water

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Article

Revisiting Soil Aquifer Treatment: Improving Biodegradation and Filtration Efficiency Using a Highly Porous Material

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Supplementary Materials

Table S1. Physiochemical and biological characteristics of the Shafdan secondary treated effluent measured during the spring (May 2018), summer (August 2018), and autumn (November 2018).

Parameter / Month	Av. Temp.[1]	pH	Bacterial Production	Bacterial Abundance	Total Organic Carbon	Total Nitrogen
	°C		μg C L^{-1} d^{-1}	Bac. mL^{-1}	mg L^{-1}	mg L^{-1}
May	24.9	7.2	172	NA	10	6
August	30.2	7.2	203	9.5×10^{8}	11	9
November	23.4	7.6	211	9.8×10^{5}	13	8

1.1. Experimental Design

Secondary treated wastewater (TWW) effluent, taken from the Shafdan infiltration ponds, was sterilized, cooled, and loaded into the different quadruplicate experimental column sets of media via peristaltic pumps set to an average infiltration flux rate that was similar to Shafdan (~1 m3 m-2 d-1). This flow rate was checked prior to the beginning of the experiment, as well as every day of the experiment, to verify consistency. After the experiment successfully commenced, each column was then individually wrapped in aluminum foil to limit the occurrence of photosynthetic activity (Figure S1, B). The overall system was then covered with aluminum foil for a second time to further minimize photosynthetic activity.

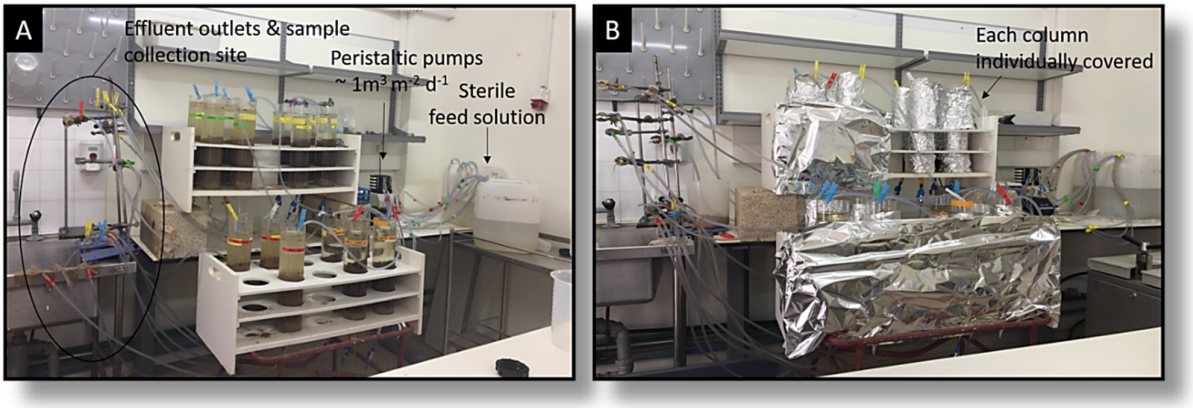

Figure S1. Photo of actual experimental design (**A**) and with the aluminum foil cover to limit the occurrence of photosynthetic activity (**B**).

1.2. Bacterial Production Rates (BP)

Media samples (~1.8 g) from each column were collected at the end of each experiment, re-suspended in 3.5 mL of sterile secondary effluent, and spiked with 90 nmol L^{-1} (final concentration) of [4,5-^{3}H]-leucine (Perkin Elmer USA, specific activity 123.8 Ci $mmol^{-1}$) as previously described by Frank et al. 2019.(Frank et al., 2019) The samples were incubated in a dark rotation for 4 h under ambient temperature. At the end of the incubation, the Leucine assimilation was terminated by adding 100 µL of 100% trichloroacetic acid (TCA) cold solution. The samples (tuff and sand) were then sonicated (Elmasonic, Singen) twice for 10 min to remove the bacterial biomass from the grain particles. The biomass extract was divided into three 1-mL aliquots, and the micro-centrifugation technique was applied.(Smith and Azam, 1992) Disintegration per minute (DPM) from each sample was read using a liquid scintillation counter (Packard Tri carb 2100). A conversion factor of 1.5 kg C mol^{-1} with an isotope dilution factor of 2.0 was used to calculate the bacterial carbon assimilation rate.(Simon and Azam, 1989).

