



1 **Use of an external organic carbon source in the removal of nitrates in Bio-sand**
2 **filters (BSF)**

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10
11 **ABSTRACT**

12 Bio-sand filters (BSF) are point of use (POU) potable water filtration systems
13 commonly used in low-income communities at household level. The principle of
14 operation is similar to that of a slow sand filter and the major difference is that they are
15 operated intermittently at the point of use. It is one of the emerging low cost
16 technologies which makes use of readily and locally available construction materials
17 but is poor in the removal of nitrates. In order to enhance the removal of nitrates
18 through de-nitrification, a modified bio-sand filter with ethanol as an external carbon
19 source at C/N ratios of 1.1 and 1.8 was investigated. In the absence of an external
20 carbon source, the nitrate removal efficiency was 32% whilst removal efficiencies at
21 C/N ratios of 1.1 and 1.8 were 44% and 53% respectively. The inflow rate reduced
22 significantly from an initial flow rate of 0.04m³/hr to 0.01m³/hr. The reduction in the
23 inflow rate was mainly due to the growth of the biological layer on the filter media. The
24 study showed that the use of an external carbon source like ethanol in biosand filtration
25 enhances the removal of nitrates in potable water.

26
27 **INTRODUCTION**

28 Bios-sand filters are intermittent slow sand filters designed for household use and
29 hence called point of use (POU) water filtration systems, with principal filtration
30 mechanisms being physical, chemical and biological. The biological mechanisms take
31 place at the top layer where a biological mat develops in the 50 to 100mm of the media.
32 The biological layer acts both as a fine filter to remove small colloidal particles,
33 dissolved impurities and at the same time immobilizes pathogens.

34
35 Although bio-sand filters are now widely applied in the treatment of water at household
36 level, few studies have been conducted on the removal of chemical contaminants.
37 Current research in BSF has mainly focussed on the removal of pathogenic organisms
38 like *Escherichia coli* (*E. coli*) and suspended solids. One chemical of major concern
39 is nitrate-nitrogen contamination (NO₃-N) in surface and ground water as it poses
40 serious health problems (Almasiri and Kaluarachchi, 2007). Methaemoglobinemia in
41 infancy is related to nitrate ingestion resulting in low oxygen in-intake and
42 consequently causing death. Furthermore, presence of nitrates in drinking water
43 results in the formation of nitrosamines in the stomach, which are carcinogenic. Nitrate
44 poisoning has been reported in livestock when concentrations exceeded 100mg/l
45 (Tredoux et al., 2000) and other problems related to nitrate in drinking water are well
46 documented in literature (Moraes 1995; Fan and Steinberg, 1996; Lin et al., 2002;
47 Forman 2004).

48
49 Main sources of NO₃-N in surface waters and groundwater aquifers include use of
50 agricultural fertilizers, animal waste disposal, wastewater effluents from conventional
51 and on-site sanitation facilities. Water supply from high nitrate concentration



52 environments needs some form of treatment or dilution with low-nitrate content water.
 53 The current design of conventional biosand filters has been proved to be poor in the
 54 removal of nitrates (Heather et al., 2010; Mahlangu et al., 2011; Kennedy et al., 2012).
 55 Physical and chemical methods such as ion-exchange, reverse osmosis, electro-
 56 dialysis, distillation, nanofiltration and activated carbon have been applied in the
 57 removal of nitrates from drinking water supplies. These methods are relatively
 58 expensive and show poor selectivity for nitrate removal with generation of brine, which
 59 is difficult to dispose (Moheseni et al., 2013). Hence there is need to explore
 60 alternative technologies like biological de-nitrification which has been proved to be
 61 efficient in complete nitrate elimination and has the advantage of producing a harmless
 62 by-product (N₂). The pathway for nitrate removal by heterotrophic bacteria is:-nitrate
 63 →nitrite→ nitric oxide → nitrous oxide →gaseous diatomic nitrogen:-
 64



