1 **Removal of antibiotics in sand, GAC, GAC sandwich and anthracite/sand**

2 **biofiltration systems**

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13 **Abstract**

14   Drinking water biofiltration offers the possibility of the removal of trace level

15 micropollutants from source water. Sand, granular activated carbon (GAC), GAC sandwich (a

16 layer of GAC loaded in the middle of sand bed), and anthracite-sand dual biofilters were set-

17 up in duplicate at bench-scale to mimic the filtration process in real drinking water treatment

18 works. During the 3-month system operation, removal of five antibiotics (amoxicillin,

19 clarithromycin, oxytetracycline, sulfamethoxazole, and trimethoprim) and overall biofilter

20 performance were evaluated. Natural surface water spiked with a mixture of the target

21 antibiotics was used as feedwater to the biofilters. Results showed that the target antibiotics

22 were substantially removed (> 90%) by GAC-associated biofilters and partially removed (≤

23 20%) by sand alone and anthracite-sand biofilters. In particular, the GAC sandwich biofilter

24 exhibited superior performance compared to sand/anthracite biofilter, and the comparisons

25 among all biofilters indicated that both adsorption and biodegradation contributed to the

26 removal of the target antibiotics in the GAC-associated biofilters. Adsorption kinetics showed

27 that sulfamethoxazole fitted with pseudo-first-order adsorption model, while trimethoprim,

28   amoxicillin, oxytetracycline and clarithromycin fitted the pseudo-second-order model. All

29   antibiotics fitted the Langmuir model according to the isotherm experiment. To date, this is the

30   first study evaluating the removal of antibiotics by GAC sandwich biofilters. Overall, this

31   research will provide useful information which can be used for optimising or updating existing

32   biofiltration processes in industry to reduce antibiotic residues from source water.

33

34   Keywords: Drinking water biofiltration; antibiotics; sand; granular activated carbon; anthracite;

35   adsorption

1. Introduction

Antibiotic residues are being detected at increasing concentrations in aquatic environments, including water bodies used as drinking water sources (Luo et al., 2014; Simazaki et al., 2015). Few studies have reported the presence of antibiotics in drinking waters in different countries and regions. In Spain, four antibiotics (azithromycin, clarithromycin, erythromycin and sulfamethoxazole) were detected at measurable levels in finished drinking water (Boleda et al., 2014). Nineteen water utilities in the U.S. have detected sulfamethoxazole and trimethoprim (< 3.0 ng/L) in both finished drinking water and tap water (Benotti et al., 2009). Thiamphenicol has been reported at a maximum of 104.3 ng/L among 32 pharmaceuticals in tap waters collected from 13 cities in China, followed by sulfamethazine (89.6 ng/L) and sulfathiazole (27.4 ng/L) (Leung et al., 2013). In general, the lack of comprehensive and systematic studies on the occurrence of antibiotics in drinking water may limit the assessment of the potential human health risks from exposure to a mixture of very low concentration antibiotic residues via drinking water throughout a lifetime (WHO, 2012). The effect of chronic, low-level exposure to antibiotics on the human body is still unknown.

Biofiltration is a natural and cost-effective drinking water treatment technology which is widely used in Europe (Zearley and Summers, 2012). Media commonly used for drinking water treatment are adsorptive media such as granular activated carbon (GAC) and non-adsorptive media such as sand and anthracite. Sand biofiltration is one of the earliest water treatment process and often regarded as an efficient and stable technology in drinking water treatment plants (DWTPs). The major component of the treatment process in sand biofilters occurs at the surface layer (known as the *schmutzdecke*) of the sand bed in which biological activities are highest (Zhu and Bates, 2013). As an energy-efficient drinking water treatment technology, sand biofiltration offers an opportunity to remove trace level micropollutants from the source water. For instance, Zearley *et al.* reported removal efficiencies of 4.2% for sulfamethoxazole and 92% for trimethoprim by lab-scale sand filtration (Zearley and Summers, 2012). Pompei *et al.* observed removals ranging from 70 – 99% of 2 µg/L of paracetamol,

63   diclofenac, naproxen, ibuprofen, benzophenone-3 and methylparaben in a sand biofiltration

64   pilot plant in Brazil (Pompei et al., 2019). GAC biofilter has also been widely used in DWTPs

65   (Suffet, 1980). Adsorption and biodegradation are main mechanisms contributing to the

