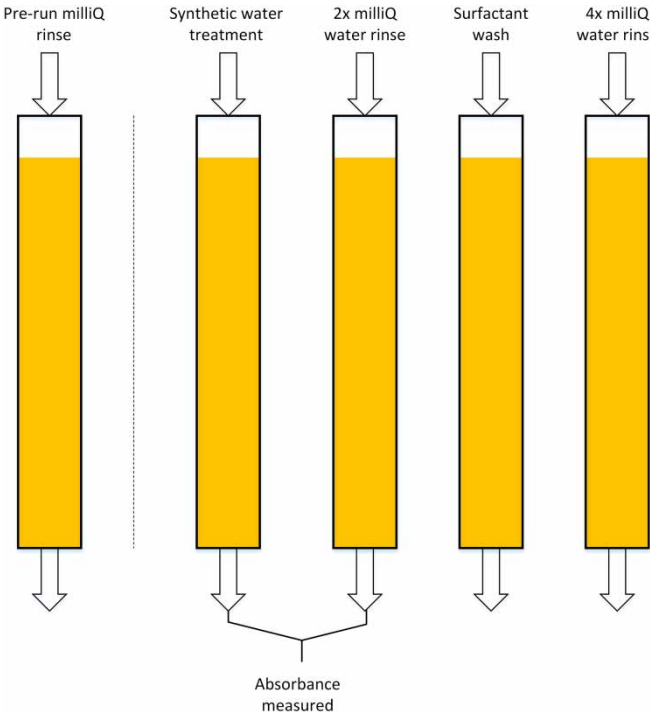

Figure 1 | The steps used to test efficacy of surfactants for the separation of *E. coli* (ATCC® 25922™) from $MO_{2.1}$ without separating $MO_{2.1}$ from the sand. The inlet solutions for the steps are: Step 0 - sterile Milli-Q water rinse; Step 1 - synthetic water; Steps 2 and 3 - Milli-Q water; Step 4 – surfactant; Steps 5–8 - Milli-Q water.

A schematic of this process is presented in Figure 1. New columns were prepared for sodium dodecyl sulfate concentrations of 0.001 and 0.0005 M and for dodecyl glucoside concentrations of 0.1, 0.01 and 0.001 M.

The solutions passing out of the columns were collected and the absorbance of the eluting solutions was measured at 600 nm. This was used to estimate the bacterial concentration of each solution and is used here as an alternate method of measuring bacterial concentrations in non-turbid solutions. To justify this method, the absorbance of bacterial solutions from 0.01 to 0.1 at 600 nm was compared with the cultivatable counts of these solutions. Colony counting was also performed on select columns. The percentage of *E. coli* inhibited by the column was determined by Equation (1),

$$\mathrm{Eq}_1 = 100\% - 100\% \left(\frac{\mathrm{Ab}_{\mathrm{outlet\ 1}} + \mathrm{Abs}_{\mathrm{outlet\ 2}} + \mathrm{Abs}_{\mathrm{outlet\ 3}}}{\mathrm{Abs}_{\mathrm{inlet\ 1}}} \right) \quad (1)$$

where Abs is the absorbance of the eluting solution at 600 nm for the solutions entering the column, inlet 1, and passing out of the column, outlets 1, 2 and 3.

Bacterial staining

Sand was heated at 600 °C in a high temperature oven for 5 hours to remove any impurities and organic matter, including dead bacteria, from the surface. 10 g of *f*-sand was prepared as above and exposed to synthetic water, 0.01 M dodecyl glucoside and synthetic water in the same manner as the columns. 2 g samples were collected after each step and were stained with a 1:500 dilution of propidium iodide in Tris buffer for 15 minutes, then rinsed twice with sterile Milli-Q water. Staining was performed on all samples, including controls of bare sand and *f*-sand, to confirm that only the dead *E. coli* was being stained. The samples were viewed on a Nikon Ti Live Cell Microscope at 40× optical zoom. A 100 ms exposure for fluorescence was used on all samples with a 195 ms exposure for the live view.

RESULTS

Mass determination of $MO_{2.1}$

The mass of $MO_{2.1}$ per gram of *f*-sand was calculated using the Beer-Lambert Law. The maximum, minimum and average masses of eluted $MO_{2.1}$ in mg per g of *f*-sand were 1.19, 0.48 and 0.79, respectively. Variation in turbidity was observed across all of the elution-type experiments. To overcome the variation between samples and batches, the absorbance at 300 nm was used as a baseline and subtracted from the absorbance of the protein peak at 280 nm.