66
 67 The biological de-nitrification technology is based on the conventional theory, that
 68 carbon is the limiting factor in the efficiency of biological de-nitrification. Heterotrophs
 69 utilises carbon from organic compounds like sugars, organic acids and amino acids as
 70 source of electrons rather than from inorganic compounds like carbon dioxide as the
 71 case in autotrophic de-nitrification. Although autotrophic nitrate removal has the
 72 advantage of not requiring an organic carbon source, it is associated with slow growth
 73 rate of autotrophic bacteria and low nitrate removal rate.
 74

75 Few studies have been conducted on the ability of the bio-sand filters in the removal
 76 of nitrates. In a study conducted in rural Cambodia by Heather et al. (2010), it was
 77 revealed that there was simultaneous nitrification and de-nitrification occurring in the
 78 bio-sand filters. However, about 85% of the biofilters under study did not meet the
 79 WHO guideline for NO₃-N in the treated effluent. The study showed that de-nitrification
 80 was predominant when the inflow into the filter was from surface water, which could
 81 be due to the high organic carbon content. Kennedy et al. (2012) studied the effects
 82 of hydraulic loading on removal of nitrates in biosand filters and the overall nitrate
 83 removal efficiency was low (16%). Mahlangu et al. (2011) established that the
 84 conventional BSF and the modified BSF of zeolites (clinoptilote) have relatively low
 85 removal rates of nitrates (37%). On the same study, other types of biofilters which
 86 include ceramic candle and bucket filters had poor removal of nitrates ranging from
 87 18% to 37 %. On certain occasions, the effluent concentration of NO₃-N was even
 88 higher than the unfiltered water and possibly due to desorption of previously adsorbed
 89 nitrates.
 90

91 Most sources of drinking water lack sufficient quantities of organic carbon for cell
 92 growth and energy source. The organic carbon acts as both a source of cellular
 93 material for biological respiration and electron donor for dissimilatory nitrate reduction.
 94 Waters with low carbon content require an external carbon source for de-nitrification
 95 to take place under anoxic conditions and nitrate is converted to gaseous diatomic
 96 nitrogen.
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98 A variety of external carbon sources like sucrose, ethanol, methanol and acetic acid
 99 have been applied in conventional slow sand filters to aid heterotrophic denitrification
 100 at C/N ratios ranging from 1 to 2.5. The studies have shown considerable
 101 improvement levels in the de-nitrification process due to the recorded high nitrate



102 removal efficiencies of about 90% (Green et al., 1994). Gomes et al. (2000) assayed
103 the influence of sucrose, ethanol, methanol and ethyl alcohol in nitrate reductase in
104 contaminated groundwater and showed very high removals with effluent
105 concentrations ranging from 0 to 5mg/l. Aslan and Cakici (2007), reported removal
106 rate of 94% for nitrate in slow sand filters when acetic acid was used as a carbon
107 source. Methanol is toxic due to some of the residual concentrations of carbonaceous
108 compounds found in the effluent and produces an excessive growth of biomass.
109 Sucrose and glucose have a tendency to form a biomass which increases turbidity in
110 the final effluent. Acetic acid and ethanol are considered to be the most suitable
111 carbon sources in removal of nitrate and no limits have been set in potable water.
112 They are also cheaper, a concept inherent with the use of bio-sand filtration
113 technology.

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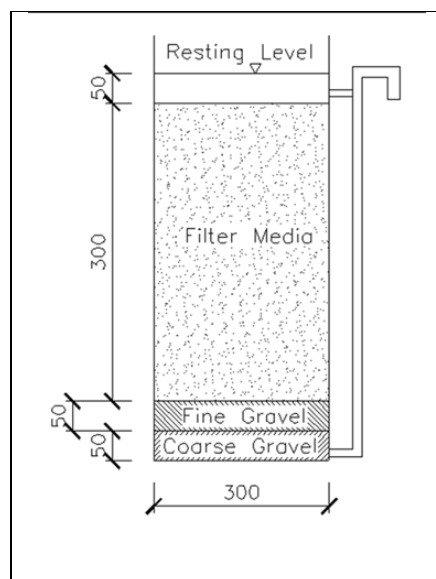
115 However, heterotrophic de-nitrification has not been investigated in bio-sand filters
116 except in the conventional slow sand filters. The aim of this study was to investigate
117 the removal of $\text{NO}_3\text{-N}$ in biosand filters with ethanol as a carbon source and to
118 establish the optimum Carbon to Nitrate (C/N) ratio for microbial activity which
119 achieves maximum removal with minimum excess carbon in the effluent.