66   removal of organic compounds during the GAC biofiltration process. The unique porous

67   surface structure and high surface area of GAC enable its capacity for the adsorption of

68   organic compounds and other non-polar contaminants; while the biofilm formed on the surface

69   of GAC can remove organics via biodegradation. It has been proven that GAC biofiltration can

70   effectively remove a number of organic micropollutants (including antibiotics) in drinking water

71   and wastewater treatment processes (Gerrity et al., 2011; Reungoat et al., 2011; Kennedy et

72   al., 2015). Gerrity *et al.* reported that 90 – 95% of sulfamethoxazole and trimethoprim were

73   removed by pilot-scale biological GAC filter (Gerrity et al., 2011). Major drawbacks of the GAC

74   biofiltration include the formation of biofilm and the continuous loading of natural organic

75   matter may block the adsorbable area and cause a reduction in the GAC media lifespan.

76   In addition to single medium biofilters, combination of different filter materials is also

77   common practice in DWTPs. The mostly commonly used dual-media configurations are

78   anthracite/sand and GAC/sand (Shirey et al., 2012). Introducing a layer of GAC to the slow

79   sand filter (GAC sandwich biofilter) to form 'super-filters' make an advanced treatment being

80   used in DWTPs worldwide. The GAC sandwich biofilter was first studied in the UK by Bauer

81   *et al.* in 1996 for the removal of pesticides (Bauer et al., 1996). As well as increasing the

82   adsorption capacity of the carbon, it enhances the already excellent biological performance of

83   the slow sand filter. Recent studies based on lab-scale GAC sandwich biofilters have shown

84   capacity to remove DEET (*N*,*N*-diethyl-m-toluamide), paracetamol, caffeine and triclosan from

85   synthetic waste water (Li et al., 2018; Li et al., 2019). To date, no research has been conducted

86   for the removal of antibiotics by bench or pilot-scale GAC sandwich biofiltration in the context

87   of drinking water treatment. Moreover, previous bench-scale biofiltration experiments often

88   use synthetic tap water as the feed (Zearley and Summers, 2012; Paredes et al., 2016), which

89   cannot represents the complexity of real environmental conditions. Considering the

90   persistence of antibiotic residues in natural water environments, it is of great importance to

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91 find an economical and effective way to reduce the risk of antibiotic resistance dissemination

92 in drinking water.

93   Four types of commonly used biofilters, including Sand, GAC, GAC sandwich, and

94 anthracite-sand, were set-up at bench-scale. Over the course of three months, this study

95 focused on the following: (1) the effectiveness in removing five antibiotics (amoxicillin,

96 clarithromycin, oxytetracycline, sulfamethoxazole and trimethoprim) by different types of

97 biofilters; and (2) the overall biofiltration process robustness. The adsorption kinetics of the

98 target antibiotics onto GAC under the experimental conditions were determined to further

99 explore the adsorption removal mechanism by GAC.

2. Materials and methods

2.1 Chemicals

Five antibiotics amoxicillin (AMOX), clarithromycin (CTM), oxytetracycline (OTC), sulfamethoxazole (SMX) and trimethoprim (TMP) were selected, representing different commonly used classes. The major physical-chemical properties of the selected antibiotics are summarised in Table S1. All standards (purity ≥99.0%) were purchased from Sigma-Aldrich, UK. HPLC grade acetonitrile and methanol were obtained from Fisher Scientific, UK. Individual stock standards were prepared in methanol at 1 mg/mL, except for amoxicillin, which was dissolved in acetonitrile/water (50:50, v/v) at 1 mg/mL. A working solution was prepared by diluting the stock solutions in ultrapure water into 1 mg/L. All the stock solutions were stored at -20 °C and working solutions were preserved at 4 °C.

2.2 Biofilter design and operation

A total of 25 L of lake water was collected twice a week from Regent's Park, London (latitude 51.525187, longitude -0.158017) from October 2017 to January 2018. Fresh feedwater was added to the system every day, while the remaining of raw water was kept at 4 °C. Biofilter feedwater was spiked with the target antibiotics at 2 µg/L, except for amoxicillin which was spiked at 5 µg/L due to the analytical method constraints. The concentration selected in this study was according to their reported removal in the literatures and the analytical method limitations (Zearley and Summers, 2012; Paredes et al., 2016; Zhang et al., 2017).

