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Removal of trace organics by MBR treatment: the role of molecular properties

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1 **Removal of trace organics by MBR treatment: the**
2 **role of molecular properties**

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14 **Abstract**

15 This study examined the relationship between specific molecular features of trace organic
16 contaminants and their removal efficiencies by a laboratory scale membrane bioreactor (MBR).
17 Removal efficiencies of 40 trace organic compounds were assessed under stable operating
18 conditions. The reported results demonstrate an apparent correlation between chemical structures
19 and the removal of trace organic contaminants by the laboratory scale MBR system. The removal
20 of all 14 very hydrophobic trace organic compounds ($\text{Log } D > 3.2$) selected in this study was
21 consistently high and was above 85%. The occurrence and types of electron withdrawing or
22 donating functional groups appears to be another important factor governing their removal by
23 MBR treatment. In this study, all hydrophilic and moderately hydrophobic ($\text{Log } D < 3.2$)
24 compounds possessing strong electron withdrawing functional groups showed removal
25 efficiency of less than 20%. In contrast, high removal efficiencies were observed with most
26 compounds bearing electron donating functional groups such as hydroxyl and primary amine
27 groups. A qualitative framework for the assessment of trace organic removal by MBR treatment
28 was proposed to provide further insights into the removal mechanisms of trace organic
29 contaminants by MBR treatment.

30 *Keywords:* membrane bioreactor (MBR), trace organic contaminants, sorption, biodegradation
31 molecular structure, hydrophobicity.

32

33 **1 Introduction**

34 Major driving forces toward water recycling today are the growing demand for water from an
35 increasing population, changing lifestyle patterns, urbanisation, and diminishing natural water
36 resources. In addition, better public awareness about environmental protection has resulted in
37 progressively more stringent wastewater quality discharge regulations. Despite the growing
38 interest in water recycling, our predictive capacity regarding the ability of treatment technologies
39 to remove specific trace organic contaminants remains very limited. This is reflected by the
40 public reluctance to accept reclaimed water for potable reuse and the fact that most water
41 recycling applications are currently still restricted to non-potable purposes.

42 Membrane bioreactors (MBRs) have recently emerged as an important technology for water
43 recycling, capable of transforming wastewater to high quality effluent suitable for various water
44 recycling applications (Atkinson, 2006). Becoming commercially available only around two
45 decades ago, MBR technology has already been well proven and can provide a superior rating
46 for most bulk water quality indicators such as pathogens, suspended solids and nutrient removal
47 compared to conventional activated sludge (CAS) treatment processes (Melin et al., 2006;
48 Visvanathan et al., 2000). However, the efficiency of MBR technology as a barrier for a range of
49 trace organic contaminants such as endocrine disrupting chemicals (EDCs), pesticides, and
50 pharmaceutically active compounds (PhACs), as well as the specific removal mechanisms
51 involved remain unclear (Clara et al., 2005; De Wever et al., 2007; Kimura et al., 2005; Qu et al.,
52 2009; Visvanathan et al., 2005; Wintgens et al., 2004). Previous studies have indicated
53 significant variation in the removal of trace organics by MBRs, ranging from near complete
54 removal for some compounds (e.g. ibuprofen and bezafibrate) to almost no removal for several
55 others (e.g. carbamazepine and diclofenac) (Clara et al., 2005; Kimura et al., 2005; Tadkaew et
56 al., 2010; Urase et al., 2005). The reasons for such variation are not yet fully understood.

57 Physicochemical properties of trace organics have been reported to significantly govern the
58 removal efficiency by MBR treatment. Biosorption of trace contaminants driven primarily by
59 hydrophobic interaction appears to be one of the key mechanisms controlling removal efficiency
60 in MBR. For instance, apparent improvement in removal efficiency of certain acidic trace
61 organics such as ibuprofen, ketoprofen, and diclofenac has been observed when MBRs are
62 operated under acidic conditions rather than neutral conditions (Tadkaew et al., 2010; Urase et

63 al., 2005). This phenomenon was explained by the speciation of the compounds from hydrophilic
64 ionic forms to much more hydrophobic forms at pH lower than their pK_a values.

65 A limited number of studies has shed some light on the effect of chemical structures on the
66 removal efficiency of trace chemicals during biological treatment processes. For example,
67 Kimura *et al.*, (2005) attributed the poor removal of clofibric acid, diclofenac, and dichloprop to
68 the presence of chlorine in their molecular structure or their relatively complicated aromatic
69 rings. Several studies have utilised the US-EPA-developed Biodegradation Probability Program
70 for Windows (BIOWIN) software package which is one of the most widely used quantitative
71 structure biodegradability relationship (QSBR) computer-based programs to estimate the
72 biodegradability of organic compounds under aerobic conditions. Lapertot and Pulgarin
73 investigated the biodegradability of 17 priority hazardous substances and suggested that the
74 primary and ultimate BIOWIN models were generally suitable for removal assessment of these
75 compounds in industrial wastewater treatment processes (Lapertot and Pulgarin, 2006). On the
76 other hand, Yu *et al.*, (2006) reported some inconsistency between the likelihood of
77 biodegradability predicted by BIOWIN and experimental data when they investigated the
78 removal efficiency of 18 pharmaceutical and personal care products at a conventional municipal
79 wastewater treatment plant (Yu *et al.*, 2006).

