Supporting Information for:

Engineering solutions to improve the removal of bacteria by bioinfiltration systems during intermittent flow of stormwater

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Tables and Figures:

Methods:

Coating sand with iron oxide. Iron oxide was coated on clean sand following the method outlined elsewhere.[1] Briefly, 200 mL of sand was mixed with 80 mL of 2.5 $FeCl_3$, baked at 110 °C until dry, heated at 550 °C for 3 h, cooled at room temperature in air, rinsed several times in deionized water, and dried at 110 °C. The dried sand was mixed with 400 mL of 2.1 M $Fe(NO_3)_3$ and 3 mL of 10 M NaOH and heated at 110 °C for 14 h. The coated sand was sieved (0.5 mm opening) to remove fine iron oxide particles, washed several times in deionized water, dried at 110 °C. The iron mineral coated in this method was identified as hematite using X-ray diffraction (data not shown). The sand and coated sands were autoclaved (121 °C, 100 kPa, 15 min) and stored in a sterile container prior to use in the column experiments. It is not reported whether autoclave changes the surface property of sand or coated sand. A previous study[2] autoclaved sand and coated sand before conducting transport experiment with bacteria.

Preparation of bacteria culture. Preserved strains were stored in 25% glycerol at -80 °C and used to culture bacteria for all experiments following method outlined elsewhere[3]. Briefly, a loop from frozen stock was streaked on tryptic soy agar (TSA) plate and incubated at 37 °C for 24 h. A single colony was transferred into 20 mL of tryptic soy broth (TSB) and incubated at 37°C for 8 h. About 20 µL of the culture was transferred into second batch of 20 mL of TSB and incubated at 37 °C for 16 h. The harvested culture was centrifuged at 5000 g for 5 min, and the pallet was rinsed twice with phosphate buffer saline (PBS) to remove growth media. The PBS solution was replaced with synthetic stormwater following centrifugation and the cells were suspended in synthetic stormwater to achieve a suspension of $0.8 – 1.8 \times 10^6$ colony forming units (cfu)/mL. The bacterial suspension was equilibrated with stormwater for 16-18 h at 4 °C and the suspension were warmed to room temperature in a water bath before using it in column experiments.

Experimental technique to drain water. The injection and subsequent mobilization of bacteria in stormwater involved inverting the column at different stages of the experiments to maintaining the direction of flow of water: (1) Injection of bacteria under saturated condition (flow from bottom to top; injection), (2) draining of pore water in the inverted column (flow from top to bottom; pause), and (3) application bacteria-free stormwater by further inverting the column (flow from bottom to top; intermittent flow). All experiments could have been conducted by injecting stormwater from top to bottom without having to invert the columns. However, after draining the column (0.5 pv in 30 min), the injection at top did not increase the water content in column because water flows through preferential flow, thereby bypassing most fractions of sand columns. This is not ideal and not representative of environmentally relevant condition, where the moisture content of bioinfiltration system increases during storm event. Additionally, preferential flow would have further complicated our results: the remobilization due to intermittent flow would be underpredicted as rewetting will not cause stormwater to access major fraction of sand column.