Effect of surfactants on $MO_{2.1}$ attachment

The effect of sodium dodecyl sulfate on the attachment of $MO_{2.1}$ to the sand was immediately apparent. At 0.01 M, the highest concentration tested, there was an 80% reduction in the amount of $MO_{2.1}$ but there was no further significant loss after the first wash. At 0.001 M, there was a gradual reduction in the amount of $MO_{2.1}$, and at 0.0005 M, the amount present was relatively stable. The

results of the interaction of sodium dodecyl sulfate and *f*-sand are presented in Figure 2.

Washing the *f*-sand with dodecyl glucoside caused a slight and immediate reduction in the amount of $MO_{2.1}$. The loss was not as significant as that observed with the sodium dodecyl sulfate, and for 0.01 and 0.001 M the mass of $MO_{2.1}$ remained above 80% of the initial value. The results are presented in Figure 3.

FUNCTIONAL SAND COLUMN TEST RESULTS

Synthetic water treatment results with sodium dodecyl sulfate in an *f*-sand column

Based on the results from part one, the 0.01 M concentration of sodium dodecyl sulfate was excluded from further investigation. The treatment results of functional sand columns with sodium dodecyl sulfate, 0.001 and 0.0005 M, are presented in Figure 4 and demonstrated that the surfactant was not effective at separating the *E. coli* from the *f*-sand. In the first treatment run, when the synthetic water was passed through the *f*-sand column, between 60% and 70% of the *E. coli* was removed. Further treatment results did not show any reduction in *E. coli* and even increased the turbidity of the water.

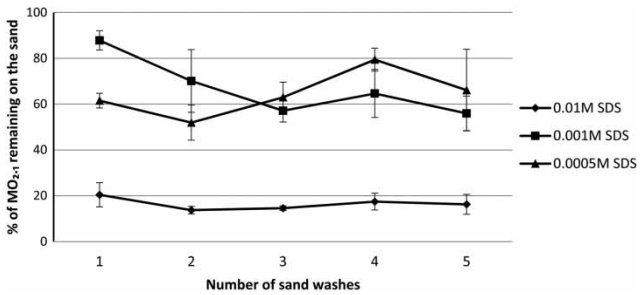

Figure 2 | Effect of sodium dodecyl sulfate (SDS) on attachment of $MO_{2.1}$ to sand used at three concentrations of 0.01, 0.001 and 0.0005 M and percentage of $MO_{2.1}$ remaining on the sand.

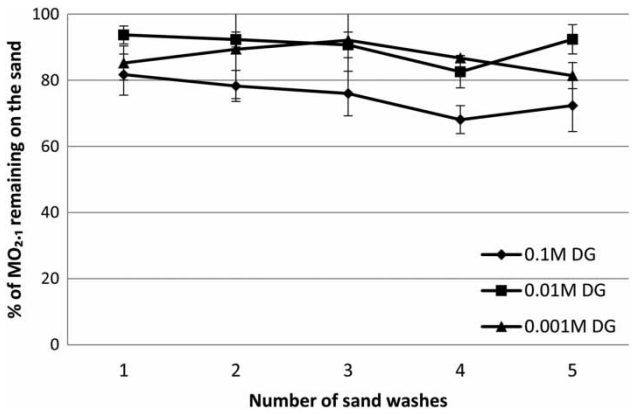

Figure 3 | Effect of dodecyl glucoside (DG) on attachment of $MO_{2.1}$ to sand used at three concentrations of 0.1, 001 and 0.001 M, and percentage of $MO_{2.1}$ remaining on the sand.

Synthetic water treatment results with dodecyl glucoside in an *f*-sand column

The dodecyl glucoside was effective at separating the *E. coli* from the *f*-sand without affecting the capacity of the *f*-sand to remove *E. coli* from water. The 0.01 M dodecyl glucoside was the most consistently effective, as shown in Figure 5. To confirm the use of UV-visible spectroscopy in bacterial estimation, colony counts were performed on the *f*-sand columns treated with 0.01 M dodecyl glucoside. For the first treatment, the percentage reduction in the *f*-sand columns determined by colony counting was 12% greater than that determined by UV-visible spectroscopy. For the second, third and fourth treatments, the difference in the values was less than 4%. When testing by UV-visible spectroscopy, the absorbance includes both viable and inhibited cells, whereas with cultivatable counts, only the living cells are recorded, indicating that UV-visible spectroscopy underestimates the

