



## **Quantification of biofilm build-up in filters when intermittently loaded with low-strength synthetic wastewater**

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Publication Date	2011

1 *Published as: Healy, M.G., Rodgers, M., Burke, P. 2011. Quantification of biofilm build-up*  
2 *in filters when intermittently loaded with low-strength synthetic wastewater. Desalination*  
3 *271(1-3): 105-110. doi:10.1016/j.desal.2010.12.024*  
4

5 QUANTIFICATION OF BIOFILM BUILD-UP IN FILTERS WHEN  
6 INTERMITTENTLY LOADED WITH LOW-STRENGTH SYNTHETIC  
7 WASTEWATER

8  
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14

15 ABSTRACT

16  
17 Accumulation of particulate matter and microorganisms present in wastewater as biofilm  
18 on the surface of filters can lead to clogging of the media. If clogging of filters occurs,  
19 they need to be temporarily decommissioned before they can be operated again. The  
20 mechanisms causing clogging may only be delineated through destructive sampling of a  
21 filter. The aim of this study was to characterise the build-up of biofilm in the upper layer  
22 below the surface of polishing filters intermittently loaded with effluent from a novel  
23 horizontal flow biofilm reactor used for the treatment of domestic-strength wastewater.  
24 Three filter media were used: crushed glass, sand, and a shallow podzolized soil. The

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

32

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

35

## 36 **1. Introduction**

37

38 In Ireland, 396,000 houses use septic tanks and percolation areas to treat their wastewater  
39 [1]. In these systems, wastewater flows into a two-chambered septic tank, where primary  
40 sedimentation and some anaerobic treatment occur, and then to a percolation area for  
41 further physical, chemical and biological treatment. If properly designed, constructed and  
42 operated, septic tank/percolation systems are capable of treating domestic wastewater to a  
43 high standard.

44

45 Half of the soils of Ireland are considered unsuitable for percolation areas in the treatment  
46 of septic tank effluent [2]. An area may be unsuitable for percolation if: 1) the saturated  
47 hydraulic conductivity of the soil is too high or too low; 2) if the bedrock or water table is

48 too close to the surface; or 3) if the site is confined. In such cases, attached growth  
49 systems, such as filters, rotating biological contactors, or suspended growth systems, such  
50 as sequencing batch reactors and activated sludge systems, are recommended [3].

51

52 A novel horizontal flow biofilm reactor (HFBR) for treating wastewater, comprising a  
53 stack of about 40 horizontal polyvinyl chloride sheets, has been developed by researchers  
54 at the National University of Ireland, Galway [4]. In this system, wastewater is  
55 intermittently pumped onto the top of the stack. Wastewater flows along each sheet from  
56 one end to the other and back again on the next underneath sheet, down through the stack.  
57 As the wastewater flows along the sheets of the stack, biofilms form and organic carbon  
58 (C), total suspended solids (TSS), nutrients and bacteria are removed. This system has  
59 been successfully used in other studies to treat domestic-strength wastewater and has  
60 achieved chemical oxygen demand (COD) and total nitrogen (Tot-N) removals of over  
61 90% [5, 6]. The effluent from this unit may be polished using intermittently-loaded  
62 filters, which are efficient in the treatment of domestic [7, 8] and agricultural wastewaters  
63 [9, 10]. Over time, due to the accumulation of hydrated extracellular polymers  
64 (exopolymers), the presence of living and dead microorganisms, or mass accumulation  
65 within the media pores, the hydraulic conductivity may gradually reduce, leading to  
66 clogging in the upper layers of the filter [11]. Clogging involves several mechanisms  
67 such as [12]: reduction of pore space by TSS and bacterial growth on entrapped or  
68 dissolved solids.

69

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

74

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

89

90 Spychała and Błażejowski [18] examined the performance of 0.3m-deep filter columns,  
91 comprising fine sand with a  $d_{10}$  of 0.1 mm over a 596-day study duration. At organic and  
92 TSS loading rates of 6 g BOD<sub>5</sub> m<sup>-2</sup> d<sup>-1</sup> and 2.7 g TSS m<sup>-2</sup> d<sup>-1</sup>, respectively, five of the