120

121 **MATERIALS AND METHODS**

122 Two bio-sand filters were investigated at household level: - one with an external
123 carbon source (BSFC) to enhance the de-nitrification process at C/N ratios of 1.1 and
124 1.8, and the other one without a carbon source (BSF). The two bio-sand filters were
125 dosed with known concentrations of ammonium nitrate which was the source of nitrate.

126



127

128 Figure 1 Schematic representation of the bio-sand filter (dimensions in mm).

129

130 **Filter construction**

131 The two bio-sand filters were constructed according to the Centre for Affordable Water
132 and Sanitation Technology guidelines (CAWST 2008). Plastic buckets 25ml in volume



133 were used and were packed with multi-media filter material. The multi-media filter bed
 134 consisted of fine sand of 0.3mm diameter and 250mm deep; sand of 0.95mm diameter
 135 and 750mm deep; gravel of 7mm diameter and 50mm deep. The South African
 136 National Standard (SANS 3001) were used to determine the particle size and grading
 137 in order to achieve the required particle size distribution of the filter media. Dewatering
 138 of the filter between charges is avoided by a vertical discharge tube that rises from 2-
 139 7 cm above the height of the filter media. The elevated outlet allows the media to
 140 remain saturated after a charge has been filtered and when water is no longer flowing
 141 from the outlet (Fig. 1). The design parameters of the filter are summarised in Table
 142 1.

143

144 Table 1: Summary of the design values used for the two filters (BSF & BSFC)

145

Design parameter	Unit	Recommended Value	Applied Value
Media depth	m	0.3-0.5	0.3
Supernatant depth	mm	50	50
Surface area	m ²	0.06	0.071
Effective size	mm	0.15-0.40	0.35
Coefficient of uniformity	---	1.5 to 3	2.64
Filtration velocity (in clean filter bed)	m/hr	0.10 to 0.6	0.17-0.63
Inflow rate	m ³ /hr	0.03 to 0.04	0.04

146

147 The filtration cycle of a biofilter is made-up of resting time (6-24hrs) and a maximum
 148 filtration time of about 2hrs (Fewster et al., 2004). The biological treatment occurs
 149 during the resting time and after this period the filter bed is drained. In this study the
 150 raw surface water was fed into the filter once a day and the resting time and filtration
 151 time were 24hrs and 2hr respectively. The filtered water was collected in a 5 litre
 152 vessel for laboratory analysis. The average inflow rate was measured from noting the
 153 start time of filtration and the time periods at which the level of the water in the
 154 receiving vessel changed by 1 litre.

155

156 The superficial velocity (v_s) is related to the surface area of the filter and is normally
 157 used in filtration computations and is also equivalent to the hydraulic surface loading
 158 divided by the surface area of the filter. For BSF, the inflow rate is not constant since
 159 the water is only poured once for a filter cycle and hence the infiltration velocity
 160 decreases with time from the start to end of cycle.

161

162 Nitrate and Carbon source dosage

163 A stock solution of ammonium nitrate (NH₄NO₃) of concentration of 190g/l was dosed
 164 to both filters (BSF and BSFC), and to achieve a dose of 25mg/l in the 25l filter volume,
 165 3.33ml of the stock solution was required. The ethanol was applied only to BSFC at
 166 C/N ratios of 1.1 and 1.8. With a molar mass of 46g/mol of ethanol (C₂H₅OH) the
 167 carbon equivalent in the ethanol was 24g/mol (52.2%). Therefore, at a nitrate dose of
 168 25mg/l and C/N ratio of 1.1 the dosage of carbon as ethanol in a 25l biosand filter was
 169 7.45ml of carbon as ethanol. Similarly, at C/N ratio of 1.8, the required dose of carbon
 170 as ethanol was 12.1ml. The surface loading of NO₃-N was calculated by multiply the
 171 concentration of nitrate with the superficial velocity (g/m².d).