1.3. Bacterial Abundance (BA)

Media were collected (~2.2 g) in sterile 15-mL falcon tubes and suspended in 6 mL of sterile secondary effluent. Each sample was supplemented with 600 µL of 50 mM ethylenediaminetetraacetic acid (EDTA) as a chelator and then vortexed prior to two cycles (10 s) of probe-sonication (Q Sonica Sonicator). Supernatant (1.7 m/L) was transferred to a sterile 2-mL cryotube (Thermo Scientific), while the media were dried and weighed for normalization to the gram. In addition, outflow samples were taken directly from the column output prior to termination of the experiment. Supernatant and outflow samples were fixed with glutaraldehyde (50% in H2O, specially purified, Sigma Aldrich), incubated at room temperature for 10 min, and stored at −80 °C. Prior to analysis with an Attune NxT acoustic focusing flow cytometer (life technologies), samples were thawed in a 37 °C water bath and diluted with phosphate buffered saline (PBS). Unstained samples, as well as samples stained with SYBR® Green (Thermo Fisher Technologies), were analyzed at a flow speed of 25 µL min-1. Staining allowed the identification of total bacteria using green

fluorescence (ex. 488 nm, em. 530 nm) over side scatter (ex. 488 nm), while the auto-fluorescence in red (ex. 488 nm, em. 695 nm) over orange (ex. 488 nm, em. 574 nm) of unstained samples was used to count phototrophic bacteria. BA of heterotrophic bacteria was thus obtained as the difference between phototrophs and total bacterial count. An example graph of the flow cytometry results is provided below (Fig. S2).

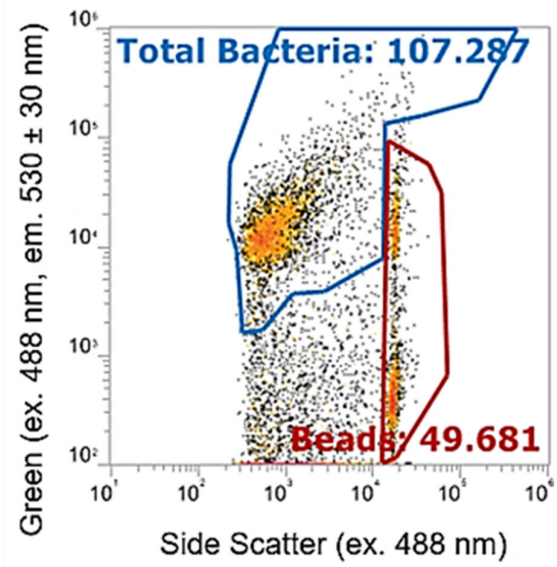

Figure S2. Representative plot of flow cytometry analysis. Selection of total bacteria in green fluorescence over side scatter. Numbers refer to events per µL, and "Beads" refers to 1-µm beads of the microsphere standard.

Results and Discussion

Table S2. Microbial and organic analysis of sand and tuff media during the different experiments, which took place in May, August, and November 2018. Numbers are shown as averages (with corresponding standard deviations STDV) of each quadruplicate set.

Method & Medium	Units	May	August	November
BP sand	µg C g^{-1} d^{-1}	0.4 ± 0.2	0.01 ± < 0.001	0.01 ± < 0.001
BP tuff	µg C g^{-1} d^{-1}	2.1 ± 0.4	0.6 ± 0.1	0.1 ± 0.04
BR sand	µg C g^{-1} d^{-1}	2.93 ± 0.2	3.67 ± 0.2	3.45 ± 0.6
BR tuff	µg C g^{-1} d^{-1}	5.30 ± 1.7	8.17 ± 0.5	9.51 ± 0.5
BCD sand	µg C g^{-1} d^{-1}	3.30 ± 0.1	3.67 ± 0.2	3.46 ± 0.6
BCD tuff	µg C g^{-1} d^{-1}	7.41 ± 0.8	8.73 ± 0.5	9.61 ± 0.5
BA attached to sand	Het × 10^8. Bac. g^{-1}	36 ± 5.5	2.0	1.9 ± 0.5
BA attached to tuff	Het. × 10^8 Bac. g^{-1}	35 ±3.1	3.7 ± 0.3	0.5 ± 0.2
TOC sand	µg C g^{-1}	33.8 ± 16.3	31.6 ± 17.1	22.8 ± 10.7
TOC tuff	µg C g^{-1}	16.8 ± 7.8	11.6 ± 0.1	45.5 ± 18.3
TN sand	µg N g^{-1}	19.2 ± 13.7	3.2 ± 1.1	18.6 ± 16.4
TN tuff	µg N g^{-1}	10.4 ± 8.9	3.5 ± 0.2	10.3 ± 1.9