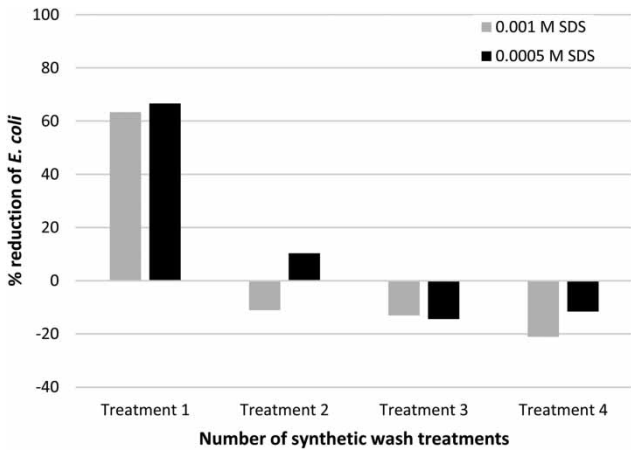

Figure 4 | Synthetic water treatments with sodium dodecyl sulfate (SDS) in an *f*-sand column.

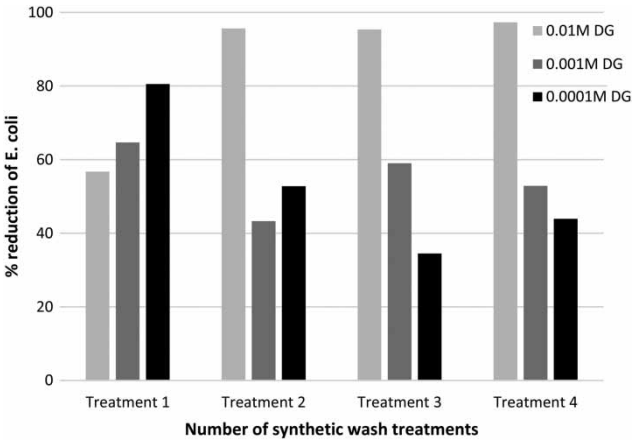

Figure 5 | Synthetic water treatments with dodecyl glucoside (DG) in an *f*-sand column.

amount of bacterial reduction. In this study, the absorbance between 0.1 and 0.001 at 600 nm was comparable to the cultivatable count taken from the solutions. However, for concentrations below 1×10^5 CFU/mL, colony counting only should be performed.

To confirm that the dodecyl glucoside was effective at separating the *E. coli* from the *f*-sand, three further columns, A1, A2 and A3, were prepared and are presented in Figure 6. For A1, the column was washed with 0.01 M dodecyl glucoside after each *E. coli* suspension, and each treatment retains the efficiency of the first run. Two synthetic water treatments were undertaken in succession for A2 and three for A3 without exposure to dodecyl glucoside between synthetic water treatments. For A2, the column was washed with dodecyl glucoside after the second treatment, and the restoration of the column is apparent on the third treatment. A fourth synthetic treatment was immediately run through the column and the efficiency was reduced. For A3, the column was run three times before it was washed with the dodecyl glucoside, and by the third treatment the *E. coli* removal dropped to below 10%. After washing with the dodecyl glucoside, the *f*-sand regained its initial efficacy.

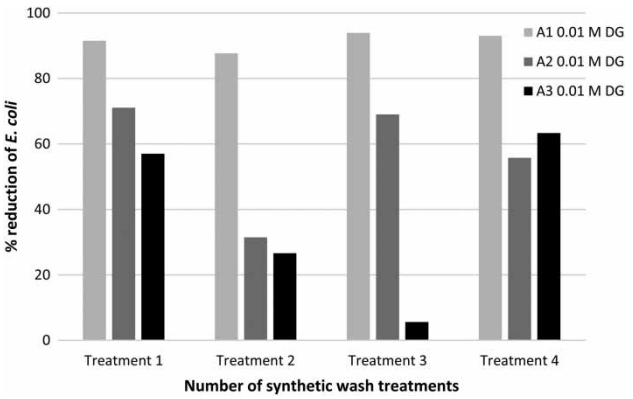

Figure 6 | 0.01 M dodecyl glucoside (DG) column tests. The order of washes is A1: *E. coli* suspension, 0.01 M dodecyl glucoside, *E. coli* susp., 0.01 M dodecyl glucoside, *E. coli* susp., 0.01 M dodecyl glucoside, *E. coli* susp., 0.01 M dodecyl glucoside. A2: *E. coli* susp., *E. coli* susp., 0.01 M dodecyl glucoside, *E. coli* susp. A3: *E. coli* susp., *E. coli* susp., *E. coli* susp., 0.01 M dodecyl glucoside, *E. coli* susp.