172



173 **Filter maturation**

174 The de-nitrification in biosand filters is biological and take place under a fixed film
175 growth process whereby the bacteria develop on the surface of the sand media. For
176 the smooth operation of the biosand filter, the water level was maintained at 50mm
177 above the fine sand. The maturation period for the full development of the biological
178 layer and acclimatising of the microorganisms to ethanol and NO₃-N environment was
179 3 weeks. The operating temperatures of the filters varied between 19°C and 20°C and
180 were not controlled in order to simulate the actual operating conditions of a biosand
181 filter at household level.

182

183 **Sample Collection and Analysis**

184 Sampling bottles were washed with distilled water before and after sampling. The
185 samples were collected at the inlet and outlet of the two biosand filters in 500ml
186 Erlenmeyer flasks and stored in a refrigerator at 4°C and analysed within 1 hour. The
187 frequency of sample collection was once a week after the 12 hr resting time.

188

189 The pH and dissolved oxygen (DO) were measured using a pH meter Model HACH
190 HQ30D (FLEXI Model). The instrument was calibrated and measurements conducted
191 in accordance with the Standard Method. The nitrate was measured by Spectroquant
192 Nitrate Photometrical Test Method using Merck Spectrophotometer PHARO100 and
193 the results were reported as NO₃-N in mg/l. The carbon source which was ethanol
194 was measured as Chemical Oxygen Demand (COD) by the MERCK Spectroquant TR
195 320 Digester (Spectroquant COD Cell Test method). The samples were digested in
196 tubes containing a mixture of chromic and sulphuric acid with silver sulphate as a
197 catalyst. After digestion samples were cooled and read on the Spectroquant
198 PHARO100 Spectrophotometer. The COD test was carried out mainly to determine
199 the amount of ethanol as a carbon source in the source water before and after the
200 filtration process.

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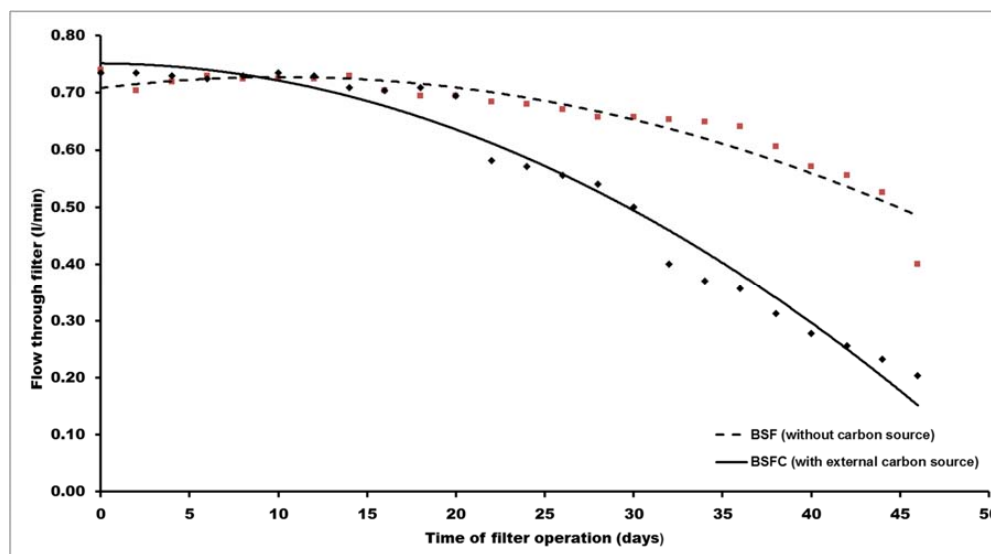
202 **RESULTS AND DISCUSSIONS**

