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Pharmaceutical sorption to lab materials may overestimate rates of removal in lab-scale bioreactors

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Abstract

Environmental contamination from pharmaceuticals has received increased attention from researchers in the past 20 years. As such, numerous lab-scale studies have sought to characterize the effects of these contaminants on various targets, as well as determine improved removal methods. Many studies have used lab-scale bioreactors to investigate pharmaceutical effects on wastewater bacteria, as wastewater treatment plants often act as reservoirs for pharmaceuticals. However, few—if any—of these studies report the specific lab materials used during testing, such as tubing or pipette tip type. In this study, the pharmaceuticals erythromycin, diclofenac, and gemfibrozil were exposed to different micropipette tips, syringe filters, and tubing types, and losses over time were evaluated. Losses to tubing and syringe filters were particularly significant and neared 100%, depending on the pharmaceutical compound and length of exposure time. Results discussed herein indicate that pharmaceutical sorption to various lab supplies results in decreases to both dosed and quantified pharmaceutical concentrations. Studies that fail to consider this source of loss may therefore draw inaccurate conclusions about pharmaceutical effects or removal efficiencies.

Keywords: Adsorption, pharmaceuticals and personal care products (PPCPs), emerging contaminants

26 **1. Introduction**

27 Pharmaceutical compounds are increasingly detected in environmental matrices around the globe
28 (Focazio et al., 2008; Xia et al., 2005). The presence of these compounds can be traced, in large part, to
29 their release from wastewater treatment plants (Margot et al., 2015). Wastewater treatment plants
30 (WWTPs) are generally not designed to remove non-biodegradable compounds like pharmaceuticals;
31 therefore, WWTPs act as reservoirs for pharmaceuticals prior to releasing them, minimally treated, to the
32 environment. Numerous pharmaceuticals have toxic effects on plants, wildlife, and microbiota at part per
33 billion concentrations (Calisto & Esteves, 2009; Kim & Aga, 2007; Sathishkumar et al., 2020).
34 Additionally, pharmaceuticals can harm wastewater-treating bacteria, potentially resulting in decreased
35 treatment efficacy as these compounds accumulate (Boonnorat et al., 2019).

36 Aerobic granular sludge (AGS) is a promising emerging biotechnology for wastewater treatment.
37 AGS consists of nitrifying, denitrifying, and phosphate-accumulating organisms that self-aggregate into
38 dense, spherical biofilms 1-2 mm in diameter. The high concentration and variety of extracellular
39 polymeric substances (EPS) in AGS confer protection from various toxins and likely provide a sorptive
40 medium for hydrophobic compound removal (Kent & Tay, 2019; Kong et al., 2015; Tay et al., 2005).
41 Altogether, granules may be capable of simultaneously treating conventional wastewater contaminants
42 while biodegrading or adsorbing recalcitrant compounds, such as pharmaceuticals. However, the body of
43 literature available on granule-driven pharmaceutical treatment is limited, and therefore more information
44 is needed on how granules respond to a wide range of pharmaceuticals.

45 The initial objective of this study was to evaluate the effects of three model pharmaceuticals on
46 lab-grown AGS. Erythromycin (ERY, antibiotic), diclofenac (DCF, NSAID), and gemfibrozil (GEM,
47 lipid regulator) are three pharmaceuticals commonly found in the environment (English et al., 2009; Fang
48 et al., 2012; Godfrey et al., 2007; Icopini et al., 2016; Louvet et al., 2010; Sathishkumar et al., 2020;
49 Schafhauser et al., 2018; Xia et al., 2005). However, few studies have investigated the interaction
50 between each compound and AGS.

51 After analysis of preliminary results, a new objective—and prerequisite—for this study became
52 apparent: understanding, quantifying, and mitigating pharmaceutical losses to various lab supplies.
53 Pharmaceutical losses to influent tubing were particularly evident. A search of the literature found only
54 one relevant publication in which pharmaceutical sorption to tubing and filter materials was tested, and
55 results were not directly applicable to the study conditions and materials described herein (Hebig et al.,
56 2014). Likewise, although multiple studies investigate AGS exposure to pharmaceuticals (Amorim et al.,
57 2014; Amorim et al., 2016; Kang et al., 2018; Kent & Tay, 2019; Kong et al., 2015; Margot et al., 2015;
58 Moreira et al., 2015; Muñoz-Palazon et al., 2021; Rodriguez-Sanchez et al., 2017; Wang et al., 2017;
59 Wang et al., 2019; Wang et al., 2018; Yu et al., 2020; Zhao et al., 2015; Zhu et al., 2013), several do not
60 report influent concentrations (Amorim et al., 2014; Kong et al., 2015; Muñoz-Palazon et al., 2021;
61 Rodriguez-Sanchez et al., 2017; Wang et al., 2017). None describe the specific materials used to ensure
62 that desired influent pharmaceutical concentrations were achieved. Several report only overall
63 pharmaceutical removal or effluent concentrations (Amorim et al., 2014; Kong et al., 2015; Muñoz-
64 Palazon et al., 2021; Rodriguez-Sanchez et al., 2017; Wang et al., 2017). After measuring significant
65 pharmaceutical losses through the influent tubing, we questioned if the removals reported were due
66 entirely to AGS-driven sorption or degradation, or if removals could partially be attributed to losses to lab
67 supplies. We therefore assessed pharmaceutical losses to various micropipette tips, tubing types, syringes,
68 and syringe filters.
69
70 **2. Methods**