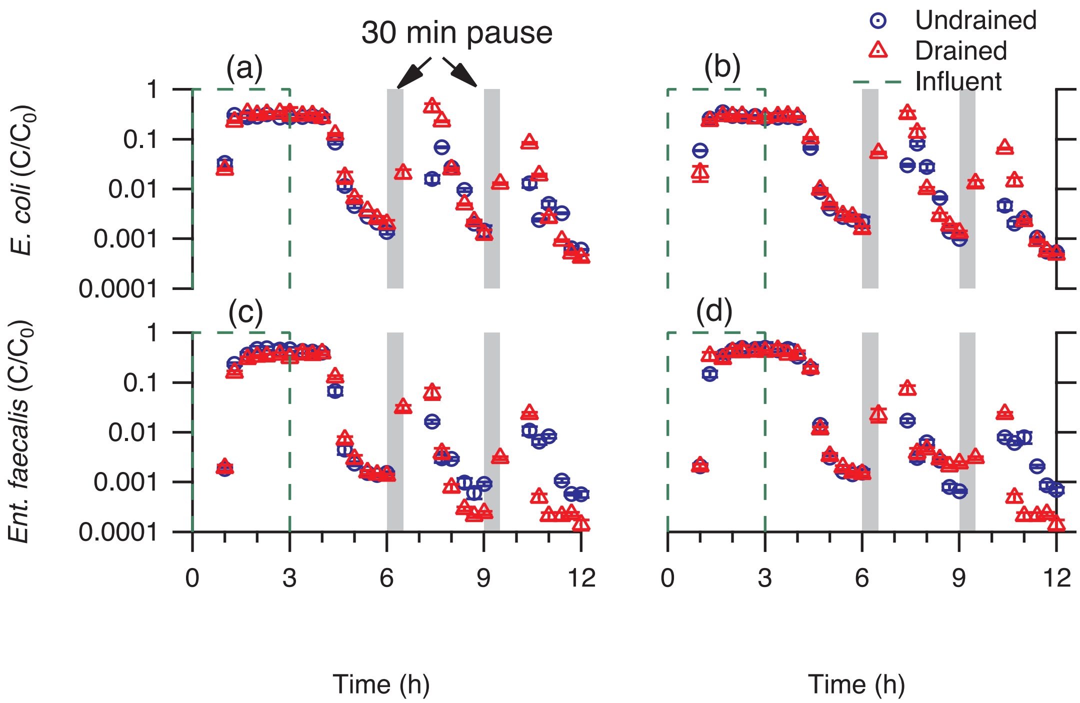

Figure S1. Results from duplicate experiments showing remobilization of *E. coli* (a-b) and *Ent. faecalis* (c-d) through undrained and drained sand column during intermittent flow. The influent concentration was 1.1 x 10^6 cfu/mL for *Ent. faecalis* and 2.6 x 10^6 cfu/mL for *E. coli.* The gray shades indicate 30 min pauses when the column was either undrained (circle) or drained (triangle). The error bar indicates one standard deviation of the mean.