Commercially available dishwashing detergent

Dodecyl glucoside is a surfactant that is widely used in commercial detergents and can be readily sourced in local supermarkets. The efficacy of a detergent mixture reported to contain between 10 and 30% v/v dodecyl glucoside was tested on the *f*-sand columns. 1% and 2% solutions were prepared, which corresponded to approximately 0.25 and 0.57 M dodecyl glucoside, respectively, and were compared against the 0.01 M v/v dodecyl glucoside solution as shown in Figure 7. The 2% solution was comparable to the 0.01 M dodecyl glucoside, whereas the 1% solution exhibited a loss of efficacy for each run. This result further confirms the use of dodecyl glucoside as an effective treatment for the removal of *E. coli* from *f*-sand.

Bacterial staining

The attachment of *E. coli* to the *f*-sand was clearly visible when viewed on the live cell microscope, as presented in Figure 8. After washing with the 0.01 M dodecyl glucoside,

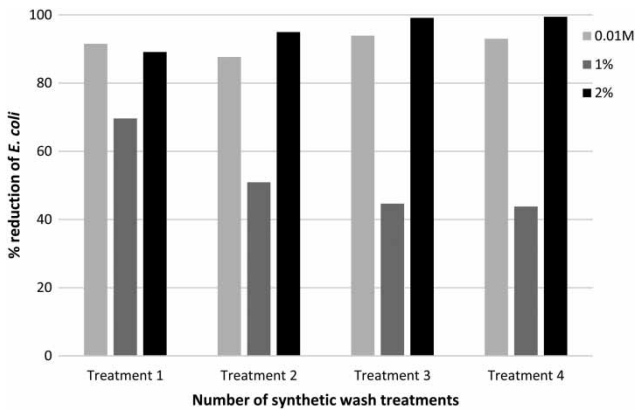

Figure 7 | Synthetic water treatments with commercially available detergent and dodecyl glucoside in an *f*-sand column.