71 **2.1 AGS Reactor Operation**

72 AGS were grown in two identical double-walled glass laboratory-scale sequencing batch reactors
73 (SBRs). Both SBRs had an internal diameter of 60 mm and working volume of 3.4 L and were operated
74 in repeating three-hour cycles: 72 minutes anaerobic feed from the bottom of the reactor, 100 minutes
75 aeration at a rate of 5 L/min, three minutes settling, and five minutes effluent discharge from a port 47 cm
76 above the bottom reactor flange. Approximately 50% of the reactor liquid was withdrawn in each cycle,

resulting in a hydraulic residence time of 6.4 hours. The solids residence time was controlled at approximately 25 ± 5 days.

An automatic timer (Chrontrol) controlled influent, effluent, and aeration pumps. Labview software (National Instruments) controlled pH (7.0 ± 0.3) and dissolved oxygen (1.75 ± 0.25 mg/L) during the aeration phase. Influent media were identical to those described by (de Kreuk & van Loosdrecht, 2004), except that the concentration of sodium acetate in the influent was increased to 10.3 mM, resulting in an organic loading rate of 2.5 g C/L*d. Both SBRs were initially seeded with AGS from an AquaNereda® treatment plant in Utrecht, The Netherlands and operated at steady state for over 200 days prior to starting experimentation.

For 30 days, the test reactor received 23 mL of pharmaceutical medium with the influent media during the feed phase, resulting in nominal influent concentrations of approximately 10 ug/L of each pharmaceutical. The pharmaceutical medium consisted of 705 ug/L each of erythromycin (TCI Chemicals), diclofenac sodium (Acros Organics), and gemfibrozil (Acros Organics) in nanopure water. Pharmaceutical properties are summarized in Table 1. Pharmaceutical stock solutions were prepared first in methanol at 1 g/L and then diluted into water; therefore approximately 7.9 mg/L methanol was also present in the influent medium. The pharmaceutical medium was protected from light to prevent photolytic degradation, prepared fresh every 8-10 days, and sampled directly to quantify pharmaceutical concentrations. Influent samples were taken from a sampling port in the tubing located at the base of the reactor and were extracted and quantified per methods detailed in section 2.2.

103 **Table 1. Physical and chemical properties of tested pharmaceuticals.**

	Erythromycin	**Diclofenac**	**Gemfibrozil**
Chemical formula	$C_{37}H_{67}NO_{13}$	$C_{14}H_{11}Cl_2NO_3$	$C_{15}H_{22}O_3$
Molecular weight (g/mole)	733.9	296.1	250.3
Water solubility (mg/L at 25°C)	2000 [a]	1418 [b]	29.1 [d]
Octanol-water partition coefficient (Log K_{ow})	3.06 [a]	4.51 [c]	4.77 [d]
Acid dissociation constant (pKa)	8.89 [a]	3.99 [c]	4.7 [d]

104 [a] (Schafhauser et al., 2018)

105 [b] (Skube et al., 2004)

106 [c] (Avdeef et al., 1998)

107 [d] (Fang et al., 2012)

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118 **2.2 Pharmaceutical compound quantification**

119 Influent and effluent samples were prepared for HPLC-QtoF-MS analyses by solid phase
120 extraction (SPE). Waters Oasis HLB cartridges (30 mg, 20 mL) were preconditioned with 1 mL methanol,
121 dried, and equilibrated with 1 mL nanopure water. 75 mL influent or 150 ml effluent sample were then
122 filtered with a 1.5 µm glass fiber filter (Hach) to remove solids and loaded on the cartridges at 10 mL/min
123 using a vacuum manifold system. Loaded cartridges were washed with 1 mL 5% vol/vol methanol in
124 water, dried for 30 minutes, then frozen at -18°C until elution (no longer than 14 days).

125 Pharmaceuticals were eluted at 5 mL/min using 2 x 5 mL methanol followed by 2 x 5 mL
126 methanol:acetone (1:1, v/v) mixture. Eluents were evaporated to dryness under a gentle nitrogen stream at
127 40°C, reconstituted in 1 mL methanol, and stored at -18C until HPLC-QtoF-MS analysis.

128 To account for possible pharmaceutical losses during the extraction process, influent and effluent
129 pharmaceutical samples were taken in duplicate. One sample from each duplicate set was pre-spiked with
130 pharmaceutical stock solution to a final nominal concentration of 10 µg/L prior to performing any
131 extraction steps. After extraction, the unspiked sample was split in half, and one half was post-spiked to a
132 final nominal concentration equal to 10 µg/L multiplied by each samples' respective concentration factor.
133 Recovery was then determined as follows:

$$Recovery = \frac{Prespike\ concentration - unspiked\ concentration}{Postspike\ concentration - unspiked\ concentration} \quad (1)$$

134 Measured pharmaceutical concentrations in unspiked samples were thus corrected for recovery of
135 each analyte. Recovery was consistently over 60% for ERY and over 95% for DCF and GEM.