93 filters became clogged after approximately 150 days (Table 1). The organic matter  
94 content, measured as mg BOD<sub>5</sub> g<sup>-1</sup> dry sand, in the uppermost sand layer was 14 mg g<sup>-1</sup>  
95 dry sand and reduced to 3 mg g<sup>-1</sup> at a depth of 0.12 m below the filter surface. Spychała  
96 and Błażejowski [18] attributed surface clogging to the presence of bacterial slimes,  
97 rather than the presence of bacteria, which only occupied 0.00112% of the pore volume  
98 in the clogging layer. Similar results were found by Rodgers et al. [15], who found  
99 elevated organic matter content and nutrients in the uppermost layer of a 0.9 m-deep  
100 stratified sand filter (*d*<sub>10</sub> of uppermost sand layer, 0.1 mm) loaded with synthetic  
101 agricultural wastewater at rates ranging from 6.5 to 76 g COD m<sup>-2</sup> d<sup>-1</sup> over a 767-day  
102 study (Table 1). Liu et al. [19] measured ATP (adenosine 5'-triphosphate) biomass in the  
103 uppermost layer of stratified sand filters intermittently loaded at a rate of approximately  
104 4.4 g COD m<sup>-2</sup> d<sup>-1</sup> with a butterfat and detergent mixture (Table 1). Before clogging  
105 occurred at 132 days, the ATP concentrations ranged between 3.65 – 13.7 μg ATP mm<sup>-2</sup>,  
106 when normalized to surface area.

107

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

116 the relative impact of loading filters with low-strength wastewater, the measured  
117 parameters were compared to similar filters loaded with high-strength wastewater.

118

## 119 **2. Materials and Methods**

120

121 Eight 0.65 m and six 0.375 m-deep laboratory filter columns containing sand, crushed  
122 glass and soil were built under a water suction of approximately 0.1 m (Fig. 1). Each of  
123 the columns had an internal diameter of 0.150 m. Six columns contained glass, five  
124 contained sand and three contained soil. Three glass columns were 0.65 m-deep and three  
125 were 0.375 m-deep. Two sand columns were 0.65 m-deep, three sand columns were  
126 0.375 m-deep, and three soil columns were 0.65 m-deep. The bottom layer of each  
127 medium was underlain by a 0.075 m layer of distribution gravel (10-20 mm in diameter).  
128 In the 0.65 m-deep glass and sand columns this was overlain by a 0.2 m layer of fine  
129 glass (0.5 to 1.1 mm in particle size) or sand ( $d_{10} = 0.15$  mm), respectively, under a 0.075  
130 m-deep distribution gravel and 0.2 m-deep layer of fine glass or sand. The top layer was  
131 0.1 m deep and comprised distribution gravel (10-20 mm in diameter). In the 0.375 m-  
132 deep glass and sand columns, a 0.1 m layer of distribution gravel (10-20 mm in diameter)  
133 overlay a 0.2 m layer of fine glass and sand, respectively. In the 0.65 m-deep soil  
134 columns, a 0.1 m layer of distribution gravel (10-20 mm in diameter) overlay a 0.475 m  
135 layer of top soil (a shallow podzolized soil sieved to less than 5 mm;  $d_{10} = 0.02$  mm). The  
136 glass, sand and soil filters were packed to average bulk densities of 1700, 1500 and 1200  
137  $\text{kg m}^{-3}$ , respectively. The base of each filter comprised a series of holes drilled in plastic  
138 stop-ends.

139

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

149

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

158

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



163 Standards [23]. Supplementary LOI measurements were made at the following depth  
164 increments: 0.06 – 0.09 m and 0.09 – 0.12 m. LOI gives an indication of biomass  
165 distribution within each column. SEM was used to compare biofilm build-up on grains at  
166 the filter surface with a virgin sample. The samples were taken using an aluminum stub  
167 coated with quick-drying silver paint. The specimens were gold-coated in an Emscope SC  
168 500 sputter coater (Emscope, Ashford, UK) and were viewed with a SEM (Model S-570,  
169 Hitachi, Tokyo, Japan).

170

### 171 **3. Results and Discussion**

172

#### 173 3.1 Water quality results

174

175 The operational regime and performance of the filters are tabulated in Tables 3 and 4,  
176 respectively. The organic loading rate on the filters was  $9.8 \text{ g COD m}^{-2} \text{ d}^{-1}$ , based on the  
177 top plan area. Throughout the study duration, statistical analysis using a paired-samples T  
178 test proved that there was no significant difference in COD removal within each set of  
179 filters for a particular medium at the 95% confidence interval ( $P=0.05$ ). The 0.65m soil  
180 filter achieved the greatest COD reduction – 65% - and produced an average COD  
181 effluent concentration of  $34.0 \pm 10.5 \text{ mg COD L}^{-1}$ . At the 95% confidence interval, there  
182 was a significant difference between the 0.65 m-deep soil, glass and sand columns. The  
183 0.65 m and 0.35 m–deep sand filters had effluent COD concentrations of  $53.8 \pm 23.7 \text{ mg}$   
184  $\text{COD L}^{-1}$  and  $54.5 \pm 21.3 \text{ mg COD L}^{-1}$ , respectively – a 45% and 44% reduction,

185 respectively. All filters produced final effluent COD concentrations that were much less  
186 than the Urban Waste Water Treatment Directive [24] value of 125 mg COD L<sup>-1</sup>.