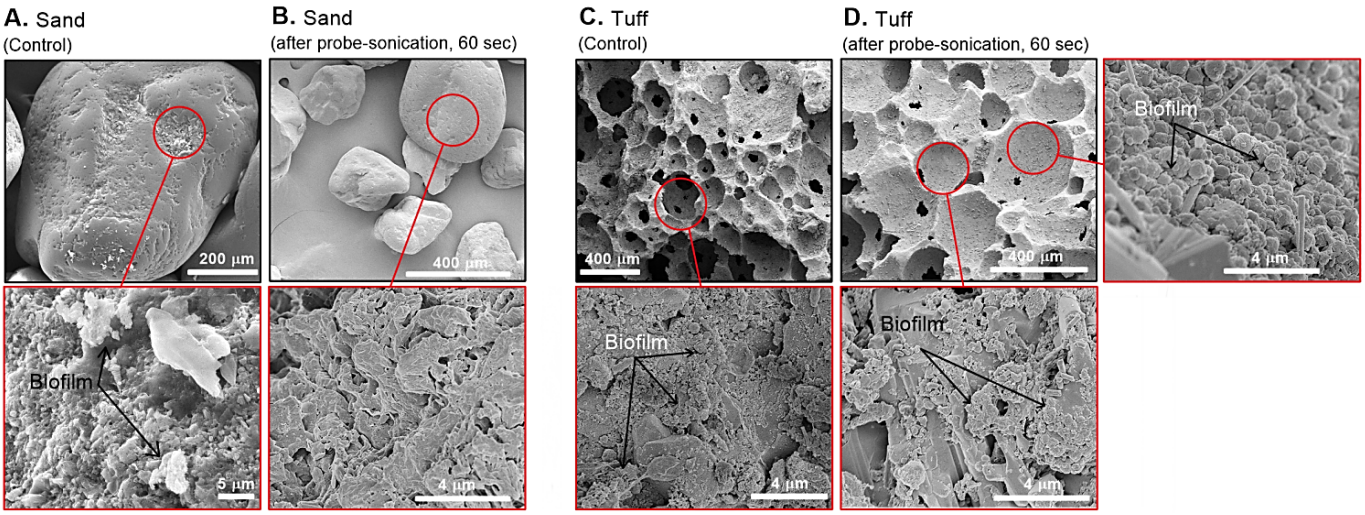

Figure S3. Scanning electron microscopy images of a sand grain taken from the SAT infiltration pond pre-sonication (**A**); and a separate sand grain after 60 s of probe-sonication, shown to be devoid of biofilm presence (**B**). In contrast to the sand grain, the tuff grain pre-sonication (**C**); and an additional tuff grain after 60 s of probe-sonication (**D**) still contained high concentrations of biofilm in its various canals.

Table S3. Outflow water quality results. Water quality results of the sterilized secondary WW (which served as the feed solution-inflow) and the captured outflow after 48 h of percolation through the columns of media, throughout the different experiments that took place in May and November 2018. Numbers are shown as averages (with STDV) of each quadruplicate set**.**

Organic material	Units	May	November
TOC in the feed solution (secondary WW effluent)	mg L^{-1}	14.6 ± 0	14.0 ± 0
TOC in sand effluent	mg L^{-1}	12.2 ± 0.2	11.6 ± 0.91
TOC in tuff effluent	mg L^{-1}	8.68 ± 1.5	9.86 ± 0.13
TN in the feed solution (secondary WW effluent)	mg L^{-1}	7.50 ± 0	6.80 ± 0
TN in sand effluent	mg L^{-1}	6.47 ± 0.31	6.10 ± 0.33
TN in tuff effluent	mg L^{-1}	2.87 ± 0.98	5.92 ± 0.16
BA in the feed solution (secondary WW effluent)	Het. Bac × 10^9 L^{-1}	3.2 ± 0.13	2.1 ± 0
BA in sand effluent	Het. Bac × 10^9 L^{-1}	3.6 ± 0.23	1.8 ± 0.68
BA in tuff effluent	Het. Bac × 10^9 L^{-1}	1.9 ± 0.65	1.3 ± 0.24