136 Mass spectrometry was performed with an Agilent 6538 UHD Accurate-Mass QtoF HPLC-MS
137 (Agilent Technologies) in positive ion mode. Chromatographic analysis methods were adapted from
138 (Sodré & Sampaio, 2020). In brief, pharmaceuticals were separated with an Agilent Eclipse Plus C18
139 column (2.1 x 100 mm) at 30°C under gradient elution. Methanol and ultrapure water, both enriched with
140 formic acid (0.1% v/v), were used as mobile phases at 0.7 mL/min. Gradient elution was achieved by
141 increasing the concentration of organic solvent from 1% initially to 95% at 10.1 minutes, then returning to

142 initial conditions and re-equilibrating for 3 minutes (total run length of 13 minutes). Sample injection
143 volume was 8 μL. Retention times for ERY, DCF, and GEM were consistently 6.6, 8.4, and 9.1 minutes,
144 respectively.

145 Analyte concentrations were determined based on a calibration curve relating peak area with
146 nominal concentration in analytical standards in both methanol and water. The instrument limits of
147 quantification were 10 μg/L for ERY and 50 μg/L for DCF and GEM. As a check for biodegradation, all
148 samples were also screened for ERY, DCF, and GEM biodegradation products using a personal
149 compound database and library (PCDL). However, degradation products were not detected in any of the
150 samples described in the following sections, and therefore pharmaceutical losses were likely abiotic in
151 nature.

152

153 **2.3 Lab supply sorption tests**

154 To identify the source of pharmaceutical losses and determine possible mitigating actions,
155 pharmaceutical losses to various micropipette tips, syringe filters, and tubing types were assessed by
156 repeated washing of each with pharmaceutical solution. The lab supplies tested are listed in Table 2.
157 Losses to different micropipette tips were quantified first after initial analyses of standards prepared with
158 Axygen tips resulted in noisy, unpredictable data (not shown). The best-performing micropipette tips
159 identified in this test were used in all future tests.

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Table 2. Lab supplies tested for pharmaceutical loss. Catalog numbers are included in parentheses.

Pipette tips	Syringe filters	Tubing (all Masterflex)
Ayxgen Maxymum Recovery (AXYTF1000LRS)	Sartorius MiniSart Regenerated Cellulose (17765K)	Tygon size 14 (06404-14)
USA Scientific TipOne (1122-1730)		Puriflex size 14 (96419-14)
Mettler-Toledo Rainin RC-1000LR (17014398)	FisherBrand PTFE membrane (09-719H)	PharmaPure size 13 and 14 (06435-13, 06435-14)
		Platinum-cured silicone size 14 (96410-14)

184 Pharmaceutical losses to syringe filters were evaluated next. Many pharmaceutical extraction
185 protocols utilize a final syringe filtration step to remove particulates after reconstituting extracted samples
186 (Camacho-Muñoz et al., 2009; Chen et al., 2012; Kang et al., 2018; Spongberg & Witter, 2008; Wang et
187 al., 2019); likewise, samples taken directly from the AGS reactor required syringe filtration before
188 analyses. In both cases, filtration volumes were small (under 2 mL) and therefore pharmaceutical loss to
189 the filtration material could have a large impact on measured concentrations. It was therefore important to
190 determine if pharmaceutical loss to syringe filters could be occurring and decreasing measured
191 concentrations. Syringes were also tested for pharmaceutical loss and losses were not detectable (data not
192 shown).

193 Lastly, pharmaceutical losses to different tubing types were evaluated after preliminary data
194 showed that pharmaceutical concentrations declined as solution travelled through influent tubing.

195 Mixed solutions of ERY, DCF, and GEM were prepared by diluting a 1 g/L stock solution in
196 methanol into nanopure water. Glassware that had recently been baked in a muffle furnace (550ºC x 5
197 hours to remove all carbon-based contaminants) was used to store all pharmaceutical solutions. Glassware
198 was also tested for pharmaceutical loss and losses were not detectable (data not shown). All
199 pharmaceutical samples taken during lab supply sorption tests were analyzed directly (i.e., not extracted).
200 Losses were calculated as follows:

$$Loss = 100\% \times \left(1 - \frac{Current\ Concentration}{Initial\ Concentration}\right) \quad (2)$$

201

202 **2.3.1 Pipette tip testing**

203 A solution containing each pharmaceutical at 100 µg/L was prepared (“pharmaceutical solution”).
204 This concentration was selected to ensure that pharmaceutical concentrations would remain detectable
205 after losses to tips. One milliliter micropipette tips from Axygen, USA Scientific, and Mettler-Toledo
206 (Table 2) were tested for losses. For each tip brand tested, two glass flasks were filled with 50 mL
207 pharmaceutical solution, and samples were immediately taken from both with fresh pipette tips. One flask

208 ("rinse") was then used to pull and discharge 1 mL solution into the pipette tip repeatedly four times. The
209 other flask ("sample") was then used to collect 1 mL of solution for analysis, thereby representing the
210 fifth usage of the pipette tip. In this way, the 0th, 5th, 10th, and 15th "washes" of each tip were sampled.
211 This method ensured that measured concentrations reflected actual pharmaceutical losses to the pipette
212 tip, not declining concentrations in the rinse flask. The test was conducted in triplicate for each pipette tip
213 except the Axygen tips, as initial analyses of standards prepared with Axygen tips showed noisy,
214 unpredictable relationships between peak area and concentration. The goal of this test, therefore, was to
215 determine if USA Scientific or Mettler-Toledo tips performed better than Axygen tips.
216