187

188 Complete TSS removal occurred in all filter columns. The 0.65 m-deep soil columns also  
189 performed best in bacteria removal and achieved an average effluent bacteria  
190 concentration of  $0.5 \times 10^6 \pm 0.2 \times 10^6$  CFU per 100 ml. The effluent NH<sub>4</sub>-N concentration  
191 from all filters was close to zero, indicating that practically complete nitrification had  
192 occurred in all filters, irrespective of their depth.

193

### 194 3.2 Soil water characteristic curves

195

196 The  $\theta_V(h)$  curves for the glass, sand and soil filters are illustrated in Figures 2, 3 and 4,  
197 respectively. The  $\theta_V(h)$  curves indicate that biofilm formation mainly occurred in the  
198 uppermost 0.03 m depth below the filter surface and gradually decreased with depth. The  
199 saturated volumetric water content,  $\theta_s$ , decreased from the surface to a depth of 0.15 m –  
200 indicating that biofilm did not extend far into the media - and ranged from 38.6% to  
201 34.2% in the glass filter, 45.9% to 39.2% in the sand filter, and 54% to 51% in the soil  
202 filter. The lack of significant increases in the removal of COD with respect to filter depth  
203 would appear to be related to the build-up of biofilm in the uppermost filter layer of each  
204 medium.  $\theta_s$  is used as an indication of biofilm build-up as it allows all the media to be  
205 compared against each other under zero suction. The extent to which biofilm permeated  
206 the filters appeared to vary between media. Generally, there was very little difference  
207 between the  $\theta_V(h)$  curves at all measured suctions greater than 0.1 m of water in the glass

208 filters, suggesting that the biofilm mainly formed in the uppermost 0.03 m filter layer  
209 (Figure 2). Relative to the glass filters, the sand and soil filters had a greater variation in  
210 water retention capacities at all measured suctions (Figures 3 and 4, respectively),  
211 suggesting that the biofilm layer penetrated further into these filter media. As all filters  
212 had the same organic and hydraulic loading regime, this suggests that media size or  
213 composition may influence clogging layer formation.

214

### 215 3.3 Other indicators of biofilm formation

216

217 The deposition of Tot-P (Figure 5) and the LOI in the upper 0.12 m sand and glass filter  
218 layers (Figure 6) appear to confirm the conclusions from the  $\theta_v(h)$  curves. Over the study  
219 duration, approximately 12 g P was applied to the sand and glass filters, whereas  
220 approximately 6 g P was applied to the soil filters. The Tot-P adsorbed in the filters over  
221 the measured depth of 0.12 m was approximately 80 mg (glass), 120 mg (sand) and 173  
222 mg (soil). The Tot-P deposition was lowest in the glass filter and ranged from  $31.8 \pm 2$  mg  
223  $\text{kg}^{-1}$  near the filter surface to  $19.2 \pm 2$  mg  $\text{kg}^{-1}$  at a depth of 0.12 m (Figure 5). The LOI  
224 ranged from  $0.43 \pm 0.09\%$  in the upper-most layer to  $0.04 \pm 0.01\%$  at a depth of 0.12 m.  
225 These values reflect the soil water characteristic curves. The LOI of virgin glass was  
226 0.04%. The greatest reduction in LOI – 71% of the overall reduction – occurred within  
227 0.01m of the glass filter surface (Figure 6). The Tot-P deposition in the upper 0.12 m  
228 layers of the sand and soil filters followed the same trend (i.e. higher concentrations at the  
229 filter surface versus lower concentrations with depth), but were more evenly distributed  
230 with depth below the filter surfaces. Tot-P deposition ranged from  $50 \pm 5$  mg  $\text{kg}^{-1}$  to

231 30.2±4 mg kg<sup>-1</sup> for the sand filter, and from 50±3 mg kg<sup>-1</sup> to 45.6±4 mg kg<sup>-1</sup> for the soil  
232 filter. Echoing the results of the  $\theta_v(h)$  curves for the sand filter (Figure 3), the LOI values  
233 for the sand filter suggested a more even distribution of biofilm in the upper 0.12 m depth  
234 than the glass filter. Measured values ranged from 0.72±0.09 % at the surface to  
235 0.34±0.02 % at a depth of 0.12 m from the filter surface. The LOI of virgin sand was  
236 0.33%. In a sand filter loaded at rates ranging from 6.5 to 76 g COD m<sup>-2</sup> d<sup>-1</sup> for a period  
237 of 767 days and dismantled after clogging (at a final organic loading rate of 18.2 g COD  
238 m<sup>-2</sup> d<sup>-1</sup> applied for 42 days), Rodgers et al. [15] measured Tot-P concentrations ranging  
239 from 1500 mg kg<sup>-1</sup> near the filter surface to 600 mg kg<sup>-1</sup> at a depth of 0.12 m.