Sand, GAC, GAC sandwich, and anthracite-sand biofilters were set-up in duplicate at bench scale. Silica sand was purchased from Mineral Marketing, UK with an effective size of 0.20 mm (size range 0.16 - 0.50 mm) and a uniform coefficient of 1.82; GAC was purchased from Chemviron Carbon, UK with an effective size of 0.72 mm (size range 0.62 - 1.60 mm) and a uniform coefficient of 1.68; anthracite was purchased from EGL Puracite, UK with an effective size of 0.90 mm (size range 0.85 - 1.46 mm) and a uniform coefficient of 1.32. Surface characteristics of the filter media are shown in Fig. S1. The media was uniformly placed into

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128 62 cm-length laboratory acrylic (Plastic Shop, UK) columns with a 36-mm inner diameter (ID)

129 and 2-mm wall thickness. In this study, depending on the effective size of sand, GAC and

130 anthracite, the ratio of the column diameter to the filter media diameter ranged from 40-180,

131 which are sufficiently large to prevent wall effects of media packing (Jiang et al., 2017). The

132 composition of biofilters is shown in Fig. 1, each column was loaded with 36 cm of filter media

133 and 5 cm of support media (0.6–3.0 mm gravel) to allow free drainage of filter effluent.

134   The biofiltration system was operated continuously at room temperature (20.3 ± 2.0 ºC)

135 for 12 weeks. A filtration rate of 0.06 m/h was used for all filters (D'Alessio et al., 2015). The

136 run was divided into three phases; phase I (maturation), when ≥ 99% reduction was achieved

137 for total coliforms and *Escherichia coli* (*E. coli*) after 4 weeks' operation; phase II (antibiotics

138 spike), from which the target antibiotics were added to the feed for 8 weeks; and phase III

139 (backwashing/cleaning) at the end of system run. Specifically, for phase II, the spike of the

140 five antibiotics started at the same time and continued for the remainder of the study. Fresh

141 feedwater spiked with antibiotics were refilled to the system every day. For the cleaning of

142 GAC sandwich, the *schmutzdecke* layer was stirred by glass rod and water above the filter

143 was then withdrawn using a syringe at the same time (Reungoat et al., 2011). The remaining

144 biofilters were backwashed for 10 min using biofilter effluents to achieve a 20 - 30% bed

145 expansion (Liu et al., 2012).

146

147 **2.3 Biofilter sampling strategy**

148   During the course of system run, duplicate influent and effluent samples were taken

149 weekly (one-time sampling) for the determination of general water quality parameters,

150 including pH, conductivity, turbidity, dissolved organic carbon (DOC), ultraviolet absorbency

151 (UV_{254}), dissolved oxygen (DO), phosphate (PO_4^{3-}), nitrite (NO_2^-) and nitrate (NO_3^-), total

152 coliforms and *E. coli*. Duplicate influents and effluents were also collected weekly (one-time

153 sampling) for antibiotic removal determination after the spike of antibiotics to the system.

154 Details on the analytical methods are provided in Section 2.5. In particular, as a slow filtration

155 rate (0.06 m/h) was used throughout this study, effluents for antibiotic analysis were collected

156   continuously until enough volume (500 - 550 mL) was achieved, while influents were collected

157   five times and mixed as one during the collection of the effluents. Prior to sampling, the flow

158   was rechecked and adjusted as needed.

159

160   **2.4 GAC adsorption kinetics**

161   In addition to the biofiltration experiment, isotherms and adsorption kinetics of the five

162   antibiotics on GAC were determined to further explore the removal mechanisms of the target

163   antibiotics. GAC used in the adsorption experiment was the same type of media used in the

164   biofiltration experiment (see Section 2.2 for details). The initial concentration of the five

165   antibiotics were set at 5 µg/L by adding stock solution to aqueous samples. For the contact

166   time test, 0.5 g GAC was added into glass bottles filled with 1 L lake water spiked with

167   antibiotics. Bottles were placed in a rotary mixer at a speed of 30 rpm. Reaction time was set

168   at 1 h, 3 h, 6 h, 9 h, 12 h, 24 h, and 48 h, respectively. For the GAC dosage test, 0.025 g, 0.05

169   g, 0.1 g, 0.25 g, 0.5 g, and 1 g GAC were added into 1 L lake water. Bottles were placed in a

170   rotary mixer at the speed of 30 rpm for 24 h. All bottles were prepared in triplicate and samples

171   process followed the analytical procedures described in Section 2.5.2. In addition, for each

172   test, lake water spiked with 5 µg/L of antibiotics without GAC addition was used as a control

173   to check the biodegradation of the target antibiotics during the experimental period. No

174   measureable biodegradation of the target antibiotics was observed in the control bottles,

175   hence, only adsorption mechanism was considered in the test.