217 **2.3.2 Syringe filter testing**

218 Regenerated cellulose syringe filters (Sartorius Minisart, 0.45 um x 25 mm) and PTFE membrane
219 syringe filters (Fisherbrand, 0.45 um x 25 mm) were used to test pharmaceutical losses. 500 µg/L
220 pharmaceutical solution was prepared and sampled directly with Mettler-Toledo tips (previously
221 identified as the best-performing micropipette tips). This concentration was selected to ensure that
222 pharmaceutical concentrations would remain detectable after losses to the high surface area material in
223 filters. A 3 mL syringe was used to flush 1.5 mL solution through each filter to flush out any organic
224 contaminants that may have been attached to the syringe filter material. The next mL filtered was then
225 collected and analyzed. Tests were conducted in triplicate with new filters and syringes.
226

227 **2.3.3 Tubing testing**

228 The tubing types listed in Table 1 were exposed to the target pharmaceuticals in two separate
229 tests. In a short-term test, 30 cm long tubing samples provided by ColeParmer (Table 2) were connected
230 to a 1 RPM peristaltic pump. The tubing residence time was approximately two minutes. PTFE tubing
231 was connected to the influent end of each type of tubing to increase the tubing length and submerged in
232 separate pharmaceutical solutions, all at approximately 705 µg/L. Reasoning for the concentration of
233 pharmaceutical solution is provided in Section 2.1. Pharmaceutical losses to PTFE tubing were assumed

234 to be minimal, as this type of tubing was also used during the pharmaceutical extraction and concentration
235 process with good recovery of each analyte. Pump heads compatible with PTFE tubing were not available
236 for this research.

237 Pumping was continuous for 24 hours. Samples were taken with Mettler-Toledo pipette tips from
238 each influent immediately prior to starting pumping, and then collected from the effluent end of each tube
239 type after 24 hours.

240 The best-performing tubing type identified in the short-term was then utilized for longer-term,
241 higher concentration testing. Triplicate 90 cm lengths of size 13 PharmaPure tubing were connected to a 1
242 RPM pump, yielding an approximate residence time of six minutes. Influent ends were submerged in
243 separate flasks containing 12.5 mg/L pharmaceutical solution (nominal concentration). Pharmaceutical
244 concentrations were increased with an eye to future testing, as it was desired that influent pharmaceutical
245 concentrations in forthcoming AGS exposure tests would be 50 μg/L. Dilution of 6.5 mL pharmaceutical
246 solution at 12.5 mg/L with 1600 mL influent media would result in the desired 50 μg/L influent
247 concentration.

248 Pumping was continuous for seven days. Influent samples were taken with Mettler-Toledo pipette
249 tips and effluent samples were collected directly from the effluent end of each tube. After seven days,
250 PTFE tubing was connected to the influent end of one tubing section, and only the PTFE portion was
251 submerged in influent solution. Influent and effluent samples were then collected for the next three days
252 to determine if sorption was occurring preferentially to the inside or outside of the tubing section.
253

254 **3. Results and discussion**

255 **3.1 Loss to lab supplies**

256 **3.1.1 Pipette tips**

257 Pharmaceutical losses to different pipette tips varied from -10 to nearly 20%, depending on the
258 compound and tip used (Fig. 1). Positive losses after washing indicate that pharmaceutical sorption to the
259 tip was still occurring; negative losses indicate that sorption decreased significantly, resulting in higher

260 pharmaceutical concentrations in the sample released from the pipette tip than were measured in the
261 initial sample. A negative loss may therefore indicate equilibrium-induced desorption.

262 Variation among replicate samples was high for both tips tested in triplicate; however, Mettler
263 Toledo (MT) tips performed more consistently when exposed to DCF and GEM, as evidenced by lower
264 standard deviation among sample replicates. After the 5th wash, DCF and GEM losses to MT tips were -
265 5.5 ± 2.6% and -9.8 ± 3.5%, respectively. DCF and GEM losses to USA Scientific tips were -6.2 ± 4.4%
266 and 11.9 ± 6.7%, respectively. ERY losses to both tip types were similar and below 1% on average.

267 Despite the high variability seen in this test, results show that pharmaceutical sorption to different
268 brands of micropipette tips may significantly influence measured sample concentrations—and the number
269 of tip uses is also likely to have a large impact. It is also difficult to conclude why certain tips perform
270 better than others. All tips' manufacturers list virgin polypropylene as the pipette tip material, and all tips
271 had similar geometries. Likewise, it is difficult to correlate the properties of each pharmaceutical (listed in
272 Table 1) with losses. ERY has the lowest octanol-water partition coefficient (K_{ow}) of the three compounds
273 tested, which may explain why ERY losses were generally lower than those for DCF or GEM. However,
274 this behavior was not consistent, as ERY and DCF losses to Axygen tips were near equal (-8% and -10%,
275 respectively), despite large differences in K_{ow} values.

276 Altogether, pharmaceutical losses due to either parameter—tip reuse or brand—may have a large
277 impact on measured concentrations, and the variability associated with pharmaceutical loss may make
278 impacts difficult to predict.