240

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

254

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

264

#### 265 **4. Conclusions**

266

267 The following conclusions may be drawn from this study:

268

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

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

282

### 283 **Acknowledgements**

284

285 This research was supported by a student fellowship from the College of Engineering and  
286 Informatics, NUI Galway. The authors acknowledge the support of the late Dr. John  
287 Mulqueen, NUI Galway; Mr. Michael Coughlan, Dept. of Microbiology, NUI Galway;  
288 Messrs. Gerry Hynes and Peter Fahy, and Ms. Mary O'Brien, Civil Engineering, NUI  
289 Galway.

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391 **Captions for figures**

392

393 Figure 1. Schematic of the laboratory filters.

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

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

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

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

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

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

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

407

408

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

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411 Reference	412 Wastewater type	413 Media type	414 Column depth (m)	415 Loading rates		416 Time to clogging days	417 Comments
				g COD m <sup>-2</sup> d <sup>-1</sup>	g TSS m <sup>-2</sup> d <sup>-1</sup>		
				418 Liu et al., 2003	419 Butterfat and detergent		
425 Rodgers et al., 2004b <sup>1</sup>	426 Agricultural	427 Sand	428 0.9	429 18.2	430 3.1	431 42 Filter previously operated at lower organic loading rates with no occurrence of clogging.	
432 Sychała and Błażejowski, 2003	433 Domestic	Sand	0.3	11.6 <sup>2</sup>	2.7	~150 Clogging due to bacterial slimes.	
EPA guidelines <sup>3</sup>	Domestic	Sand	0.61-0.91	9.3	3.9		

431 <sup>1</sup> Organic loading rate quoted is final loading rate before clogging. Filter was operational for 725 days before final loading application commenced.

432 <sup>2</sup> Organic concentration reported in paper = 71 mg BOD L<sup>-1</sup>. BOD<sub>5</sub>/COD ratio estimated as 0.5 [25].

433 <sup>3</sup> US EPA [14]. Calculations based on a typical flow of 24 L m<sup>-2</sup> d<sup>-1</sup> with a septic tank effluent COD and TSS concentration of 389 and 163 mg L<sup>-1</sup>, respectively [3].

434 Table 2. Composition of synthetic wastewater used to simulate domestic wastewater<sup>1</sup>

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436	Component	Amount (g)
437		
438	Glucose	18
439	Yeast	2.7
440	Dried Milk	10.8
441	Urea	2.7
442	NH <sub>4</sub> Cl	5.4
443	Na <sub>2</sub> PO <sub>4</sub> .12H <sub>2</sub> O	9
444	KHCO <sub>3</sub>	4.5
445	NaHCO <sub>3</sub>	11.7
446	MgSO <sub>4</sub> .7H <sub>2</sub> O	4.5
447	FeSO <sub>4</sub> .7H <sub>2</sub> O	0.18
448	MnSO <sub>4</sub> .H <sub>2</sub> O	0.18
449	CaCl <sub>2</sub> .6H <sub>2</sub> O	0.27
450	Bentonite	3.6

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451 <sup>1</sup> Diluted to 90 litres

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

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460	Days of operation (d)	525
461	Filter hydraulic loading rate ( $L\ m^{-2}\ d^{-1}$ )	100
462	Average organic loading rate ( $g\ COD\ m^{-2}\ d^{-1}$ )	9.9
463	Average influent COD concentration ( $mg\ L^{-1}$ )	$99.2\pm 13.4$
464	Average TSS loading rate ( $g\ TSS\ m^{-2}\ d^{-1}$ )	2.2
465	Average influent TSS concentration ( $mg\ L^{-1}$ )	$22.4\pm 13.5$

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489 Table 4. Performance of laboratory filters.

