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QUANTIFICATON OF BIOFILM BUILD-UP IN FILTERS WHEN INTERMITTENTLY LOADED WITH LOW-STRENGTH SYNTHETIC WASTEWATER

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ABSTRACT

Accumulation of particulate matter and microorganisms present in wastewater as biofilm on the surface of filters can lead to clogging of the media. If clogging of filters occurs, they need to be temporarily decommissioned before they can be operated again. The mechanisms causing clogging may only be delineated through destructive sampling of a filter. The aim of this study was to characterise the build-up of biofilm in the upper layer below the surface of polishing filters intermittently loaded with effluent from a novel horizontal flow biofilm reactor used for the treatment of domestic-strength wastewater. Three filter media were used: crushed glass, sand, and a shallow podzolized soil. The

parameters used to measure biofilm build-up were: soil water retention, total phosphorus (Tot-P) content and loss on ignition (LOI). The LOI and Tot-P deposition near the filter surface were lowest in the glass filters. Soil water retention curves indicated that biofilm formation mainly occurred in the uppermost 0.03 m depth below the filter surface and gradually decreased with depth. This indicates that measurements of volumetric water content using time domain reflectometry probes may be used as an *in situ* proxy for measurements that would normally require the destructive sampling of a filter.

Keywords: horizontal flow biofilm reactor; filtration; scanning electron microscopy; surface clogging; biofilm; soil water characteristic curve.

1. Introduction

In Ireland, 396,000 houses use septic tanks and percolation areas to treat their wastewater [1]. In these systems, wastewater flows into a two-chambered septic tank, where primary sedimentation and some anaerobic treatment occur, and then to a percolation area for further physical, chemical and biological treatment. If properly designed, constructed and operated, septic tank/percolation systems are capable of treating domestic wastewater to a high standard.

Half of the soils of Ireland are considered unsuitable for percolation areas in the treatment of septic tank effluent [2]. An area may be unsuitable for percolation if: 1) the saturated hydraulic conductivity of the soil is too high or too low; 2) if the bedrock or water table is too close to the surface; or 3) if the site is confined. In such cases, attached growth systems, such as filters, rotating biological contactors, or suspended growth systems, such as sequencing batch reactors and activated sludge systems, are recommended [3].

A novel horizontal flow biofilm reactor (HFBR) for treating wastewater, comprising a stack of about 40 horizontal polyvinyl chloride sheets, has been developed by researchers at the National University of Ireland, Galway [4]. In this system, wastewater is intermittently pumped onto the top of the stack. Wastewater flows along each sheet from one end to the other and back again on the next underneath sheet, down through the stack. As the wastewater flows along the sheets of the stack, biofilms form and organic carbon (C), total suspended solids (TSS), nutrients and bacteria are removed. This system has been successfully used in other studies to treat domestic-strength wastewater and has achieved chemical oxygen demand (COD) and total nitrogen (Tot-N) removals of over 90% [5, 6]. The effluent from this unit may be polished using intermittently-loaded filters, which are efficient in the treatment of domestic [7, 8] and agricultural wastewaters [9, 10]. Over time, due to the accumulation of hydrated extracellular polymers (exopolymers), the presence of living and dead microorganisms, or mass accumulation within the media pores, the hydraulic conductivity may gradually reduce, leading to clogging in the upper layers of the filter [11]. Clogging involves several mechanisms such as [12]: reduction of pore space by TSS and bacterial growth on entrapped or dissolved solids.

The occurrence of clogging is a function of the organic and TSS loading rates applied to the filter [9, 13] and the US EPA has set guidelines for the operation of single-pass and recirculating sand filters ([14]; single-pass: 22 g biochemical oxygen demand (BOD_5) m^{-2} d^{-1}, effective size, d_{10}, 0.25-1 mm; recirculation: 22 g BOD_5 m^{-2} d^{-1}; d_{10}, 1-5 mm).

The presence of a clogging layer may be characterised in terms of physico-chemical parameters, such as organic matter and nutrients, or physical parameters, such as water retention capacity [15]. The effects of biomass build-up may be shown by the soil-water characteristic curve, $\theta_V(h)$ [16], which is a graph of the volumetric water content, θ_V, against the pore-water suctions, h, imposed, and is dependent on the texture and structure of the media. The presence of biofilm growth may also result in a higher air entry value (the point at which air becomes continuous in a soil), greater water retention capacity (as biofilm is hydrophilic), and lower field-saturated hydraulic conductivity [15]. In filters loaded with domestic septic tank effluent, greywater septic tank effluent and tapwater, Siegrist [17] attributed the most significant changes in water content near the infiltration surface to the pore size reduction due to biomass build-up and, after 62 months of operation, the water contents in the upper 0.04 m layer for tapwater and domestic septic tank effluent were 0.26 and 0.36, respectively. Rodgers et al. [15] obtained similar results.