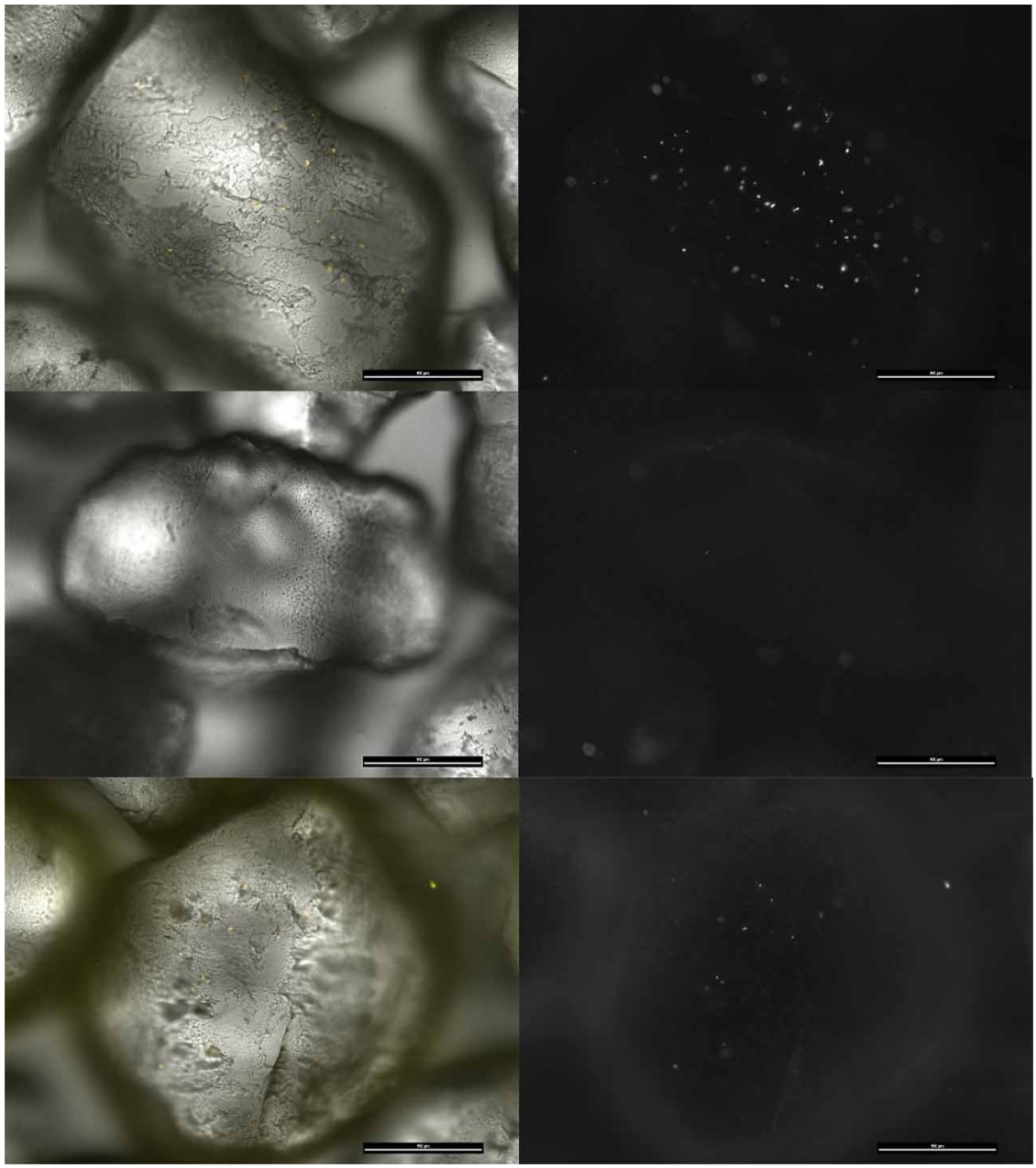

Figure 8 | Top: untreated sand, unused *f*-sand; middle: *f*-sand with *E. coli, f*-sand washed with 0.01 M dodecyl glucoside; bottom: washed *f*-sand with reattached *E. coli.* Scale bar 100 μm.

the *E. coli* was no longer visible on the *f*-sand. After further exposure to the bacterial solution, the *f*-sand attached and inhibited more *E. coli*. For each image, the left-hand image corresponds to the superimposed fluorescence-photo image, and the right-hand image is the fluorescence image only. The untreated sand and unused *f*-sand did not display any fluorescence.

DISCUSSION

$MO_{2.1}$ is a potent inhibitor of a range of bacterial and viral organisms (Broin *et al.* 2002). The key mechanism of action of $MO_{2.1}$ involves the interference and absorbance of the polypeptide through the hydrophobic regions of bacterial phospholipid membranes, reported in *E. coli* to

cause lysis and cell death (Suarez *et al.* 2005; Shebek *et al.* 2015). When $MO_{2.1}$ is attached to *f*-sand, the bacterial attachment prevents any further bacterial attachment, and the aim of this study was to develop a method of renewing the *f*-sand. Kwaambwa *et al.* (2010) examined the attachment of the coagulant protein to the oxide layer on an III face of a cut silicon crystal, reporting that saturation occurred at 5.5 mg/m^2. Rinsing with water or exposing the surface to a 2×10^{-3} M sodium dodecyl sulfate solution did not cause the protein to separate from the silica surface. In a similar experiment, the coagulant protein was shown to adsorb onto the surface of aluminium oxide (Kwaambwa *et al.* 2015). However, in that study, the coagulant protein could be removed by rinsing with water after exposure to concentrations of sodium dodecyl sulfate below the critical micelle concentration (Kwaambwa *et al.* 2015). Additionally, washing the surface with a 9×10^{-3} M solution of the cationic surfactant cetyltrimethylammonium bromide caused all of the protein to be removed (Kwaambwa *et al.* 2015).