279

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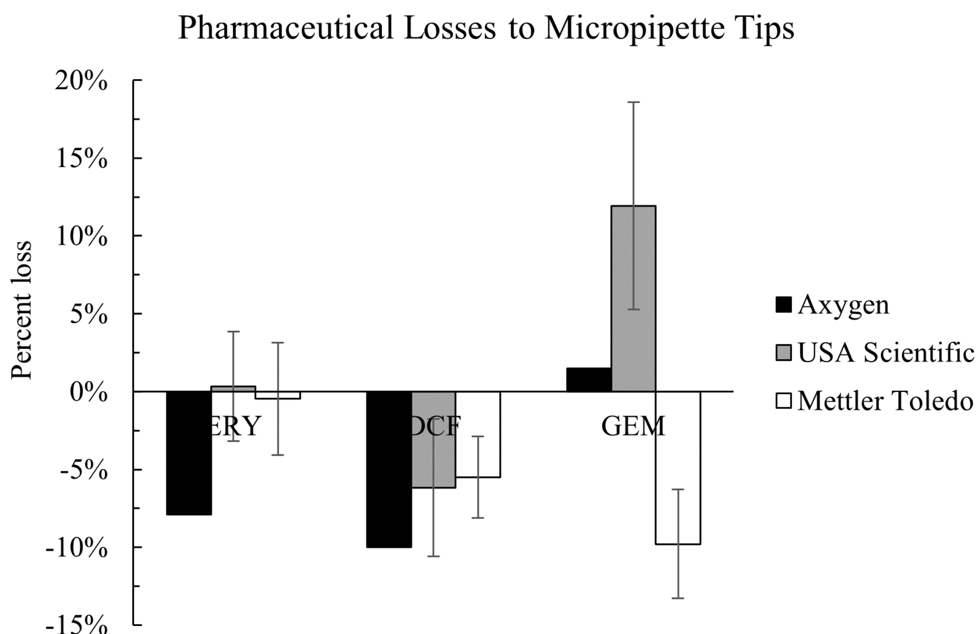
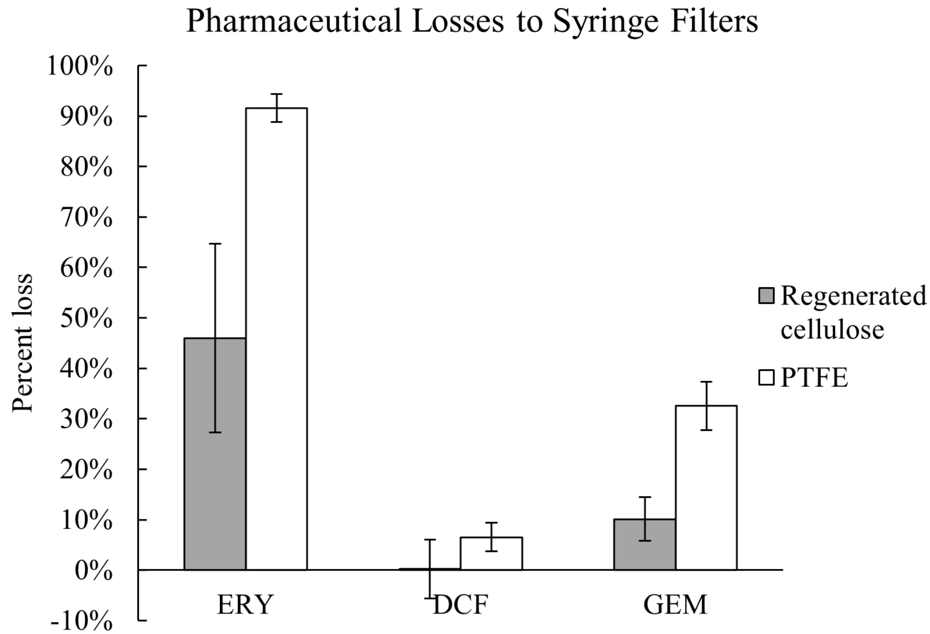


281 **Fig. 1** Pharmaceutical losses to different brands of 1 mL micropipette tips after five washes. Mettler-
282 Toledo tips performed best of the three brands tested, as losses to these tips were less than those to other
283 brands and variability within replicates was smallest. As previously mentioned, Axygen tips were not
284 tested in triplicate and therefore standard deviation bars are not included for these data

285

286 **3.1.2 Syringe filters**

287 PTFE filters performed much worse than regenerated cellulose (RC) filters during syringe filter
288 testing (Fig. 2). ERY losses to PTFE filters were 92 ± 2.8%; losses to RC filters had greater variability
289 (46 ± 19%) but were still significantly less than those to PTFE filters ($p = 0.03$). DCF losses to both filter
290 types were not significantly different ($p = 0.09$), but GEM losses to PTFE filters were again greater than
291 those to RC filters ($p = 0.002$). All losses to filters greatly exceed those to pipette tips, excepting DCF
292 loss to RC filters (0.2 ± 5.8%). These results further demonstrate that the use of specific lab supplies may
293 have a non-trivial impact on pharmaceutical quantification.