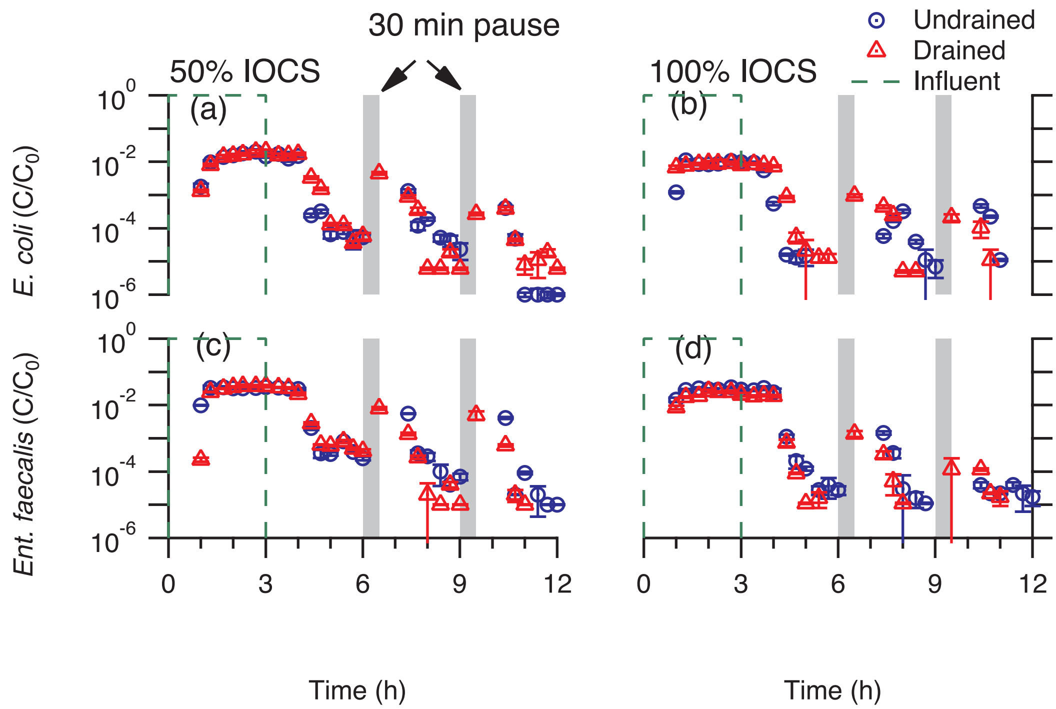

Figure S2. Mobilization of (a-b) *E. coli and* (c-d) *Ent. faecalis* in column packed with 50% IOCS (a, c), and 100% IOCS (b, d). The scale of y-axis is magnified for clarity. The gray shades indicate 30 min pauses when the column was either undrained (circle) or drained (triangle). **The error bar indicates one standard deviation of the mean.**

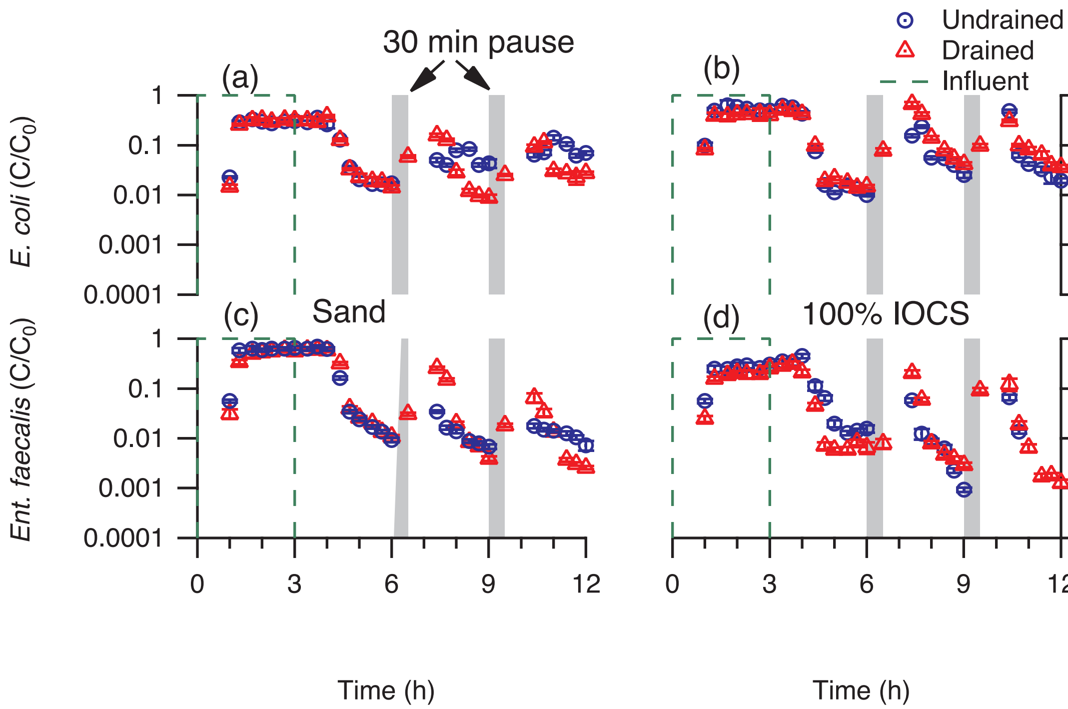

Figure S3. Removal and remobilization of (a-b) *E. coli* and (c-d) *Ent. faecalis* in stormwater containing 20 mg C L^{-1} of NOM. The gray shades indicate 30 min pauses when the column was either undrained (circle) or drained (triangle). **The error bar indicates one standard deviation of the mean.**