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491	Media	Depth	% Removals		
492		m			
493			COD	TSS	Heterotrophs
494					
495	Glass	0.65	56.2	100	81.8
496		0.375	42.4	100	79.7
497	Sand	0.65	45.3	100	85.3
498		0.375	44.4	100	79.6
499	Soil	0.65	65.4	100	92.6

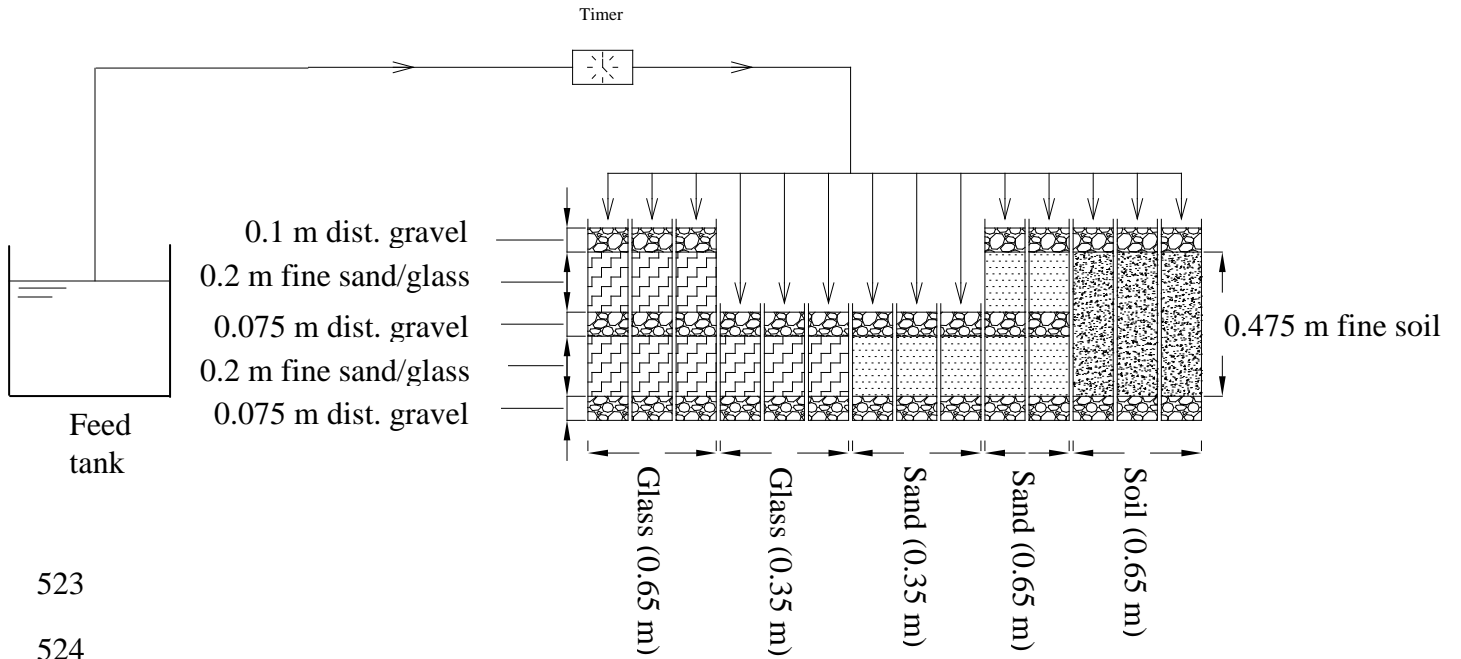
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521 Figure. 1.

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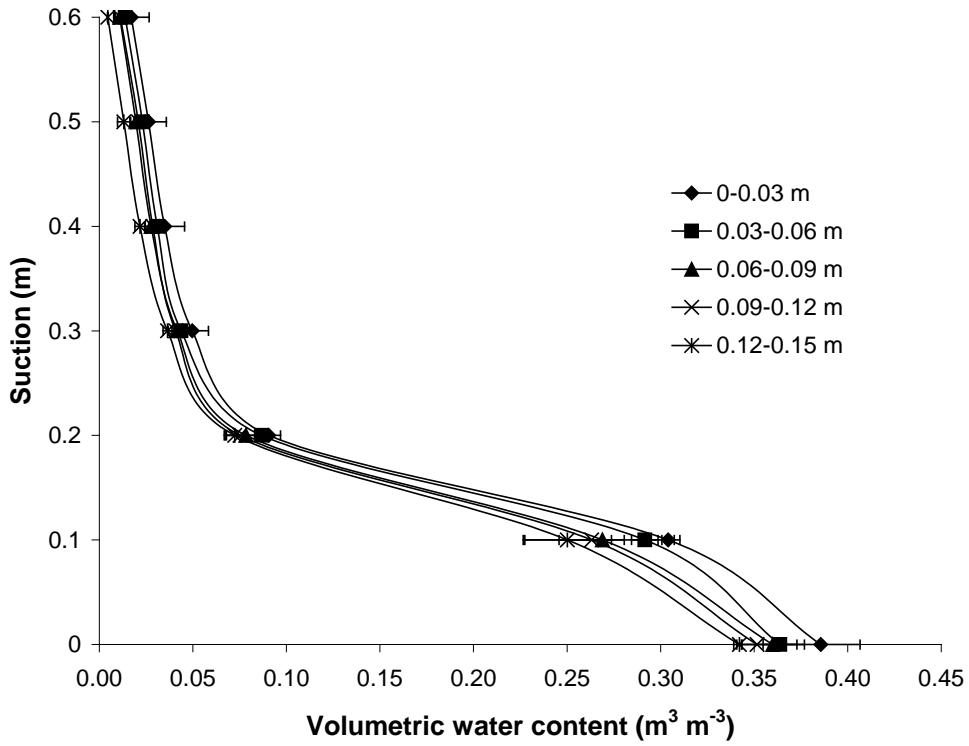
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538 Figure 2.