Spychała and Błażejewski [18] examined the performance of 0.3m-deep filter columns, comprising fine sand with a d_{10} of 0.1 mm over a 596-day study duration. At organic and TSS loading rates of 6 g BOD_5 m^{-2} d^{-1} and 2.7 g TSS m^{-2} d^{-1}, respectively, five of the filters became clogged after approximately 150 days (Table 1). The organic matter content, measured as mg BOD_5 g^{-1} dry sand, in the uppermost sand layer was 14 mg g^{-1} dry sand and reduced to 3 mg g^{-1} at a depth of 0.12 m below the filter surface. Spychała and Błażejewski [18] attributed surface clogging to the presence of bacterial slimes, rather than the presence of bacteria, which only occupied 0.00112% of the pore volume in the clogging layer. Similar results were found by Rodgers et al. [15], who found elevated organic matter content and nutrients in the uppermost layer of a 0.9 m-deep stratified sand filter (d_{10} of uppermost sand layer, 0.1 mm) loaded with synthetic agricultural wastewater at rates ranging from 6.5 to 76 g COD m^{-2} d^{-1} over a 767-day study (Table 1). Liu et al. [19] measured ATP (adenosine 5'-triphosphate) biomass in the uppermost layer of stratified sand filters intermittently loaded at a rate of approximately 4.4 g COD m^{-2} $d^{-1}$with a butterfat and detergent mixture (Table 1). Before clogging occurred at 132 days, the ATP concentrations ranged between 3.65 – 13.7 μg ATP mm^{-2}, when normalized to surface area.

To ensure enhanced removal of TSS, organics and nutrients, HFBRs can be used in conjunction with a tertiary treatment system. This paper investigates the use of three types of filters – glass, sand, soil – in tertiary treatment. Specifically, the aim of this paper was to characterise biofilm development after 525 days in intermittently-loaded filters polishing low-strength effluent from a laboratory HFBR unit treating domestic-strength wastewater. The measured parameters were: 1) soil water characteristic curve; 2) total phosphorus (Tot-P); and 3) organic matter. Scanning electron microscopy (SEM) was used to compare biofilm build-up in the clogging layer versus virgin material. To assess the relative impact of loading filters with low-strength wastewater, the measured parameters were compared to similar filters loaded with high-strength wastewater.

2. Materials and Methods

Eight 0.65 m and six 0.375 m-deep laboratory filter columns containing sand, crushed glass and soil were built under a water suction of approximately 0.1 m (Fig. 1). Each of the columns had an internal diameter of 0.150 m. Six columns contained glass, five contained sand and three contained soil. Three glass columns were 0.65 m-deep and three were 0.375 m-deep. Two sand columns were 0.65 m-deep, three sand columns were 0.375 m-deep, and three soil columns were 0.65 m-deep. The bottom layer of each medium was underlain by a 0.075 m layer of distribution gravel (10-20 mm in diameter). In the 0.65 m-deep glass and sand columns this was overlain by a 0.2 m layer of fine glass (0.5 to 1.1 mm in particle size) or sand (d_{10} = 0.15 mm), respectively, under a 0.075 m-deep distribution gravel and 0.2 m-deep layer of fine glass or sand. The top layer was 0.1 m deep and comprised distribution gravel (10-20 mm in diameter). In the 0.375 m-deep glass and sand columns, a 0.1 m layer of distribution gravel (10-20 mm in diameter) overlay a 0.2 m layer of fine glass and sand, respectively. In the 0.65 m-deep soil columns, a 0.1 m layer of distribution gravel (10-20 mm in diameter) overlay a 0.475 m layer of top soil (a shallow podzolized soil sieved to less than 5 mm; d_{10} = 0.02 mm). The glass, sand and soil filters were packed to average bulk densities of 1700, 1500 and 1200 kg m^{-3}, respectively. The base of each filter comprised a series of holes drilled in plastic stop-ends.

The influent wastewater used in this experiment was the final effluent from a laboratory HFBR treating synthetic domestic-strength wastewater. The synthetic wastewater was made up daily (after Odegaard and Rusten [20] and with composition as in Table 2) and pumped onto the top sheet of the HFBR. The final effluent from the HFBR was collected daily in a sump and was intermittently loaded, via spiral distribution manifolds, onto the surfaces of the filters. The pump was operational for 5 minute durations each hour and, throughout the 525-day study period, a hydraulic loading rate of 100 L m^{-2} d^{-1} was applied to all filter columns. Influent and effluent water samples were tested at least twice per week in accordance with the Standard Methods [21].

At the end of the 525-day study period, loading was suspended and the columns were dismantled. The physical and chemical properties of each media in the uppermost 0.15 m layer were examined. Two intact media samples were taken at each 0.03 m incremental depth below the filter surface and the $\theta_V(h)$ was determined for each depth using the sand box method (Eijkelkamp Agrisearch Equipment Ltd., The Netherlands). The sand box method involves the application of incremental water suctions to a soil, contained in a stainless steel core, 10^{-4} m^3 in volume, and positioned on Blokzijl sand, via an adjustable suction device.

Tot-P, an indicator of the abundance of organic matter, was tested (after [22]) at the following depth increments: 0-0.01, 0.02 – 0.03, 0.05 – 0.06, and 0.09 – 0.12 m. Mass loss on ignition (LOI) was carried out at 0.01 m-depth increments to a depth of 0.06 m below the filter surface in the sand and glass filters in accordance with the British

Standards [23]. Supplementary LOI measurements were made at the following depth increments: 0.06 – 0.09 m and 0.09 – 0.12 m. LOI gives an indication of biomass distribution within each column. SEM was used to compare biofilm build-up on grains at the filter surface with a virgin sample. The samples were taken using an aluminum stub coated with quick-drying silver paint. The specimens were gold-coated in an Emscope SC 500 sputter coater (Emscope, Ashford, UK) and were viewed with a SEM (Model S-570, Hitachi, Tokyo, Japan).