80 Although the connection between chemical structure and removal efficiency seems highly
81 plausible, studies to develop a capacity to predict the removal efficiency of trace organic
82 contaminants by MBR treatment processes based on a range of molecular parameters are still
83 limited. Because of the involvement of the many diverse and complex functional groups, the
84 connection between chemical structure and removal efficiency has not yet been thoroughly
85 examined in the literature. In fact, several previous attempts to identify a definitive relationship
86 between the structures of trace organic contaminants and their removal efficiencies during CAS
87 and MBR treatment have been unsuccessful (Joss *et al.*, 2005; Radjenovic *et al.*, 2007).

88 This study aimed to elucidate the connection between specific molecular features of trace
89 organic contaminants and their removal efficiencies by a laboratory scale MBR. The MBR
90 system was operated under stable conditions for an extended period to allow for a systematic
91 examination of the removal of 40 trace organic contaminants at environmentally relevant
92 concentrations. Hydrophobicity and molecular structures of the selected trace organic

93 compounds were carefully delineated and correlated to their removal efficiencies. Key factors
94 governing the removal efficiencies of trace organic contaminants were identified and reported.

95 **2 Materials and methods**

96 *2.1. Laboratory scale MBR system*

97 A laboratory-scale MBR system was used in this study. Detailed description of this MBR system
98 is available elsewhere (Tadkaew et al., 2010). The system consisted of a glass reactor, a
99 continuous mixer, two air pumps, a pressure sensor, and influent and effluent pumps. Two
100 ZeeWeed-1 (ZW-1) submerged hollow fibre ultrafiltration membrane modules supplied by
101 Zenon Environmental (Ontario, Canada) were used in this set-up. The membrane has a nominal
102 pore size of 0.04 μm . Each module has an effective membrane surface area of 0.047 m^2 . A
103 Neslab RTE 7 equipped with a stainless steel heat exchanging coil was used to maintain a
104 constant temperature in the MBR. A personal computer was used to control the permeate
105 peristaltic pump to operate on a 14 minute suction and 1 minute off cycle to provide relaxation
106 time to the membrane modules. Flow rate of the influent pump was matched with that of the
107 permeate pump to maintain a constant reactor volume. The continuous mixer was used to ensure
108 homogeneous conditions of the mixed liquor and to prevent the settling of biomass.

109 *2.2. Synthetic wastewater*

110 A synthetic wastewater simulating municipal sewage was used to ensure a stable feeding rate
111 throughout the experiment. Concentrated stock solution was prepared and stored in a refrigerator
112 at 4 °C. It was then diluted with MilliQ water on a daily basis to make up a feed solution
113 containing glucose (400 mg/L), peptone (75 mg/L), KH_2PO_4 (17.5 mg/L), MgSO_4 (17.5 mg/L),
114 FeSO_4 (10 mg/L), and sodium acetate (225 mg/L). This composition was based on a previous
115 study (Zhang et al., 2006).

116 *2.3. Trace organic compounds*

117 In this study, 40 organic compounds were selected to represent four major trace organic groups
118 of concern in water reuse applications – namely pesticides, pharmaceutically active compounds,
119 steroid hormones, and other endocrine disrupting chemicals. The selection of these model trace

120 organic compounds was also based on their widespread occurrence in domestic sewage and their
121 diverse physicochemical properties (e.g. hydrophobicity and molecular weight). The effective
122 hydrophobicity of these compounds varies significantly as reflected by their Log D values at pH
123 8 (see supplementary data) which is typical of an activated sludge reactor (Wells, 2006). The
124 most hydrophilic compound is enalapril with Log D at pH 8 of -1.21 and the most hydrophobic
125 compound is nonylphenol with Log D at pH 8 of 6.19 . All selected trace organic compounds
126 were of analytical grade. A combined stock solution was prepared in pure acetonitrile. The trace
127 organic stock solution was kept in a freezer and was used within less than a month.

128 *2.4. Analytical techniques*

129 The analysis of the model trace organics was based on a previously reported method (Tadkaew et
130 al., 2010; Vanderford and Snyder, 2006). Analytes were extracted using 5 mL, 500 mg solid
131 phase extraction hydrophilic/lipophilic balance (HLB) cartridges (Waters, Millford, MA, USA).
132 Samples were spiked with a solution containing 50 ng of an isotopically labeled version of each
133 analyte. The sample was then loaded onto the cartridges at 15 mL/min, after which the cartridges
134 were rinsed with 5 mL of reagent water and dried with a stream of nitrogen for 30 min. Loaded
135 cartridges were stored at $-4\text{ }^{\circ}\text{C}$ in sealed bags until elution and analysis.