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204 **Flow rates**

205 Initial flow rates in the control filter BSF started from 0.04m³/hr and declined to
206 0.03m³/hr by end of experiment, whilst in BSFC which received carbon source the flow
207 rate reduced from 0.04m³/hr to 0.01m³/hr (Fig. 2). The reduction in flow rates was
208 comparable to studies conducted on Bio-sand filters by Kubare & Haarloff et al.,
209 (2010) and Kennedy et al., (2012). The declining in the filtration rate was due to filter
210 clogging and was substantial when the biological layer was fully mature. The reduction
211 in the flow rate was more pronounced in the filter dosed with an external carbon source
212 (BSFC) compared to one without carbon (BSF). Therefore, there was more growth of
213 the biomass in the biofilter with an external carbon source due to the favourable
214 environment conducive for growth of heterotrophic bacteria. Conventional surface
215 cleaning will not remove the biomass at the bottom layers. Consequently, a household
216 would require more filters to meet the daily water demand as well as increasing the
217 resting period in BSFC to reduce excessive growth of biomass. Overall, the filtration
218 velocity ranged from 0.17m/hr to 0.63m/hr and typical filtration rates for BSF range
219 from 0.16 to 1.1m/hr (Elliot et al. 2008; Kubare & Hannoff 2010).

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223 Figure 2: Variation of flow rates in the filters with and without carbon source

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225 Changes in pH and DO

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227 The pH and DO are important physiochemical parameters in the removal of nitrates in
228 BSF. There was no significant change in the pH of the influent and effluent water for
229 both filters (BSF and BSFC). Overall, there was a slight decrease in pH from 8.6 to 6.8
230 and such a pH range would favour the de-nitrification process since maximum de-
231 nitrification rates are achieved at pH range of 7 to 8.5. Whereas for pH values smaller
232 than 6 and larger than 8.5 would result in a sharp decrease in the de-nitrification
233 activities. However pH may increase during de-nitrification because the reduction of
234 nitrate to gaseous nitrogen with organic substrate as an electron donor results in the
235 production of carbon dioxide and oxygen hydroxide (OH⁻), which may react to form a
236 bicarbonate (HCO₃⁻) and carbonate (CO₃²⁻) (Drtil et al. 1995; Wang et al., 1995). With
237 regards to water quality guidelines, the pH values were within the acceptable South
238 African (2015) guideline limits of 5.0 to 9.7.

238

239 The overall reduction of DO in the filter with an external carbon source was 65% with
240 average inflow and outflow concentrations of 8.23mg/l and 2.94mg/l respectively.
241 However, the reduction in dissolved oxygen was less in the filter without an external
242 carbon source (50%). The reduction in the DO is due to the oxygen demand by aerobic
243 and nitrifying bacteria at the top of the filter bed.

244

245 Nitrate Removal Rates

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247 The nitrate removal mechanisms during heterotrophic de-nitrification are bacterial
248 respiration and bacterial synthesis (Mohseni-Bandp et al., 2013). The de-nitrification
249 will take place at the bottom of the filter bed where there is less oxygen (anoxic
250 conditions). William and Beresford (1986) concluded that nitrification and de-
251 nitrification happen simultaneously in zones where there are short distances between
252 the aerobic and anaerobic zones. The same scenario is depicted in biosand filters
due to the short filtration length of approximately 0.3-0.5m (Elliot, CWAST).