3. Results and Discussion

3.1 Water quality results

The operational regime and performance of the filters are tabulated in Tables 3 and 4, respectively. The organic loading rate on the filters was 9.8 g COD m^{-2} d^{-1}, based on the top plan area. Throughout the study duration, statistical analysis using a paired-samples T test proved that there was no significant difference in COD removal within each set of filters for a particular medium at the 95% confidence interval (P=0.05). The 0.65m soil filter achieved the greatest COD reduction – 65% - and produced an average COD effluent concentration of 34.0±10.5 mg COD L^{-1}. At the 95% confidence interval, there was a significant difference between the 0.65 m-deep soil, glass and sand columns. The 0.65 m and 0.35 m–deep sand filters had effluent COD concentrations of 53.8±23.7 mg COD L^{-1} and 54.5±21.3 mg COD L^{-1}, respectively – a 45% and 44% reduction, respectively. All filters produced final effluent COD concentrations that were much less than the Urban Waste Water Treatment Directive [24] value of 125 mg COD L^{-1}.

Complete TSS removal occurred in all filter columns. The 0.65 m-deep soil columns also performed best in bacteria removal and achieved an average effluent bacteria concentration of $0.5 \times 10^6 \pm 0.2 \times 10^6$ CFU per 100 ml. The effluent NH_4-N concentration from all filters was close to zero, indicating that practically complete nitrification had occurred in all filters, irrespective of their depth.

3.2 Soil water characteristic curves

The $\theta_V(h)$ curves for the glass, sand and soil filters are illustrated in Figures 2, 3 and 4, respectively. The $\theta_V(h)$ curves indicate that biofilm formation mainly occurred in the uppermost 0.03 m depth below the filter surface and gradually decreased with depth. The saturated volumetric water content, θ_s, decreased from the surface to a depth of 0.15 m – indicating that biofilm did not extend far into the media - and ranged from 38.6% to 34.2% in the glass filter, 45.9% to 39.2% in the sand filter, and 54% to 51% in the soil filter. The lack of significant increases in the removal of COD with respect to filter depth would appear to be related to the build-up of biofilm in the uppermost filter layer of each medium. θ_s is used as an indication of biofilm build-up as it allows all the media to be compared against each other under zero suction. The extent to which biofilm permeated the filters appeared to vary between media. Generally, there was very little difference between the $\theta_V(h)$ curves at all measured suctions greater than 0.1 m of water in the glass filters, suggesting that the biofilm mainly formed in the uppermost 0.03 m filter layer (Figure 2). Relative to the glass filters, the sand and soil filters had a greater variation in water retention capacities at all measured suctions (Figures 3 and 4, respectively), suggesting that the biofilm layer penetrated further into these filter media. As all filters had the same organic and hydraulic loading regime, this suggests that media size or composition may influence clogging layer formation.

3.3 Other indicators of biofilm formation

The deposition of Tot-P (Figure 5) and the LOI in the upper 0.12 m sand and glass filter layers (Figure 6) appear to confirm the conclusions from the $\theta_V(h)$ curves. Over the study duration, approximately 12 g P was applied to the sand and glass filters, whereas approximately 6 g P was applied to the soil filters. The Tot-P adsorbed in the filters over the measured depth of 0.12 m was approximately 80 mg (glass), 120 mg (sand) and 173 mg (soil). The Tot-P deposition was lowest in the glass filter and ranged from 31.8±2 mg kg^{-1} near the filter surface to 19.2±2 mg kg^{-1} at a depth of 0.12 m (Figure 5). The LOI ranged from 0.43±0.09% in the upper-most layer to 0.04±0.01% at a depth of 0.12 m. These values reflect the soil water characteristic curves. The LOI of virgin glass was 0.04%. The greatest reduction in LOI – 71% of the overall reduction – occurred within 0.01m of the glass filter surface (Figure 6). The Tot-P deposition in the upper 0.12 m layers of the sand and soil filters followed the same trend (i.e. higher concentrations at the filter surface versus lower concentrations with depth), but were more evenly distributed with depth below the filter surfaces. Tot-P deposition ranged from 50±5 mg kg^{-1} to

30.2±4 mg kg^{-1} for the sand filter, and from 50±3 mg kg^{-1} to 45.6±4 mg kg^{-1} for the soil filter. Echoing the results of the $\theta_V(h)$ curves for the sand filter (Figure 3), the LOI values for the sand filter suggested a more even distribution of biofilm in the upper 0.12 m depth than the glass filter. Measured values ranged from 0.72±0.09 % at the surface to 0.34±0.02 % at a depth of 0.12 m from the filter surface. The LOI of virgin sand was 0.33%. In a sand filter loaded at rates ranging from 6.5 to 76 g COD m^{-2} d^{-1} for a period of 767 days and dismantled after clogging (at a final organic loading rate of 18.2 g COD m^{-2} d^{-1} applied for 42 days), Rodgers et al. [15] measured Tot-P concentrations ranging from 1500 mg kg^{-1} near the filter surface to 600 mg kg^{-1} at a depth of 0.12 m.