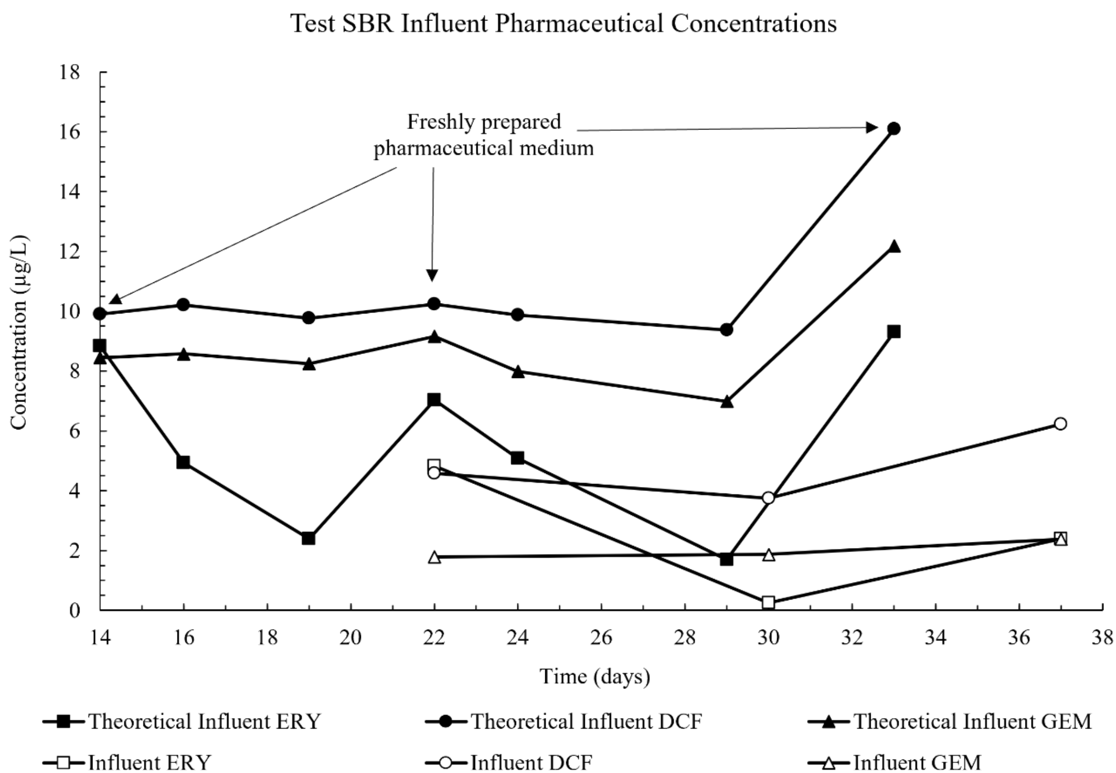
294
295 **Fig. 2** Pharmaceutical losses after flushing 1.5 mL through RC or PTFE syringe filters. Regenerated
296 cellulose filters far outperformed PTFE filters, as indicated by much smaller losses to these filters
297

298 **3.2 Pharmaceutical dosing into AGS sequencing batch reactor**

299 Fig. 3 shows the influent and "theoretical influent" concentrations into the test sequencing batch
300 reactor (SBR). Theoretical concentrations were calculated by measuring the concentration of each
301 pharmaceutical in the pharmaceutical medium, and then multiplying it by the influent dilution factor of
302 0.014. For all pharmaceuticals, theoretical influent (TI) concentrations are much greater than actual
303 influent concentrations, which indicates a loss of each compound somewhere in the influent tubing to the
304 reactor.

305 Both DCF and GEM TI concentrations are relatively constant for the test period, excepting day
306 33, which may be an outlier; this may indicate that sorption of DCF and GEM to the Tygon tubing
307 submerged in the pharmaceutical medium was minimal. Most sorption of these compounds likely
308 occurred to platinum-cured silicone ("silicone") tubing located past the feedstock bottle. Actual influent
309 concentrations of DCF and GEM were consistently 2.5 and 4.7 times lower than TI concentrations,
310 respectively.

311 Conversely, TI ERY concentrations decreased over time and recovered each time fresh
312 pharmaceutical medium was prepared, indicating ERY sorption to the Tygon tubing in the pharmaceutical
313 medium. ERY also adsorbed to silicone tubing located past the feedstock bottle, as evidenced by up to
314 seven times lower ERY concentrations in the actual influent.
315