176

177   **2.5 Analytical techniques**

178   2.5.1 General water quality parameters

179   Determination of general water quality parameters followed standard methods (APHA)

180   (Baird et al., 2012). Table S2 lists the methods and instruments used in this study. Influents

181   and effluents were filtered through a 0.45 µm mixed cellulose esters membrane (Millipore, UK)

182   for the analysis of DOC, phosphate, nitrite and nitrate. DOC was determined by a Shimadzu

183   TOC-L machine (UK) following manufacturer's procedures; while phosphate, nitrite and nitrate

184 were analysed by Ion chromatography (IC, Dionex ICS 1100, US). A standard membrane

185 filtration method was used for the enumeration of *E. coli* and coliform bacteria following ISO

186 9308-1:2014.

187 2.5.2 Quantification of Antibiotics

188 Solid phase extraction (SPE) was used to extract target antibiotics from aqueous

189 samples. Prior to extraction, samples were filtered through a 0.45 μm membrane filter

190 (Millipore, UK) and acidified to pH 3.0 with hydrochloric acid. Na_2EDTA was added to samples

191 at 0.5 g/L. Then, all samples were subjected to SPE using 500 mg Oasis HLB (hydrophilic

192 lipophilic balance) cartridges (Waters, UK). Lake water spiked with 2 μg/L of antibiotic mixture

193 was used to control for SPE recovery. Details of SPE protocol are summarised in Fig. S2.

194 Samples were reconstituted to a final volume of 500 μL with 0.1% formic acid in acetonitrile-

195 water (50:50, v/v). A matrix-matched calibration method was used throughout this study (Ben

196 et al., 2008; Grujic et al., 2009). The lake water was free of the selected antibiotics and thus

197 was used to prepare the matrix-matched standards in order to minimise the influence of

198 interfering substances from the environmental sample. An Accela 1100 HPLC system coupled

199 to a LTQ ion-trap mass spectrometer (Thermo Finnigan LTQ) was used for the detection of

200 target antibiotics. The optimised mass spectrometer parameters are summarised in Table S3.

201 Mean recoveries and limit of detection (LOD) for the antibiotics are reported in Table S4.

202

203 **2.6 Data analysis**

204 Removals of DOC, turbidity, UV_{254}, total coliforms, *E. coli* and antibiotics were

205 calculated based on influent and effluent concentrations. Mean and standard deviation were

206 calculated in Microsoft Excel 2016. One-way analysis of variation (ANOVA) and Pearson

207 correlation analysis were performed using OriginPro 2018. All figures were generated by

208 OriginPro 2018.

3. Results and discussion

3.1 Biofilter performance

The main physico-chemical characteristics of the lake water is summarised in Table S5. The lake water is slightly alkaline, with pH ranging from 7.85 - 8.35. The average turbidity, DO and DOC in the lake water during this study were 0.54 ± 0.07 NTU, 7.32 ± 1.49 mg/L and 3.82 ± 0.31 mg/L, respectively. The biofiltration system was operated continuously for 12 weeks, which generated a total of 12 batch samples. The overall performances of the sand biofilter (SB1 and SB2), GAC biofilter (GB1 and GB2), GAC sandwich biofilter (GSB1 and GSB2) and anthracite-sand biofilter (ASB1 and ASB2) are discussed in this section.

Generally, microbiological activities were more dynamic in phase I by the evidence of greater oxygen consumption/pH drop (Fig. S3), and DOC removal (Fig. S4). The levels of DO present in the effluent were above the recommend value of 3 mg/L for slow sand filtration (Fig. S3-c), suggesting an aerobic environment in the aqueous phase throughout the biofilter columns (Huisman and Wood, 1974). The GSB was found to be the most effective in removing turbidity-causing particles (Fig. S3-d). By contrast, ASB exhibited the worst performance in terms of turbidity reduction. Nitrate concentration increased in the effluent of SB and ASB during phase I and II (Fig. S3-e), while decreased in the effluent of GB and GSB. As no chemical oxidant was included in the filtration process, biological nitrification was the main causative agent for the increase of nitrate concentration in the effluents. The involvement of GAC in GB and GSB may contribute to the removal of nitrate through adsorption (Zhang et al., 2015). Bacterial communities attached to the filter media may affect the extent of nitrification during biofiltration (White et al., 2012), resulting in the differences in nitrate concentration in the effluents. After biofiltration, phosphate remained stable (ranged between 0.31 mg/L and 0.35 mg/L) in phase I and increased moderately to 0.41 mg/L (on average) in phase II (Fig. S3-f), with no statistical difference ($p > 0.05$). The increased phosphates in the effluents may be due to algal respiration which converts algal phosphorus to inorganic phosphorus (Pompei et al., 2017). No phosphate was found in the lake water samples in