Figures 7 and 8 show the SEM analysis for the glass and sand surface filter layers, respectively, at the end of the 525-day study period on virgin samples of media. SEM analysis was conducted on the soil filters, but, due to the nature of the soil granules, the results were indistinguishable. SEM analysis showed organic deposits that were in accordance with the indirect quantitative $\theta(h)$ and loss on ignition results. The figures indicate varying degrees of biofilm build-up, although they were not as pronounced as the *schmutzdecke* of biofilm measured at the filter surface by Rodgers et al. [15]. In virgin glass and sand (Figures 7 and 9, respectively), the grains were clearly distinguishable, but, after 525 days of operation, they are indistinguishable. This confirms that the clogging layer is a surface phenomenon. Although the organic and inert materials were high below the surface of the upper-most filter layers (Figures 2-6), Figures 7 and 8 indicate that the clogging layer developed as a *schmutzdecke* (a surface biological layer) on the surface.

Although the results from this study indicate that organic and particulate materials will build up in filters loaded with low-strength effluent, after 525 days of operation, no substantial filter clogging occurred. As biofilm is hydrophilic, measurements of the volumetric water content using time domain reflectometry (TDR) are a good way to determine *in-situ* measurements of biofilm build-up, and can be used as an indication of the 'state' of a filter. Although the small diameter of the columns used in this study (0.15 m) precluded such measurements, the variation in the $\theta_V(h)$ curves are correlated with the volumetric water content [15], and exhibit the same trend as the physical measurements of biofilm build-up.

4. Conclusions

The following conclusions may be drawn from this study:

1. Biofilm formation in intermittently-loaded sand, glass and soil polishing filters occurs mainly in the uppermost 0.12 m-deep filter layer.
2. The degree to which nutrients are deposited in a filter media depends on the applied organic loading rate.
3. On the basis of soil water retention, Tot-P and LOI measurements, the biofilm did not appear to penetrate as deep into the glass filters as in the sand and soil filters. This may indicate that media size and composition may also be controlling factors in biofilm formation.

4. As soil water retention measurements were analogous to measured parameters, which can only be quantified through destructive sampling of a filter, measurements of the volumetric water content using TDR are a good way to determine *in-situ* measurements of biofilm build-up, and can be used as an indication of the ‘state’ of a filter.

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Captions for figures

Figure. 1. Schematic of the laboratory filters.

Figure 2. Soil-water characteristic curve for the glass filters.

Figure 3. Soil-water characteristic curve for sand filters.

Figure 4. Soil-water characteristic curve for the soil filters.

Figure 5. Deposition of Tot-P (mg kg^{-1} of filter media) in the upper 0.12 m from the filter surface.

Figure 6. Mass loss on ignition to a depth of 0.15 m from the filter surface in the sand and glass filters.

Figure 7. Scanning electron microscopy (SEM) photography on a sample of the surface virgin glass layer *(left)* and on a sample of the glass layer *(right)* after 525 days of operation.

Figure 8. Scanning electron microscopy (SEM) photography on a sample of the surface virgin sand layer *(left)* and on a sample of the sand layer *(right)* after 525 days of operation.

Table 1. Performance of intermittently-loaded filters prior to clogging.

Reference	Wastewater type	Media type	Column depth (m)	Loading rates g COD m^{-2} d^{-1}	g TSS m^{-2} d^{-1}	Time to clogging days	Comments
Liu et al., 2003	Butterfat and detergent	Sand	0.61	~ 4.4		131	Single layer fine sand
			0.61	~ 4.4		131	0.3m coarse sand overlain 0.3m fine sand
			0.92	~ 4.4		245	3 layers – pea gravel, coarse and fine sand.
Rodgers et al., 2004b[1]	Agricultural	Sand	0.9	18.2	3.1	42	Filter previously operated at lower organic loading rates with no occurrence of clogging.
Spychała and Błażejewski, 2003	Domestic	Sand	0.3	11.6[2]	2.7	~150	Clogging due to bacterial slimes.
EPA guidelines[3]	Domestic	Sand	0.61-0.91	9.3	3.9		

[1] Organic loading rate quoted is final loading rate before clogging. Filter was operational for 725 days before final loading application commenced.
[2] Organic concentration reported in paper = 71 mg BOD L^{-1}. BOD_5/COD ratio estimated as 0.5 [25].
[3] US EPA [14]. Calculations based on a typical flow of 24 L m^{-2} d^{-1} with a septic tank effluent COD and TSS concentration of 389 and 163 mg L^{-1}, respectively [3].

Table 2. Composition of synthetic wastewater used to simulate domestic wastewater[1]

Component	Amount (g)
Glucose	18
Yeast	2.7
Dried Milk	10.8
Urea	2.7
NH_4Cl	5.4
$Na_2PO_4.12H_2O$	9
$KHCO_3$	4.5
$NaHCO_3$	11.7
$MgSO_4.7H_2O$	4.5
$FeSO_4.7H_2O$	0.18
$MnSO_4.H_2O$	0.18
$CaCl_2.6H_2O$	0.27
Bentonite	3.6