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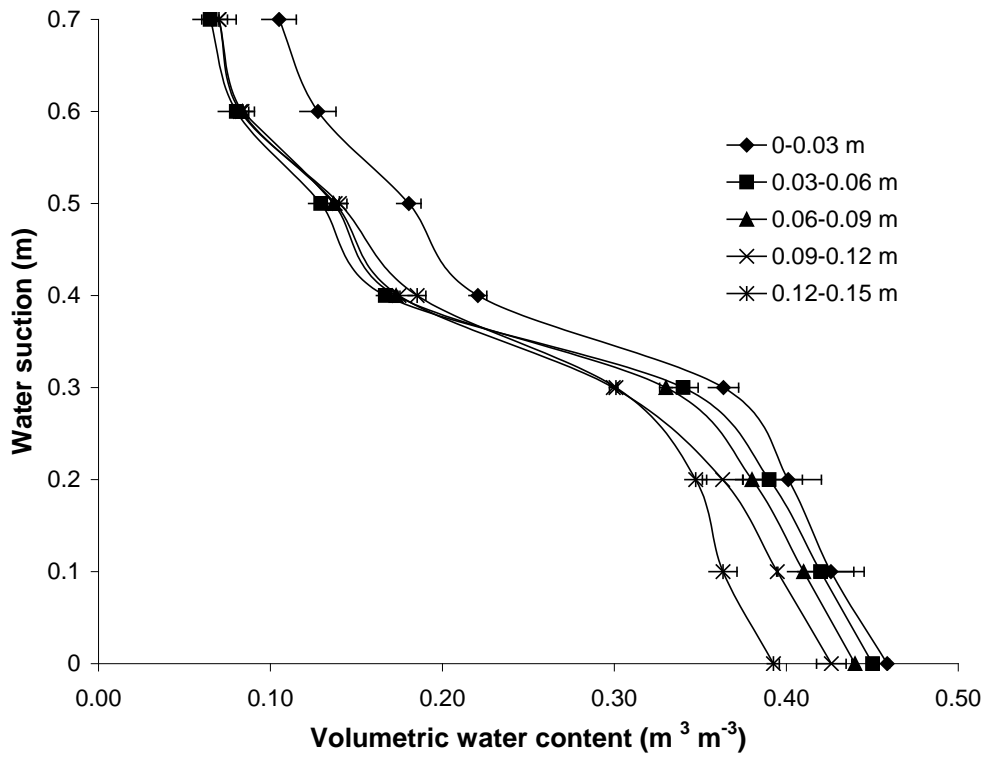
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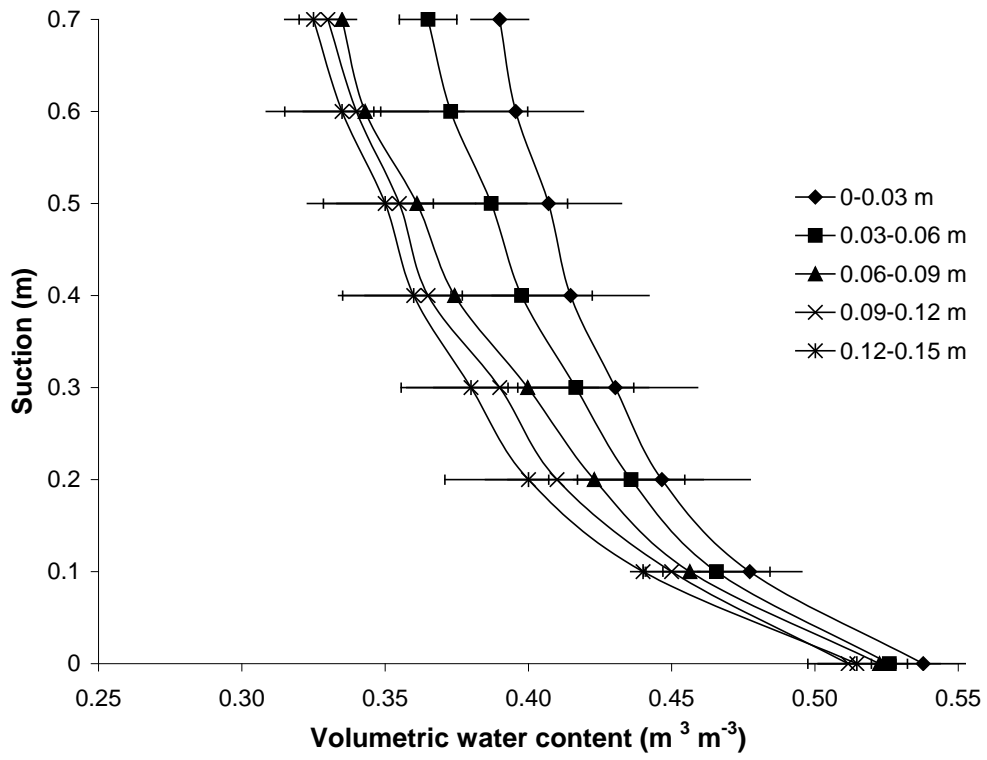
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563 Figure 3.  
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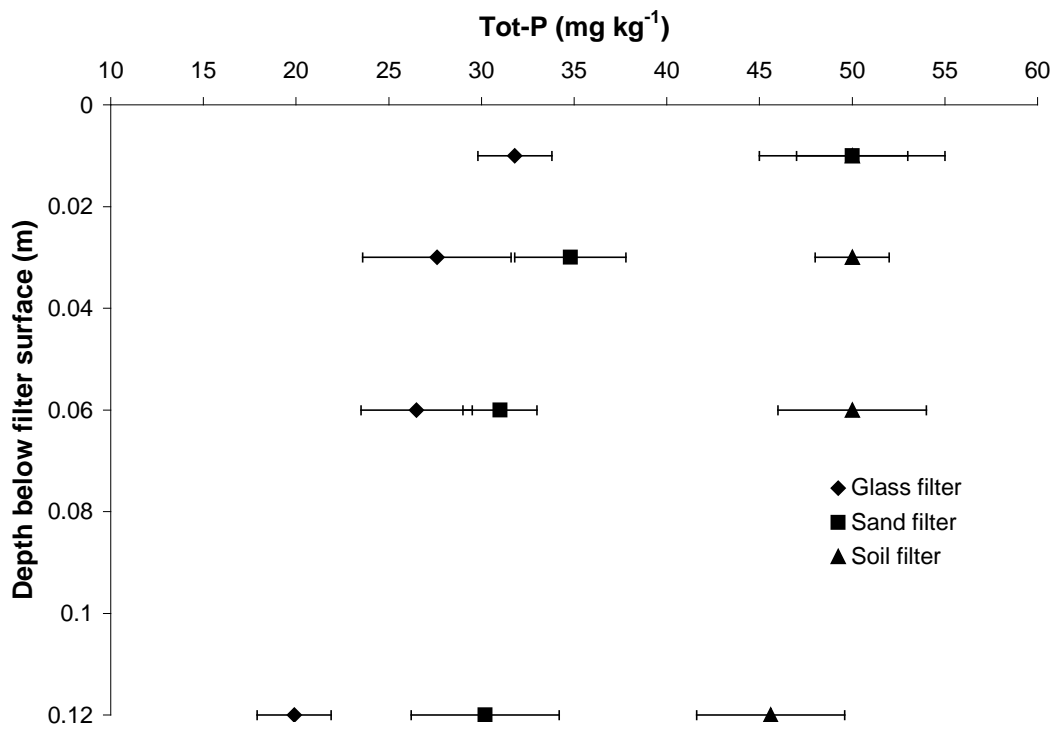