236   January, which may be due to the seasonal variation and result in the absence of phosphate

237   in the influent and SB in phase III. Removal profiles of total coliforms and *E. coli* are presented

238   in Fig. S5. After four weeks' operation, both total coliforms and *E. coli* achieved 2-log removal

239   by all of the biofilters, indicating that a matured biofilm has been established. The removal

240   slightly fluctuated between week 5 to 11, probably due to the detachment of biofilm from the

241   media surface. The removals of total coliforms and *E. coli* were consistently above 90%, which

242   was within the typical removal range of between 90% and 99.9% required by conventional

243   slow sand filters (NDWCH, 2000).

244   A general trend of a decreasing removal of DOC from phase I to II was observed across

245   all types of biofilters (Fig. 2A). The finding is in agreement with Gibert's research, where the

246   DOC removal progressively declined over time (Gibert et al., 2013). GAC-associated filters

247   revealed significantly greater DOC removal efficiencies ($p < 0.001$) than SB and ASB during

248   the operational period of 3 months, attributed to the adsorption of organic matter by the porous

249   GAC structure. The introduction of a GAC layer to the sand filter showed a similar initial DOC

250   removal compared to the whole GAC-bed filter, although a reduced removal percentage (by

251   11%) was observed by the sandwich filter at the later stage of the system run. This is

252   somewhat expected, as the dosage of GAC in GSB was lower than in the whole GAC bed and

253   the sorption capacity of GAC gradually declines over time (Gibert et al., 2013). Mean removals

254   of DOC were 21 ± 8% and 14 ± 9% by SB and ASB, respectively. Previous research has also

255   reported that by using anthracite-sand dual media (similar grain size with this study), partial

256   DOC removal (< 27%) was observed and after 6-months of operation, the removal plateaued

257   at 7.6% (Zhang et al., 2017). Campos *et al.* found an average of 23% DOC removal by full-

258   scale drinking water slow sand filters (Campos et al., 2002). The trends for the reduction of

259   UV_{254} absorbance in biofilters were consistent with DOC removal (Fig. 2B).

260   In DWTPs, biofilters are backwashed, which involves pumping water backwards

261   through the filter media, periodically to restore the hydraulic capacity as the filters are usually

262   used to remove both DOC and particles (Basu et al., 2016). In this study, biofilter

263   backwashing/cleaning had a significant impact on pH, conductivity, DO, turbidity, nitrate, total

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264 coliforms and *E. coli* (Fig. S3 and S5), while DOC was not sensitive to backwashing/cleaning

265 (Fig. S6). These observations are similar to those reported by Emelko *et al.*, in full-scale

266 biofilters, allowing conventional performance parameters to be optimised without

267 compromising DOC removal (Emelko et al., 2006).

268

269 **3.2 Overview of target antibiotics removal**

270   The overall mean removal of the five antibiotics over the entire course of the

271 experiment were $20 \pm 19\%$ by SB, $97 \pm 2\%$ by GB, $97 \pm 3\%$ by GSB, and $18 \pm 9\%$ by ASB.

272 Fig. S7 shows the average removal of antibiotics by the duplicate biofilters. GB and GSB

273 exhibited considerably superior performance in eliminating all five antibiotics compared to

274 sand or anthracite media biofilters. TMP and OTC were removed significantly more effectively

275 by SB ($55 \pm 24\%$, $p < 0.01$) and ASB ($33 \pm 12\%$ on average, $p < 0.05$), respectively. The

276 remaining three antibiotics showed comparable removal rates by the SB and ASB. The

277 dynamitic concentration changes of the five antibiotics over time are shown in Fig. 3. No

278 obvious trend of increasing or decreasing removal was found for antibiotics over the two-

279 month study period. Details of individual antibiotic are discussed below.