[1] Diluted to 90 litres

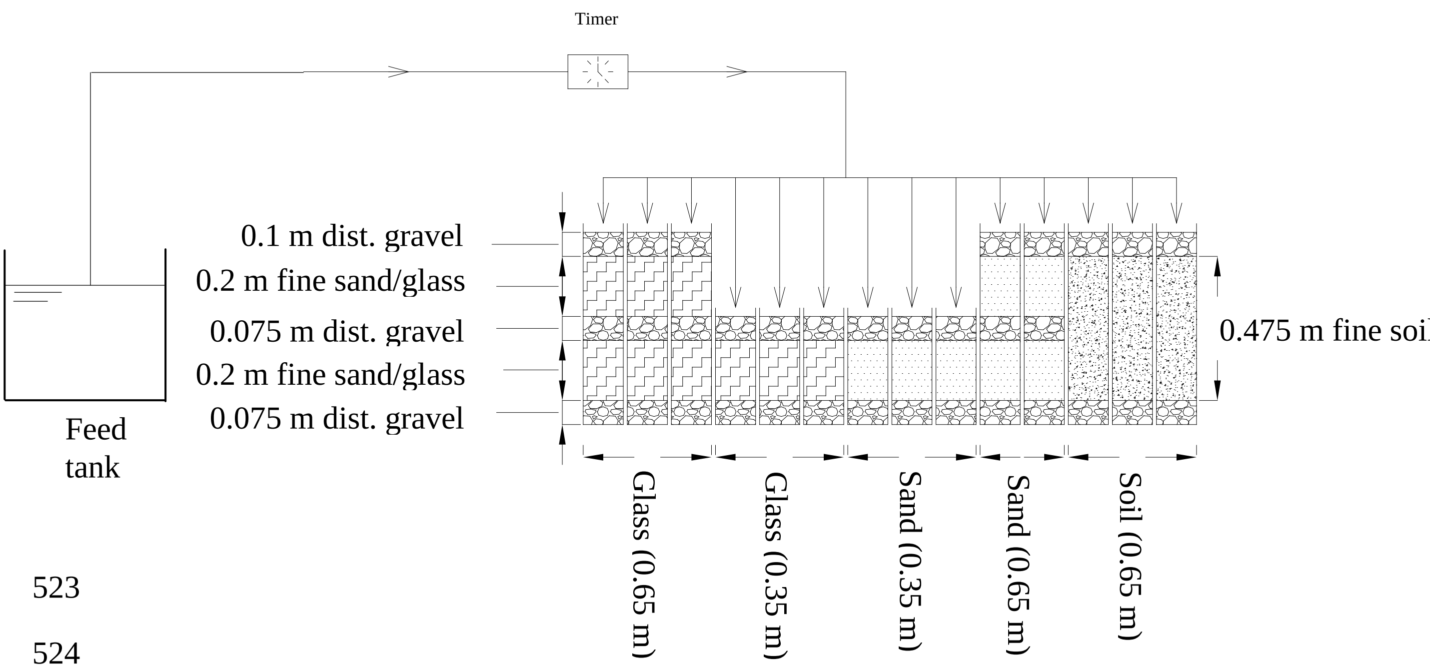
Table 3. Operational parameters, water quality parameters and loading rates for the laboratory filters.

Days of operation (d)	525
Filter hydraulic loading rate (L m^{-2} d^{-1})	100
Average organic loading rate (g COD m^{-2} d^{-1})	9.9
Average influent COD concentration (mg L^{-1})	99.2±13.4
Average TSS loading rate (g TSS m^{-2} d^{-1})	2.2
Average influent TSS concentration (mg L^{-1})	22.4±13.5

Table 4. Performance of laboratory filters.

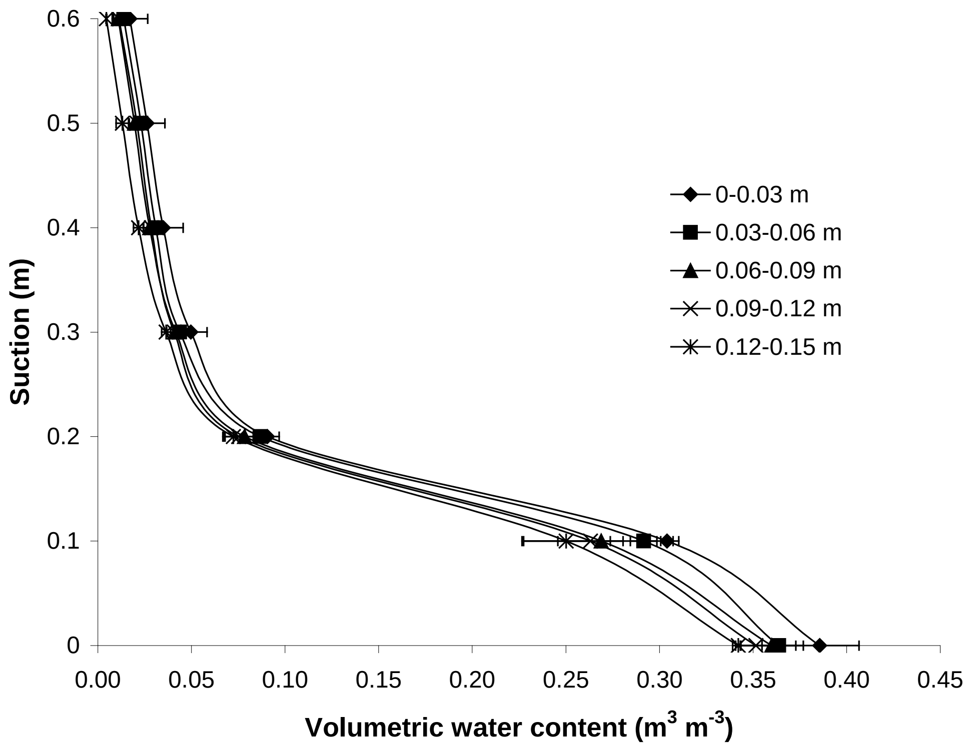
Media	Depth m	% Removals		
		COD	TSS	Heterotrophs
Glass	0.65	56.2	100	81.8
	0.375	42.4	100	79.7
Sand	0.65	45.3	100	85.3
	0.375	44.4	100	79.6
Soil	0.65	65.4	100	92.6

Figure. 1.