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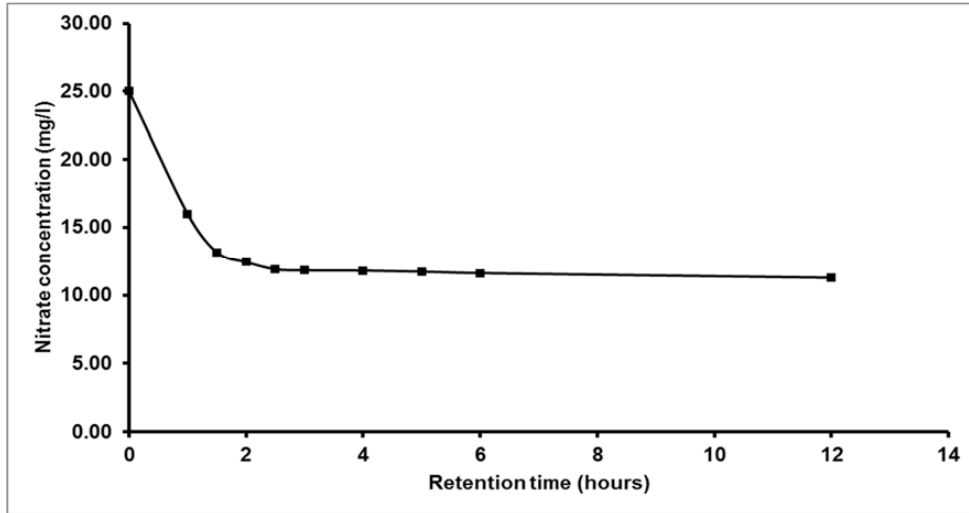
254 Heterotrophic bacteria need organic carbon as the electron donor and as the source
255 of carbon, whilst getting their oxygen by removing bound oxygen from nitrate (NO_3^-)
256 which is in the water being treated and the nitrate acts as the electron acceptor. As a
257 result of this process, the removal rate of nitrates in the filter without external carbon
258 source (BSF) was $30\% \pm 0.04$ (Table 2) and Mahlangu et al. (2011) reported a rate of
259 37% in similar filters. In the filter with an external carbon source (BSFC) the nitrate
260 removal rate was $44\% \pm 0.03$ at C/N ratio of 1.1 and $53\% \pm 0.02$ at C/N ratio of 1.8.
261 Overall the nitrate removal rate was higher with the use of an external carbon source
262 at higher C/N ratio of 1.8 (Table 3). However, the effluent nitrate concentration was
263 between 16 to 19mg/l but still above the recommended guideline values in potable
264 water.

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266 The failure to achieve effluent nitrate guideline values even though pH was optimum
267 could be due to high DO. Optimum de-nitrification occurs under anoxic conditions
268 when oxygen levels are depleted (low redox) and nitrate becomes the primary oxygen
269 source for heterotrophic bacteria. In general it has been observed that a dissolved
270 oxygen concentration of more than 0.2mg/l reduces the rate of de-nitrification
271 significantly (Jorgensen and Sorensen, 1984). High levels of dissolved oxygen were
272 recorded ranging between 2.9mg/l to 8.2mg/l and hence were higher than the optimum
273 values for de-nitrification.

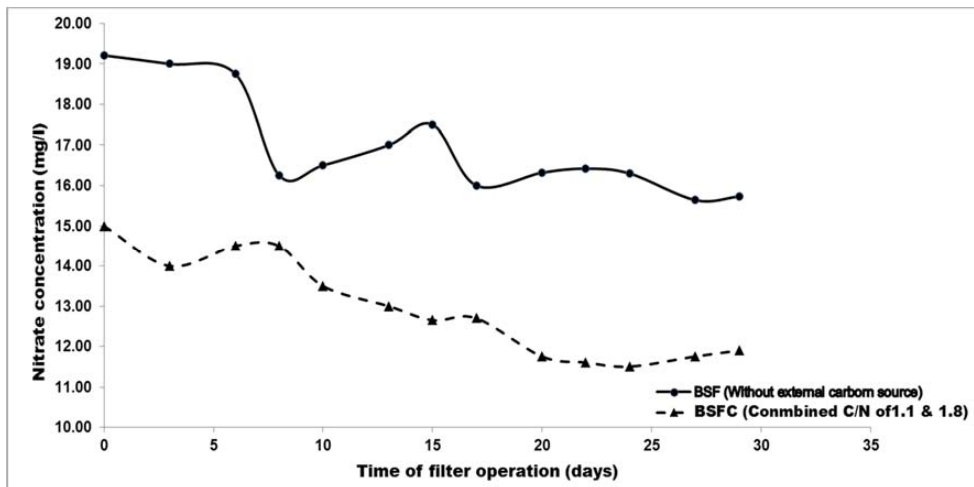
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275 Reducing the DO concentration in biosand filter will enhance the nitrate removal
276 efficiency but will compromise the aerobic microbial activity at the top layer. A feasible
277 alternative will be to increase the filter depth so as to create an anoxic zone at the
278 bottom or to increase the resting period of the filter. Bio-sand filters are designed with
279 a filtration time of 2 hrs and resting period of 12 to 24 hours (CAWST, 2007; Elliott et
280 al., 2008). The resting time provides the contact time for microbial removal and de-
281 nitrification processes and thus a long resting time will be desirable from this
282 perspective. However, too long a resting period may reduce the viability of the
283 biological layer because the survival of the microorganisms relies on the periodic
284 inflow of source water for nutrients (Baumgartner et al., 2007). Additionally, too long
285 a resting period will reduce the water production rate and thus fail to satisfy household
286 water requirements. Therefore careful selection of the resting period is vital in order
287 to balance these competing objectives. In this study a resting time of 12hrs was used
288 and nitrate concentrations measured during this period showed a rapid removal rate
289 during the first 1.5 hrs and no significant removal thereafter (Fig. 3). Therefore,
290 increasing the resting period more than the 12 hours will not have any significance in
291 the nitrate removal. However, results for the entire operational period indicates low
292 removal at the beginning (40%) and thereafter the rate increased to 53%, and this
293 illustrates the importance of maturation period. The variation of nitrate concentrations
294 for the entire operational period are illustrated in Fig. 4.