280

281 **3.3 Removal of antibiotics by sand and anthracite-sand dual biofilters**

282   Over the antibiotic-spiking operational period, similar SMX effluent concentrations

283 were found in SB and ASB, with a mean value of 1.88 ± 0.11 µg/L for SB and 1.76 ± 0.15 µg/L

284 for ASB, respectively (Fig. 3). In general, SMX showed very limited removal ($< 15\%$) in the

285 system, which was similar to the removal of 4.1% reported in previous laboratory sand

286 biofiltration study (Zearley and Summers, 2012). The removal of TMP in SB ranged from 39%

287 to 75% at the earlier stage (5-8 weeks) after antibiotics spike and from 60% to 87% at the later

288 stage (9-11 weeks), with the lowest effluent concentration of 0.27 µg/L found in week 10

289 samples. TMP was reported to be readily biodegradable (removal ranged from 50% to 92%)

290 in a previous sand biofilter study (Zearley and Summers, 2012). These results suggest that

291 conventional sand biofilter is effective for the elimination of TMP during drinking water

292   treatment. Removal of TMP in the SB was significantly higher ($p < 0.01$) than that in the ASB

293   (Fig. 3). For comparisons, mean effluent concentration of TMP was 0.89 ± 0.48 µg/L and 1.74

294   ± 0.18 µg/L for SB and ASB, respectively. A much higher (> 75%) TMP removal in anthracite-

295   sand dual media biofilters was observed by Zhang *et al.*, when 2-year-old media from a

296   drinking water treatment facility were used (Zhang et al., 2017). As TMP was found to be

297   readily biodegradable in sand biofilters under the identical operational conditions, it is likely

298   that 3 months were not sufficient to develop an effective biofilm on the surface of anthracite

299   for biodegrading TMP. ASB showed slightly better removal of AMOX than the SB. The mean

300   value of AMOX effluent concentration was 4.32 ± 0.25 µg/L for SB and 4.05 ± 0.32 µg/L for

301   ASB, respectively (Fig. 3). OTC was removed more effectively by the ASB than other

302   antibiotics, ranging from 19% to 58%. The highest and lowest OTC effluent concentrations

303   were found in SB (1.87 µg/L in week 10 sample) and ASB (0.85 µg/L in week 5 sample),

304   respectively. Similar to SMX, both the SB and ASB showed limited removal for CTM, with the

305   effluent concentrations ranging from 1.51 µg/L (25% removal) in ASB to 2.00 µg/L (no removal)

306   in SB. This observation is in agreement with the results reported by Nakada *et al.*, where no

307   removal was observed for CTM during sand filtration in a pilot-scale plant (Nakada et al., 2007).

308   The removal of TMP was considerably reduced ($p < 0.01$) from 69% to 7% after backwashing

309   of SB. By contrast, removals of the remaining antibiotics were not affected ($p > 0.05$), which

310   is possibly due to the already low removal rates before backwashing occurred. Fig. S8 shows

311   the percentage removal of the target antibiotics by SB and ASB.

312   For the non-adsorptive media, biodegradation is the only significant removal

313   mechanism for the antibiotics from the feedwater (Reungoat et al., 2011; Zearley and

314   Summers, 2012). The antibiotics exhibited different levels of biodegradability in this study.

315   Based on the mean removal by SB and ASB, the antibiotics were classified as follows (Zearley

316   and Summers, 2012): SMX and CTM had removals of less than 15% and were classified as

317   recalcitrant to biodegradation, AMOX and OTC had removals between 15% and 50% and

318   were classified as having slow biodegradation rates (moderately biodegradable), and TMP

319 had removals between 50 and 85% (only in SB) and was classified as being readily

320 biodegradable.