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589 Figure 4.  
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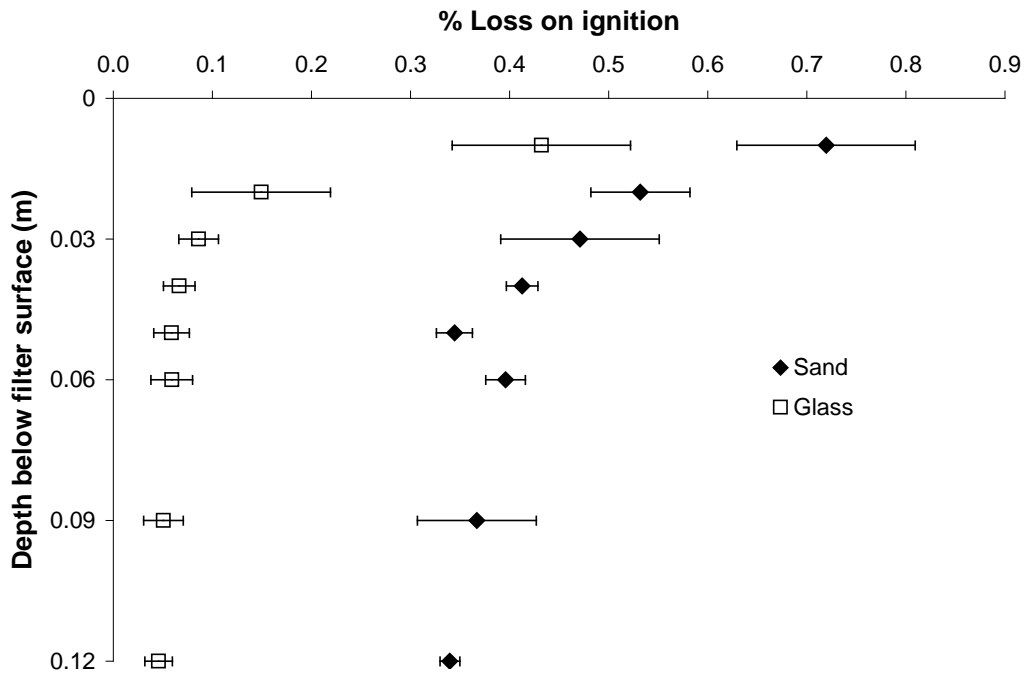
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615 Figure 5.  
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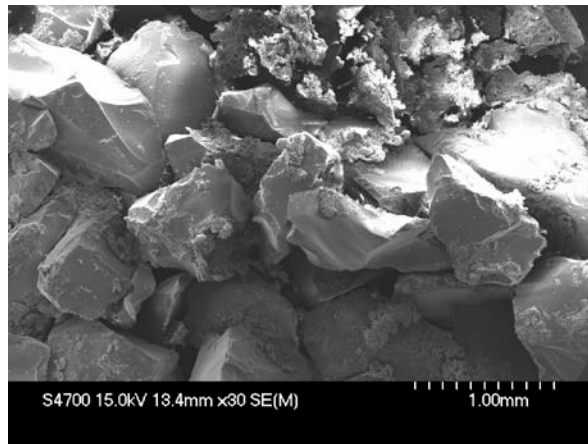
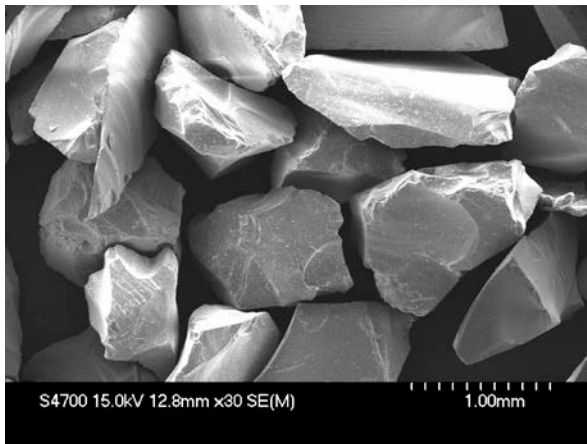
641 Figure 6.