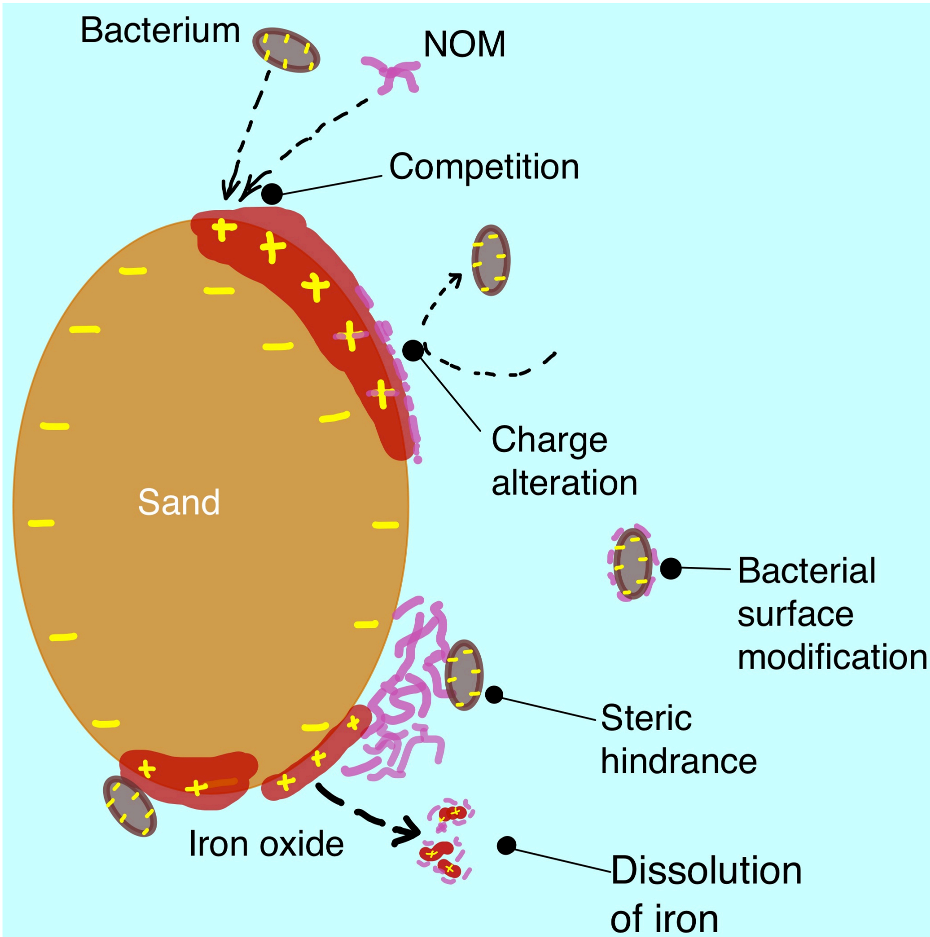

Figure S4. NOM can influence the attachment of bacteria on geomedia by (1) competing with bacteria for same attachment site, (2) altering positive surface charge of iron oxide or other minerals, (3) blocking attachment sites (steric hindrance), (4) modifying bacterial hydrophobicity, and (5) dissolving and stabilizing minerals such as iron oxide.

References

1. Benjamin, M. M.; Sletten, R. S.; Bailey, R. P.; Bennett, T., Sorption and filtration of metals using iron-oxide-coated sand. *Water Research* 1996, *30*, (11), 2609-2620.
2. Bolster, C. H.; Mills, A. L.; Hornberger, G. M.; Herman, J. S., Effect of surface coatings, grain size, and ionic strength on the maximum attainable coverage of bacteria on sand surfaces. *Journal of Contaminant Hydrology* 2001, *50*, (3–4), 287-305.
3. Wang, L.; Xu, S.; Li, J., Effects of Phosphate on the Transport of Escherichia coli O157:H7 in Saturated Quartz Sand. *Environmental Science & Technology* 2011, *45*, (22), 9566-9573.