321   Sand biofiltration works through a slime layer that accumulates above the sand surface

322 (known as the *schmutzdecke*) and within the upper layers of the sand bed (Huisman and Wood,

323 1974; Campos et al., 2002; Unger and Collins, 2008). The *schmutzdecke* layer is highly

324 biologically active and has been shown to have the ability to biodegrade many trace level

325 micropollutants (Wang et al., 2007; Maeng et al., 2011; Zearley and Summers, 2012;

326 Dalahmeh et al., 2018). The biodegradation rate of antibiotics is possibly affected by their

327 initial concentration in the raw water. It was hypothesised that SMX degradation would only

328 occur if a threshold concentration of 0.3 µg/L was exceeded (Gruenheid et al., 2008). This

329 was further confirmed by a laboratory column experiment, where a higher concentration of

330 SMX (4 µg/L) in the feed water can be removed more effectively than at 0.25 µg/L

331 (Baumgarten et al., 2011). This is due to the adaptation of biomass for degradation proceeded

332 faster at elevated concentration of SMX. Furthermore, the sorption of antibiotics onto the

333 *schmutzdecke*, or within the filter column might also contribute to their removal (Rooklidge et

334 al., 2005). For hydrophilic antibiotics (AMOX, OTC, SMX and TMP), their removal through

335 sand filtration might also attribute to the removal of the fine suspended particles adsorbing to

336 these compounds (Huisman and Wood, 1974; Nakada et al., 2007). The filter substrate plays

337 an important role in determining bacterial community composition. Although seeded from the

338 same source water, compared to anthracite, microorganisms are prone to colonise on the

339 surface of sand due to its smaller particle size and larger surface area (Wang et al., 2007).

340 Furthermore, the different elemental compositions of sand and anthracite surfaces may also

341 have influenced bacterial attachment (Wang et al., 2007). These factors could explain to a

342 certain extent the variations of removal efficiencies for individual antibiotic throughout this

343 study.

344

3.4 Removal of antibiotics by GAC and GAC sandwich biofilters

The GB and GSB showed consistent and stable removals for all of the antibiotics over time. Fig. S9 shows the removal efficiencies of the antibiotics by the two types of biofilters. The overall mean removals of the five antibiotics during the whole operational period were 97% for both GB and GSB. Except for AMOX, the effluent concentrations of antibiotics well all below 0.07 µg/L. GSB achieved considerably higher removals ($p < 0.01$) than the conventional sand filter, indicating the applicability of GSB in eliminating trace organic compounds from drinking water. This observation is in agreement with the results reported by Li *et al.*, where an average removal of 95% was observed for DEET, paracetamol, caffeine, and triclosan in sandwich biofilters with various GAC layer depths (Li et al., 2018). Comparing the results obtained from SB and GSB, it can be assumed that the removal of target antibiotics in the sandwich biofilter could be attributed to both adsorption by the GAC layers and biodegradation within the *schmutzdecke* layer. The contribution of biodegradation could not be elucidated in the sandwich biofilter since the overall removal of antibiotics was similar to that obtained in the pure GAC column. However, it is worth mentioning that the GAC sandwich seems a cost-effective option compared to full GAC contactors (Bauer et al., 1996). Antibiotic removal was not affected by the cleaning/backwashing of GAC and GAC sandwich biofilters in this study.

3.5 Relationship between general water quality parameters and antibiotics in effluents

Pearson correlation analysis showed that the removal of antibiotics is significantly correlated to nutrient levels, i.e. organic carbon and nitrate in the effluents (Table 1). Nitrification occurs in both sand and GAC biofilters (Nakhla and Farooq, 2003; de Vera et al., 2018). It has been reported that effluents from wastewater treatment plants (WWTPs) containing antibiotics have the potential to disrupt nitrification/denitrification processes in aquatic ecology (Costanzo et al., 2005). For instance, Costanzo found that the bacterial denitrification rates were reduced significantly after short term exposure to clarithromycin and amoxicillin at 1000 µg/L (Costanzo et al., 2005); antibiotics such as oxytetracycline have been shown to inhibit the nitrification process in surface water (Klaver and Matthews, 1994).

373 Significant positive correlations between the antibiotics (except for TMP) and nitrate in the

374 effluents indicated the possible impact of antibiotics on the nitrification/denitrification

375 processes. In agreement with this observation, Watkinson *et al.* identified nitrate as a potential

376 indicator to predict total antibiotic concentration in effluent in a case study of WWTP

377 (Watkinson et al., 2007). However, the extent of the interference exerted by antibiotics on

378 nitrification/denitrification was not clear as low exposure level was used in the present study.

379 A mass balance study with regards to nitrogen should be included in future study. A limitation

380 is that the present research only investigated the non-metabolised parent form of the target

381 antibiotics, therefore, in the context of antibiotic pollution risks, removal rates presented can

382 be overestimated as metabolites may remain pharmaceutically active in the filtered water

383 (Mompelat et al., 2009). Additional research is needed to identify the dominant antibiotic

384 biodegradation by-products and clarify the relevant pathways in drinking water biofilters to

385 understand the degradation mechanisms before substantial conclusions can be made.