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Figure 3: Reduction of nitrate relative to resting period in the filter with an external carbon source. Values of the nitrate are the average of the C/N ratio of 1.1 and 1.8.



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Figure 4: Variation of nitrate concentrations for the entire operational period

Denitrification Rate

The denitrification rate was computed as:

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$$R_{dn} = \frac{1}{t}(C_{in} - C_{out})$$

308 Where:-

309 R_{dn} = denitrification rate (M/L³T)

310 C_{in} = influent nitrate (M/L³)

311 C_{out} = effluent nitrate (M/L³).

312



313 The denitrification rate for BSF and BSFC were 3.66gNO₃-N/m³.d and 5.44gNO₃-
 314 N/m³.d respectively and these rates are lower than those reported by Aslan et al.,
 315 (2007) in slow sand filters ranging between 8.1 and 29.2 gNO₃-N/m³.day at filtration
 316 rates between 0.015 and 0.06 m/h.

318 Table 2: Nitrate removal efficiency at C/N=1.1 ant at influent nitrate concentration
 319 of 25 mg/l

320

Sampling interval (Days)	BSF (No external Carbon Source)		BSFC C/N=1.1 (With external carbon source)	
	Effluent Nitrate (mg/l)	Removal Efficiency	Effluent Nitrate (mg/l)	Removal Efficiency
0	19.21	23%	15.00	40%
2	19.00	24%	14.00	44%
5	18.75	25%	14.50	42%
7	16.25	35%	14.50	42%
9	16.50	34%	13.50	46%
12	17.00	32%	13.00	48%
14	17.50	30%	12.65	49%

321

322 Table 3: Nitrate removal efficiency at C/N=1.8 ant at iinfluent nitrate
 323 concentration of 25 mg/l

324

Sampli ng interval (Days)	BSF (No external Carbon Source)		BSFC (With external carbon source) C/N=1.8	
	Effluent Nitrate (mg/l)	Removal Efficiency	Effluent Nitrate (mg/l)	Removal Efficiency
17	16.00	36%	12.70	49%
20	16.32	35%	11.75	53%
22	16.42	34%	11.60	54%
24	16.30	35%	11.50	54%
27	15.64	37%	11.75	53%
29	15.73	37%	11.90	52%

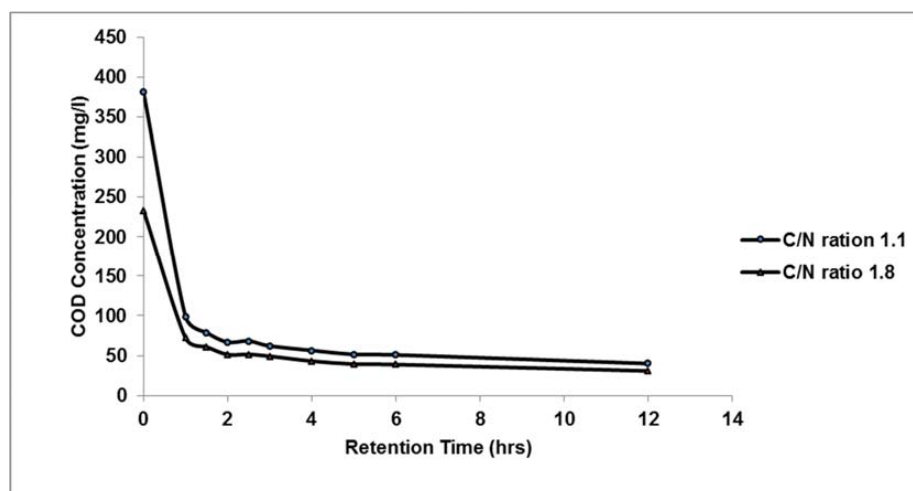
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326 **Residual COD in effluent**