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Figure 7.



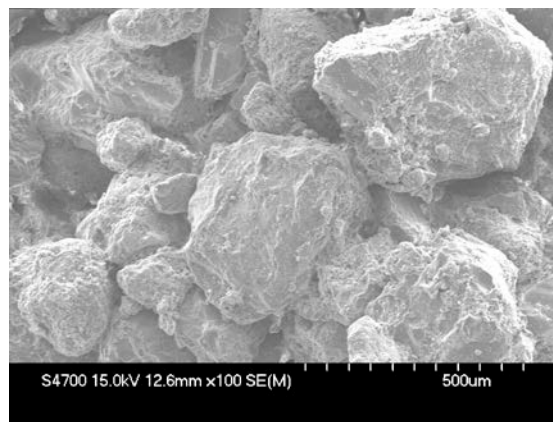
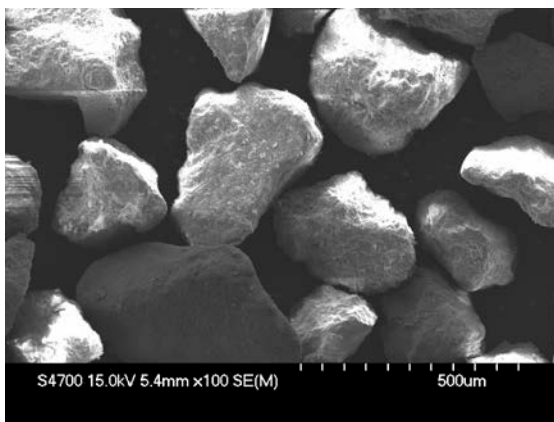
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696 Figure 8.

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