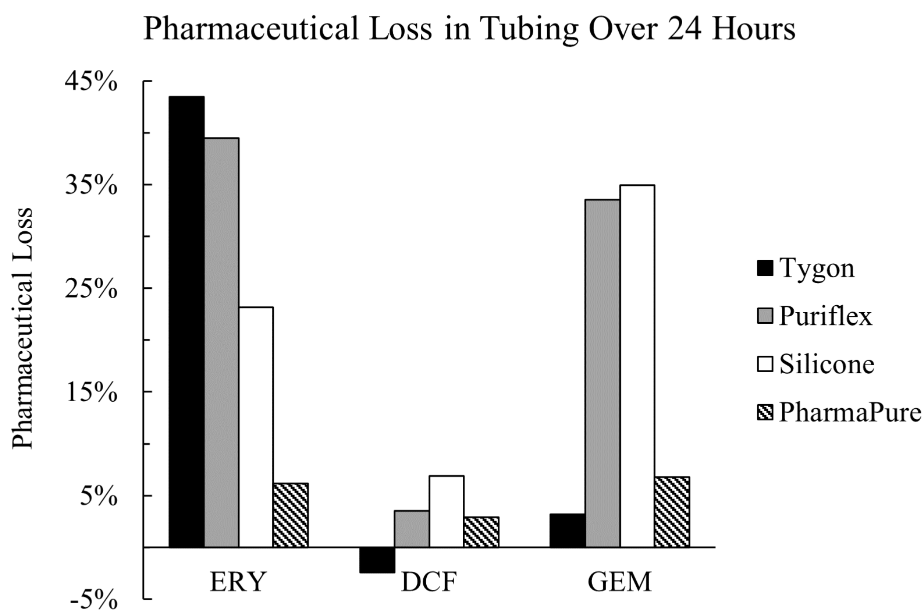
316

317 **Fig. 3** Theoretical influent and actual influent pharmaceutical concentrations to the test AGS SBR.
318 Theoretical influent concentrations (solid lines) were calculated by measuring pharmaceutical
319 concentrations in the feed medium and then multiplying those by a dilution factor. Theoretical influent
320 concentrations should equal actual influent concentrations (dashed lines); the discrepancies shown here
321 indicate losses of all three compounds to the influent tubing to the reactor

322

323 **3.2.1 Sorption to tubing**

324 Pharmaceutical losses to different tubing types in short-term tests are shown in Fig. 4. Loss of
325 each pharmaceutical to the silicone tubing roughly imitated actual influent results shown in Fig. 3: DCF
326 loss was smallest, followed by ERY, then GEM. Likewise, pharmaceutical losses to Tygon tubing
327 followed the pattern seen in theoretical influent samples in Fig. 3, with large ERY losses, near zero DCF
328 losses, and fairly small GEM losses. PharmaPure tubing performed best of the tubing types tested, with
329 only 6, 3, and 7% of ERY, DCF, and GEM lost from influent to effluent over 24 hours, respectively.
330 Again, losses to pipette tips were generally less than those to tubing.
331



332

333 **Fig. 4** Pharmaceutical losses to different brands of size 14 tubing after 24 hours of constant exposure to
334 705 μg/L pharmaceutical solution. Losses to PharmaPure tubing were generally smallest and most
335 consistent of the four tubing types tested, as ERY, DCF, and GEM losses were all under 7%
336

337 Fig. 5 shows results from a longer-term test with PharmaPure tubing. In this test, the influent end
338 of each tubing replicate was submerged in pharmaceutical medium until day seven, after which PTFE
339 tubing was connected to the influent end and submerged instead. Immediately after this change, effluent
340 GEM concentrations increased to near equal influent ones. GEM may have adsorbed preferentially to the

outer, uncoated portion of the submerged tubing, causing lower concentrations at the tubing-liquid interface to enter the tubing, despite higher concentrations in the bulk influent liquid. Upon removing the tubing from the liquid, higher bulk GEM concentrations were then able to enter and exit the tubing, unchanged. This may have occurred due to the different surface properties of PTFE and PharmaPure tubing—in addition to chemical resistance, PTFE is known to be generally smoother and less porous than other materials (McKeen, 2012), and therefore may have provided less surface area for GEM sorption than the outer surface of the PharmaPure tubing.

DCF sorption was minimal throughout testing, with effluent concentrations consistently not statistically different from influent ones, except for the initial exposure on day zero ($p = 0.13$-0.3).

Likewise, despite declining ERY concentrations throughout the entire test, influent and effluent ERY concentrations were not significantly different at any time point ($p = 0.37$-0.94). This indicates that, from day zero to seven, ERY sorption was occurring solely to the outer, uncoated portion of the PharmaPure tubing, because additional losses to the inner portion would have resulted in different influent and effluent concentrations. Sorption was also great enough to decrease bulk influent liquid concentrations, not just those at the liquid-tubing interface. Interestingly, connecting PTFE tubing to the influent end of the PharmaPure tubing did not stop ERY loss. ERY concentrations continued to decline, albeit at a slightly lower rate, from day eight to 11. The reasons for this are unknown—ERY may have adsorbed to the PTFE tubing. Degradation products were not detected in any samples, and therefore losses were likely abiotic. Results from this test also highlight the importance of testing materials under lab-relevant conditions: the existing study of pharmaceutical sorption to tubing soaked small pieces of tubing in pharmaceutical solution, and therefore was unable to distinguish if sorption occurred to the inside or outside of tubing types (Hebig et al., 2014).

Although the residence time of pharmaceutical solution in the size 13 PharmaPure tubing was approximately three times longer than that of the size 14 tubing, and the concentration of pharmaceutical solution tested was much higher, similar loss trends in both short- and long-term tests were observed when PTFE tubing was submerged. DCF and GEM losses were near zero from day eight to 11. However,

367 although influent and effluent ERY concentrations were equal on days eight and 11, approximately 54%
368 of ERY was lost from the influent on day eight to the effluent at day 11. It is possible that the increased
369 residence time, combined with higher ERY concentrations, resulted in greater ERY sorption to the
370 submerged PTFE tubing, which may explain why ERY concentrations continued to decline. Altogether,
371 results suggest that DCF and GEM sorption to tubing is not highly concentration- or residence time-
372 dependent, but ERY sorption likely is. It is also worth noting that, although submerged PTFE tubing
373 appeared to partially mitigate pharmaceutical losses, losses to PTFE syringe filters were high. This may
374 be due to the increased surface area of the membrane within each syringe filter.