In our study, the *f*-sand sample washed with a sodium dodecyl sulfate solution above the critical micelle concentration had an 80% reduction in the amount of $MO_{2.1}$ attached to the sand. The next sample was just below this and also showed a loss of $MO_{2.1}$. All of the samples were exposed to concentrations above the critical aggregation concentration. It is likely that the 0.01 M sodium dodecyl sulfate was able to overcome the electrostatic adsorption of most of the $MO_{2.1}$ polymers; however, some strongly bonded polypeptides remained. The third sample, which was below the critical micelle concentration but still above the critical aggregation concentration, did not exhibit a loss of $MO_{2.1}$. Kwaambwa *et al.* (2010) reported that the 2×10^{-3} M sodium dodecyl sulfate solution exposed to a cut silicon crystal co-adsorbed onto the silica surface with a ratio of four molecules per one *M. oleifera* protein molecule.

When the 0.001 and 0.0005 M sodium dodecyl sulfate were run through the *f*-sand column, it became apparent that the surfactant did not affect the attachment of the *E. coli*. Here, the formation of surfactant-$MO_{2.1}$ complexes and the solubilising of organic contaminants previously attached to the sand surface contribute to the increase in turbidity of the eluting solutions. The increase in turbidity of the outlet solutions was the most obvious on the first surfactant wash, where the eluting solution was visibly turbid.

Studies examining the effect of dodecyl glucoside on $MO_{2.1}$ have not been undertaken previously; however, reports of other non-ionic surfactants were encouraging. While all of the *f*-sand samples washed with dodecyl glucoside showed an immediate loss of $MO_{2.1}$, they remained stable around 80% for the subsequent washes. When the dodecyl glucoside solutions were run through the *f*-sand column, the high percentage reduction of bacteria for both 0.01 and 0.001 M dodecyl glucoside was unsurprising, as the destabilising mechanism is similar to that which occurs when phospholipid membranes are solubilised by detergents (Helenius & Simons 1975; le Maire *et al.* 2000). Bacterial membranes are made of a complex and dynamic lipid bilayer (Seddon *et al.* 2004) and gram negative bacteria possess a high density of anionic lipids (Shebek *et al.* 2015). The use of detergents to solubilise bacterial lipid membranes has been thoroughly investigated and covered by a three-stage hypothesis addressed by le Maire *et al.* (2000). In stage one, the free detergent molecules distribute into the phospholipid membrane. In stage two, the phospholipid membranes co-exist at a thermodynamic equilibrium comprised of phospholipids and detergent. Eventually, the detergent–detergent interactions destabilize the membrane structure, causing it to fragment. This leads to stage three, where, upon exposure to more detergent, the phospholipids become fully solubilised into the detergent micelles. In our study it is assumed that this three-stage phenomenon is what is causing the *E. coli* to separate from the $MO_{2.1}$. As the *E. coli* membrane was already disrupted by the $MO_{2.1}$, the hydrophobic phospholipids would readily solubilise into the dodecyl glucoside micelles and be removed from the *f*-sand column, leaving behind the $MO_{2.1}$. Since the dodecyl glucoside did not interfere with the attachment of the majority of the $MO_{2.1}$, this method allowed the reuse of the *f*-sand.

The amount of *E. coli* which the *f*-sand removed varied significantly between experimental runs. In some cases, it was 99%; however, in other runs it was as low as 57%. This was likely due to the sensitivity of the spectroscopy as a measurement method. When it was compared with colony counting, it was observed that the difference between the two methods was greater when the *f*-sand had lower efficacy, and the spectroscopy method underestimated the amount of bacteria removed when compared with direct colony

counting. It was still a useful measurement for this type of study, as it was a quick way of determining whether the system could be regenerated. For further scale-up studies, colony counting or similar methods should be employed.

CONCLUSIONS

Bacterial contamination of water is a significant problem and many people rely on untreated water for drinking purposes. $MO_{2.1}$, found within the seed of *Moringa oleifera,* can be readily adsorbed onto the surface of silica sand particles, which can then be used for the attachment and inhibition of bacteria such as *E. coli* in contaminated water. The attached bacteria prevent any further attachment and the *f*-sand is no longer effective for water treatment. The main findings from this study are as follows. 0.01 M dodecyl glucoside, a non-ionic surfactant, is effective at separating the *E. coli* from the $MO_{2.1}$, allowing the reuse of the *f*-sand. Sodium dodecyl sulfate, an anionic surfactant, causes the $MO_{2.1}$ to separate from the sand surface. A commercially available detergent containing 10–30% dodecyl glucoside was also effective at separating the *E. coli* from the sand.

The results demonstrated in this work offer a potentially sustainable, reusable process to combat bacterial contamination of water.

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