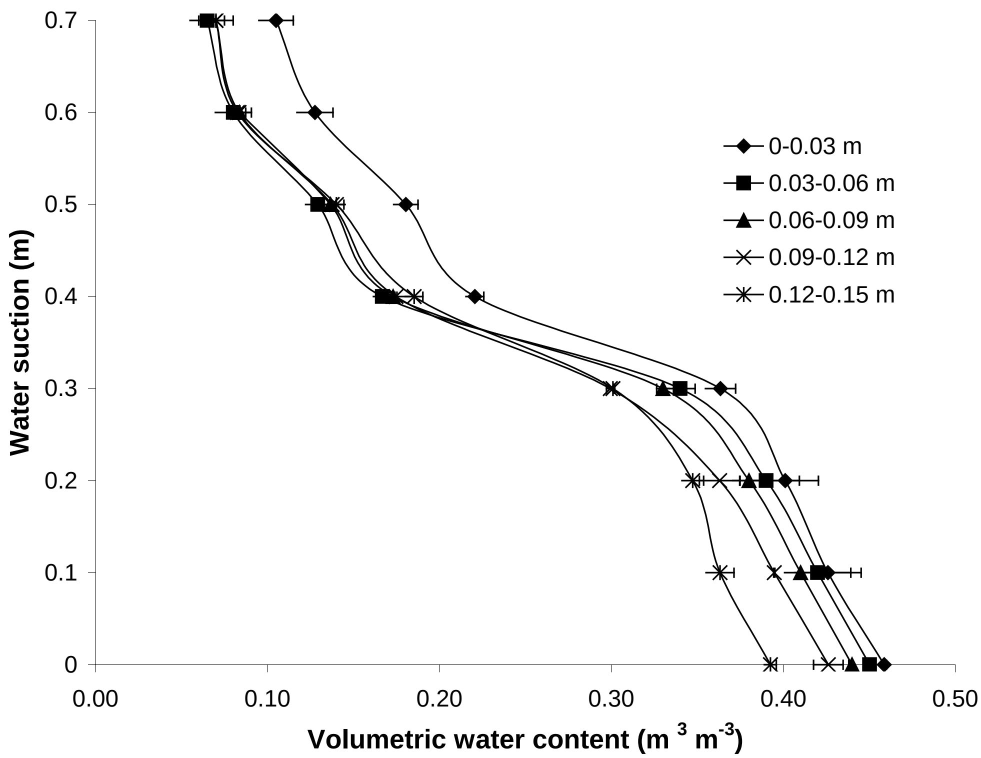
Timer
0.1 m dist. gravel
0.2 m fine sand/glass
0.075 m dist. gravel
0.2 m fine sand/glass
0.075 m dist. gravel
Feed tank
0.475 m fine soil
Glass (0.65 m)
Glass (0.35 m)
Sand (0.35 m)
Sand (0.65 m)
Soil (0.65 m)

Figure 2.



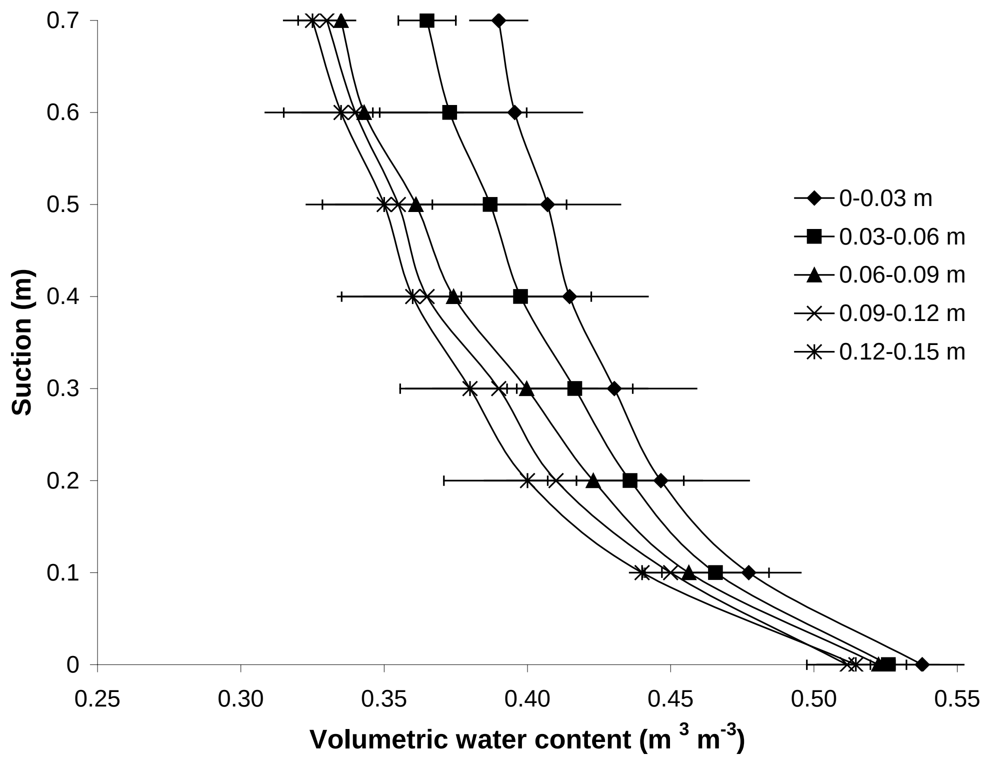
0-0.03 m
0.03-0.06 m
0.06-0.09 m
0.09-0.12 m
0.12-0.15 m
Suction (m)
0.6
0.5
0.4
0.3
0.2
0.1
0
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35
0.40
0.45
Volumetric water content (m3 m-3)