375 It is difficult to conclude why certain tubing types sorb more pharmaceuticals than others. The
376 surface roughness and pore size distribution of each tubing material are likely to have a large impact on
377 the sorptive properties of each (Yang et al., 2019); however, these data are not publicly available from
378 Masterflex. Different functional groups on the tubing material itself may have also impacted sorption
379 (Yang et al., 2019), though this data is also unavailable. Furthermore, the chemical properties of the
380 pharmaceuticals tested do not always correlate with observed sorptive behaviors. For example, GEM has
381 the highest K_{ow} and lowest molecular weight of the pharmaceuticals tested (Table 1), which should
382 translate to a higher affinity for carbonaceous materials and an ability to penetrate pores more easily in
383 each tubing type (Kose, 2010), resulting in greater sorption. However, GEM losses to tubing were not
384 consistently higher than those of other pharmaceuticals. Similarly, ERY has the largest molecular weight
385 and lowest K_{ow} of the compounds tested (Table 1), but long-term losses to PharmaPure tubing far
386 exceeded those of DCF or GEM (Fig. 5). Altogether, these results indicate that review of materials'
387 chemical and physical properties alone may not be sufficient for rigorous experimental designs—testing
388 is necessary to confirm compatibility.
389

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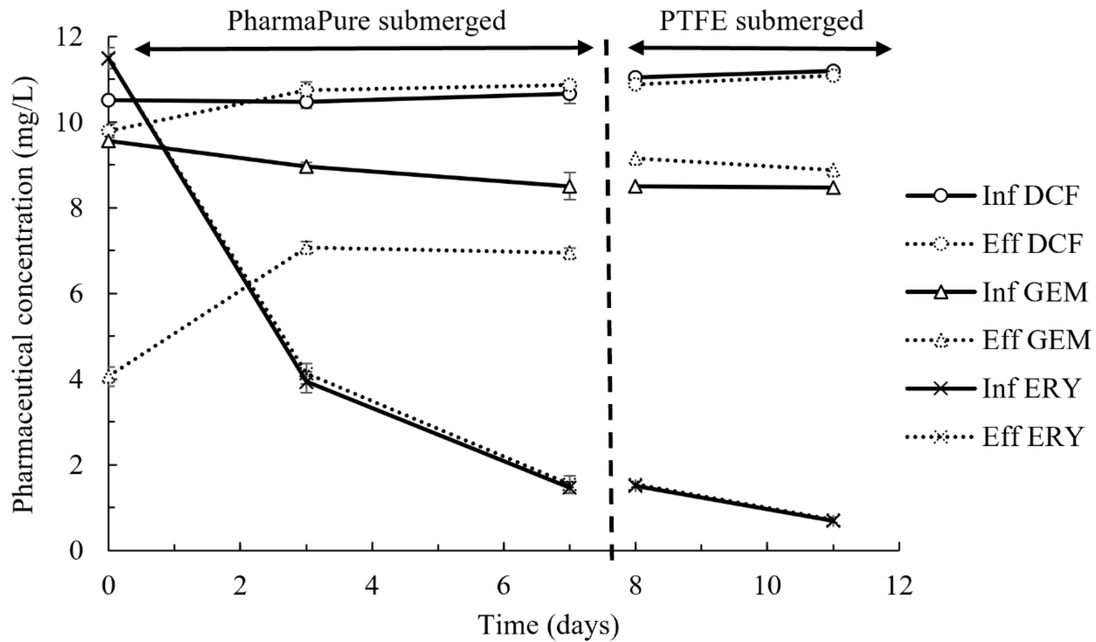

391 **Fig. 5** Results of a longer-term pharmaceutical exposure test with size 13 PharmaPure tubing and 12.5
392 mg/L pharmaceutical solution. Error bars represent standard deviation of triplicate samples and are at
393 times smaller than points. DCF losses were negligible over time and GEM losses stopped after PTFE
394 tubing was submerged in the influent pharmaceutical solution. ERY losses were largest when PharmaPure
395 tubing was submerged, but continued to decline slightly after PTFE tubing was submerged in the influent
396 solution

397

398 ## 4. Conclusions

399 ERY, DCF, and GEM losses due to adsorption to various tubing materials, brands of 1 mL pipette
400 tips, and syringe filter materials were quantified and were significant. Depending on the pharmaceutical
401 and the material, losses were at times over 90%. In particular, pharmaceutical sorption to the influent
402 tubing to a test AGS reactor greatly reduced the concentrations of pharmaceuticals dosed.

403 Results from these tests show that failure to consider pharmaceutical adsorption to various lab
404 supplies may result in overestimation of pharmaceutical removal rates in bioreactors, as well as

underestimation of pharmaceuticals in environmental samples. This issue is particularly problematic in cases where pharmaceutical removal is attributed solely to adsorption to biological materials, as is often the case in experiments with aerobic granular sludge: many studies assume that decreasing aqueous concentrations of the tested compounds are due to adsorption to AGS (Amorim et al., 2016; Kent & Tay, 2019; Kong et al., 2015; Margot et al., 2015; Wang et al., 2018; Yu et al., 2020; Zhao et al., 2015). However, results from the tests discussed herein indicate that it is essential to first confirm that various lab materials do not significantly sorb the dosed contaminants—otherwise, concluding that AGS, or other biological materials, are the sorptive medium may be inaccurate.

5. Declarations

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The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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