386

387 **3.6 Antibiotics adsorption onto GAC**

388 3.6.1 Adsorption kinetics

389 Fig. 4 shows the adsorption of the five antibiotics by GAC in surface water samples.

390 The optimal contact time was 12 h for TMP, 24 h for SMX, OTC and CTM, and 48 h for AMOX,

391 when each antibiotic had reached an equilibrium concentration and > 90% removal was

392 achieved. From Fig. 4, experimental adsorption capacity (q_e) of the five antibiotics in surface

393 water was 0.0099, 0.0099, 0.0091, 0.0097, and 0.0096 mg/g for SMX, TMP, AMOX, OTC and

394 CTM, respectively. Further kinetic modelling of the adsorption removal of antibiotics onto GAC

395 was carried out using Lagergren pseudo-first-order and pseudo-second-order equations,

396 which are two types of models that have been widely used to describe the adsorption of

397 pollutions from water onto adsorbents (Hameed and Rahman, 2008; Simonin, 2016; Li et al.,

398 2018). Details on the calculations of kinetic modelling are provided in the Supporting

399 Information (page 12).

400   Table 2 lists the results of the rate constants for different antibiotics. The results

401   suggest that for TMP, AMOX, OTC and CTM, the pseudo-second-order adsorption

402   mechanism was predominant, indicating that the rate of the adsorption is controlled by the

403   adsorption reaction at the liquid/solid interface in the GAC (chemisorption process) (Ho and

404   McKay, 1999; Hameed and Rahman, 2008). SMX had a better fit with the pseudo-first-order

405   model, suggesting that the adsorption is a diffusion-controlled process (Simonin, 2016).

406   3.6.2 Adsorption isotherms

407   The impact of GAC dosage on antibiotic removal is shown in Fig. S10. Data from the

408   batch experiments were fitted to the Freundlich and Langmuir isotherm models to evaluate

409   the adsorption characteristics of the five antibiotics on GAC. Details are provided in the

410   Supporting Information (page 13). Isotherm parameters are summarised in Table 3. Real data

411   fitted better with the Langmuir model ($R^2 > 0.9099$) than the Freundlich model, especially for

412   AMOX and CTM. Based on these results, GAC has q_m values (maximum/saturation adsorption

413   capacity) ranging from 0.0072 mg/g for CTM to 0.0269 mg/g for TMP. This could be explained

414   by the hydrophobicity of CTM as the difference in adsorption capacities is related to the

415   hydrophobicity of a compound (Nam et al., 2014). Considering the antibiotic spike

416   concentration (2 and 5 µg/L), the amount of GAC loaded (approximately 50 g in the sandwich

417   filter) and the filtration rate used (0.06 m/h), theoretically, it would take approximately 1~2

418   years until the GAC is saturated in the sandwich filter (surface sand cleaning is required

419   regularly). This is consistent with practice in DWTPs, as it typically requires years of operation

420   until GAC sorption capacity becomes exhausted.

4. Conclusion

Biofiltration has the potential to be an effective process for controlling trace level antibiotic contaminations in drinking water. During 3-months of system operation, GAC-associated biofilters exhibited a considerably superior performance in eliminating all of the five antibiotics and the mean removal was 97 ± 2%. In contrast, much lower removals were observed for the sand and anthracite-sand dual biofilters, where an average of 20 ± 19% and 18 ± 9% of antibiotics were removed from the feedwater, respectively. Considering costs and maintenance of GAC contactors, the GAC sandwich biofilter represents a more promising process for drinking water treatment. Biofilter backwashing/cleaning showed no effect on DOC removal. GAC adsorption kinetics showed that sulfamethoxazole fitted with pseudo-first-order adsorption model, suggesting that the adsorption is a diffusion-controlled process. Trimethoprim, amoxicillin, oxytetracycline and clarithromycin fitted the pseudo-second-order model, indicating that the adsorption is controlled by the surface reaction. The applicability of the Langmuir model suggests a uniform adsorption of the antibiotics on the GAC surface. Overall, this research will provide useful information which can be used for optimising or updating existing biofiltration processes in industry to reduce antibiotic residues from drinking water.

Declaration of Competing Interests

No conflict of interest declared.

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