Figure 3.



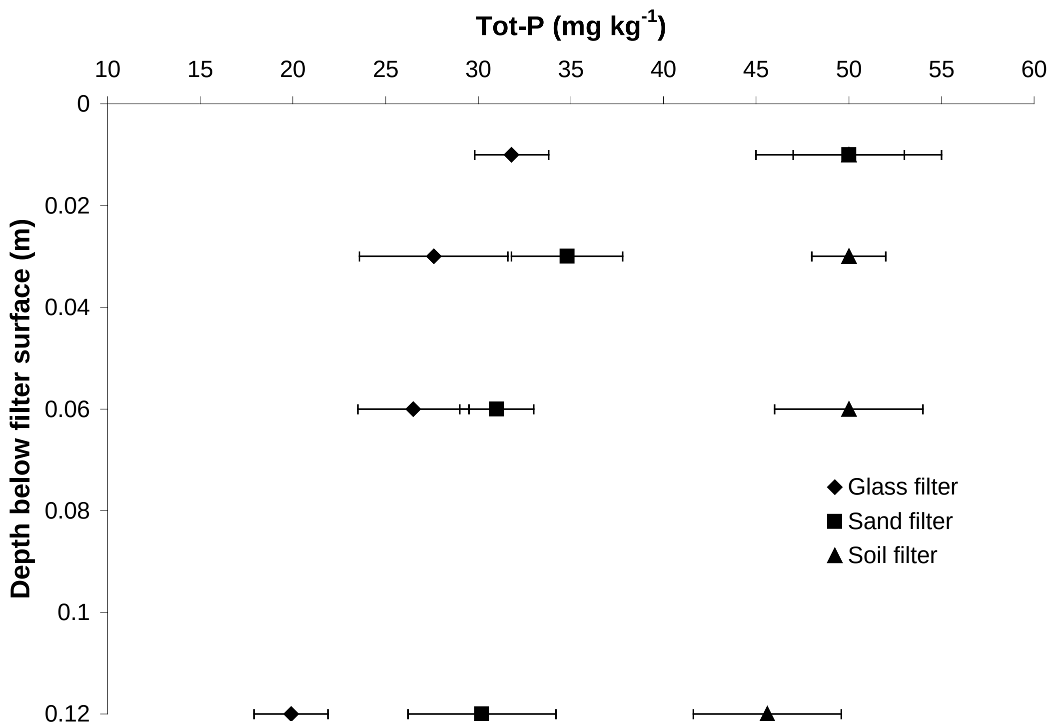
Water suction (m)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0.00
0.10
0.20
0.30
0.40
0.50
Volumetric water content (m 3 m-3)
0-0.03 m
0.03-0.06 m
0.06-0.09 m
0.09-0.12 m
0.12-0.15 m

Figure 4.



Suction (m)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0.25
0.30
0.35
0.40
0.45
0.50
0.55
Volumetric water content (m 3 m-3)
0-0.03 m
0.03-0.06 m
0.06-0.09 m
0.09-0.12 m
0.12-0.15 m

Figure 5.



Tot-P (mg kg-1)
10
15
20
25
30
35
40
45
50
55
60
Depth below filter surface (m)
0
0.02
0.04
0.06
0.08
0.1
0.12
Glass filter
Sand filter
Soil filter

Figure 6.