327 The residual ethanol measured as COD in filters with an external carbon source varied
 328 between 25mg to 35mg/l. Overall, the removal efficiency of COD at C/N ratio of 1.1
 329 and 1.8 was 89% and 90% respectively. There was rapid COD removal in the first 2
 330 hours and became constant as the resting period increases and hence there is no
 331 significant benefit with longer resting periods. The same trend is depicted with nitrate
 332 removal which concluded that the de-nitrification process takes place in the first 2
 333 hours when the COD is utilised in the process. However, the COD concentrations in
 334 the effluent were higher than the guideline values, and such high level of COD
 335 concentrations may be toxic to human health and increases disinfection by-product
 336 formation potential. This present a major health challenge in the use of an external



337 carbon source in the removal of nitrates in potable water and there is a need to explore
 338 post-treatment methods to remove the residual carbon in biosand filters.
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Figure 5: Reduction of COD relative to resting period in the filter with an external carbon source

Table 4: COD removal Efficiency at C/N = 1 and C/N=1.8

Sampling interval (days)	Influent COD (mg/l)	Effluent COD (mg/l)	COD Removal Efficiency (%)	Sampling interval (days)	Influent COD (mg/l)	Effluent COD (mg/l)	COD Removal Efficiency (%)
	C/N = 1.1				C/N = 1.8		
0	233.52	26.85	88.50				
2	233.52	25.30	89.17				
5	233.52	25.61	89.03				
7	233.52	23.98	89.73				
9	233.52	24.77	89.39				
12	233.52	25.10	89.25				
14	233.52	26.36	88.71				
17	382.12	35.54	90.70	17	382.12	35.54	90.70
20	382.12	36.10	90.55	20	382.12	36.10	90.55
22	382.12	35.86	90.62	22	382.12	35.86	90.62
24	382.12	35.42	90.73	24	382.12	35.42	90.73
27	382.12	35.40	90.74	27	382.12	35.40	90.74
29	382.12	35.18	90.79	29	382.12	35.18	90.79

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CONCLUSIONS

Bio-sand filtration enhanced by an ethanol as an external carbon source has potential in the removal of nitrates in potable water at household level. The average nitrate removal efficiency in biosand filter with ethanol as an external carbon source at C/N



352 ratios of 1.1 and 1.8 was 44% and 53% respectively. Although the nitrate
 353 concentration levels in effluent exceeded the recommended guidelines, the
 354 technology is capable of limiting nitrate in drinking water. Increasing the resting period
 355 more than 12 hours will not have any significance in the nitrate and COD removal
 356 rates since these two processes take place in the first 2 hours. Dissolved Oxygen
 357 concentration in the effluent reduced significantly due to aerobic oxidation and
 358 nitrification which took place, simultaneously on the upper layer of the filter. The
 359 reduced DO low levels promoted heterotrophic de-nitrification at the bottom of the
 360 biosand filter. However, the DO levels were still above optimum values for de-
 361 nitrification, and also the residual COD concentrations were above the water quality
 362 guidelines.

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364 The flow rates reduced with time throughout the whole experiment due to the growth
 365 of the biological layer and clogging of the filter media and as a result the yield of the
 366 biofilter was reduced. The flow rate reduction was higher in the filter with an external
 367 carbon source and was substantial when the biological layer was fully mature. Overall,
 368 the study concluded that there is high potential in the use of POU filters enhanced with
 369 an external carbon source in the removal of nitrates through heterotrophic
 370 denitrification. The major challenge on the use of an external carbon source is the
 371 high residual COD concentration, which may pose a health risk.

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