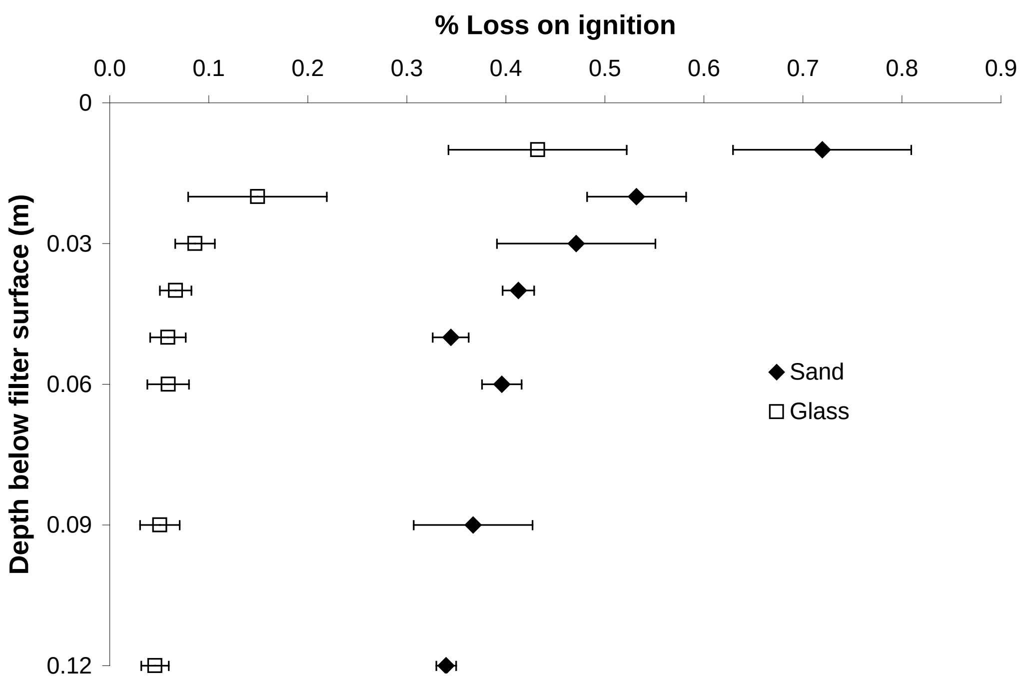

% Loss on ignition
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
0
0.03
0.06
0.09
0.12
Depth below filter surface (m)
Sand
Glass

Figure 7.

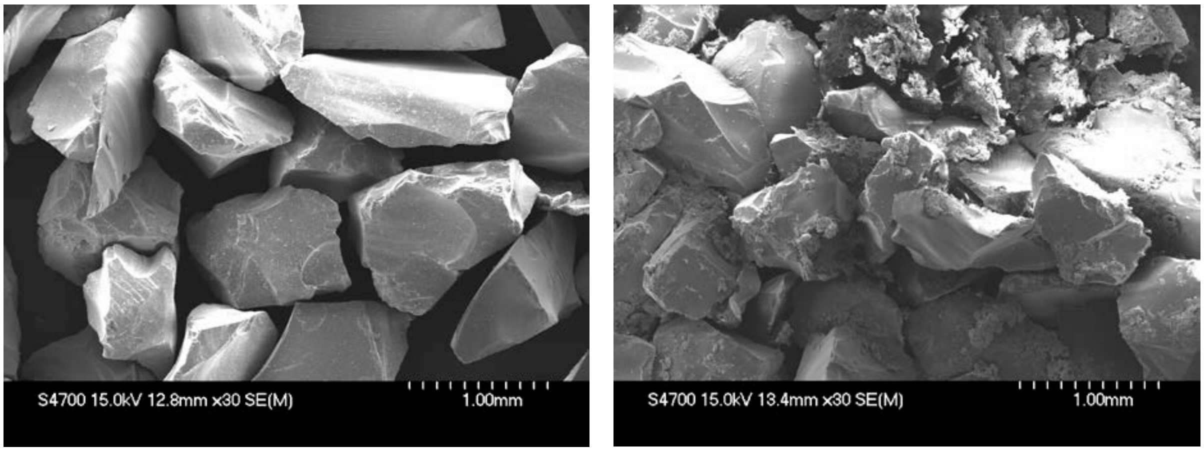
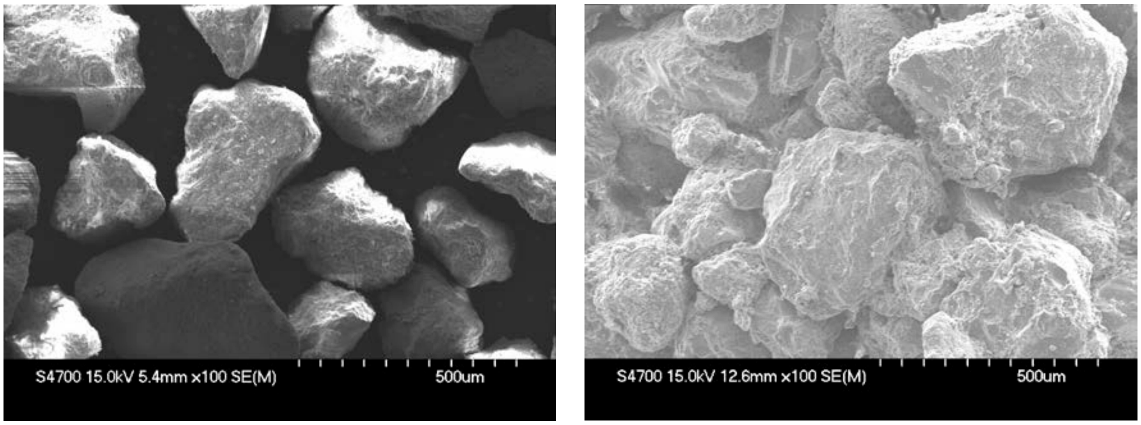

S4700 15.0kV 12.8mm x30 SE(M)
1.00mm
S4700 15.0kV 13.4mm x30 SE(M)
1.00mm

Figure 8.


S4700 15.0kV 5.4mm x100 SE(M)
500um
S4700 15.0kV 12.6mm x100 SE(M)
500um