136 Analytes were separated using an Agilent (Palo Alto, CA, USA) 1200 series high performance
137 liquid chromatography (HPLC) system equipped with a 150 x 4.6 mm, 5 μm particle size, Luna
138 C18 (2) column (Phenomenex, Torrence CA, USA). Mass spectrometry was performed using an
139 API 4000 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA, USA)
140 equipped with a turbo-V ion source employed in both positive and negative electro-spray modes.
141 Steroid hormones were analysed using an atmospheric pressure chemical ionisation method and
142 all other compounds were analysed using an electro-spray ionisation method. For each analyte
143 and internal standard a precursor ion and two product ions were monitored for reliable
144 confirmation. Relative retention times of the analyte and isotopically labeled internal standard
145 were also monitored to ensure correct identification (Vanderford and Snyder, 2006).

146 Standard solutions of all analytes were prepared at 1, 5, 10, 50, 100, 500 and 1000 ng/mL. A
147 relative response ratio of analyte/internal standard over a 1 – 1000 ng concentration range was
148 generated enabling quantification with correction for losses due to ion suppression and

149 incomplete SPE recovery. All calibration curves had a correlation coefficient of 0.99 or better.
150 The limit of reporting was determined using an s/n ratio of greater than 10.

151 Conductivity and pH were measured using an Orion 4-Star Plus pH/conductivity meter. Total
152 organic carbon (TOC) and total nitrogen (TN) were analysed using a Shimadzu TOC/TN-V_{CSH}
153 analyser. TOC analysis was conducted in non-purgeable organic carbon (NPOC) mode. Samples
154 were kept at 4°C until analysed and calibrations were performed in the range between 0 and 1000
155 mg/L and 0 to 100 mg/L for TOC and TN, respectively. Mixed liquor suspended solid (MLSS)
156 and mixed liquor volatile suspended solid (MLVSS) contents in the MBR were measured in
157 accordance to the Standard Methods for the Examination of Water and Wastewater (Clescerl et
158 al., 2005).

159 *2.5. MBR experimental protocol*

160 The MBR was seeded with activated sludge from the Wollongong sewage treatment plant, NSW,
161 Australia. After the initial start-up process, which lasted about 2 months, a small amount of
162 sludge was regularly extracted from the reactor to keep the sludge age at approximately 70 days.
163 The hydraulic retention time was set at 24 hours, corresponding to a permeate flux of 4.3 L/m²h.
164 The MBR temperature and dissolved oxygen content were kept constant at 20.0±0.1°C and 2±1
165 mg/L, respectively. Performance of the MBR system with regard to basic water quality
166 parameters was then monitored for an extended period of more than four weeks.

167 Once stable operation had been achieved, trace organic contaminants were continuously
168 introduced into the feed solution to make up a concentration of approximately 2 µg/L of each
169 selected compound. The investigation with trace organics was over a period of four weeks during
170 which no sludge was withdrawn from the reactor. The feed solution was kept in a stainless steel
171 reservoir at controlled room temperature (20±2 °C). Feed and permeate samples were taken twice
172 a week in duplicate and solid phase extraction was conducted immediately for subsequent trace
173 organic analysis. Removal efficiency was calculated as $R = 100 \times (1 - C_{Eff} / C_{Inf})$, where C_{Eff} and
174 C_{Inf} are effluent (permeate) and influent concentrations (ng/L) of the trace organic compound,
175 respectively. It is noted that complete degradation of an organic compound may follow different
176 pathways and undergo several steps. Therefore, the term removal here does not necessarily
177 indicate complete degradation of the trace organics, but rather a loss of the specific trace

178 chemical molecule. In many cases, stable intermediates or ‘metabolites’ may be produced, but
179 detailed consideration of these intermediates is beyond the scope of the current study.

180 **3 Results and discussion**

181 *3.1. Performance stability of the MBR*

182 In this study, synthetic feed solution was used to ensure a consistent influent composition. The
183 MBR showed stable and good performance with respect to all key water quality parameters. The
184 stable performance continued even following the introduction of the trace organic contaminants
185 to the feed solution. A notable exception, however, was a significant decline in the removal of
186 total nitrogen (TN) immediately after the introduction of the trace organic contaminants from
187 almost complete removal to as low as 60%. The decrease in TN removal can be explained by the
188 introduction of acetonitrile, the solvent used to introduce the trace organics, to the influent. The
189 MBR system used in this study was operated under aerobic conditions and therefore is not
190 expected to have any biological denitrification capacity. The synthetic feed solution was
191 deficient in nitrogen, and therefore, the initial high TN removal observed here could be attributed
192 to the conversion of dissolved organic nitrogen to biomass, which would then be retained by the
193 membrane. Because acetonitrile was used as a carrying solvent for the introduction of the trace
194 organic contaminant cocktail into the feed solution, the introduction of trace organic
195 contaminants into the feed solution resulted in a significant increase in TN in the influent from
196 12 mg/L to approximately 49.5 mg/L. This was assumed to be the main reason for the observed
197 decrease in TN removal. The increase in nitrogen content of the feed water did not exert any
198 discernible impact on any other biological performance indicators of the MBR system. There
199 was a slight increase in the MLSS content in the reactor from 8.6 g/L to 10.0 g/L over the
200 duration of the experiment of approximately one month while the MLVSS/MLSS ratio remained
201 constant at approximately 0.9. Other basic performance parameters including TOC removal
202 efficiency (98%), pH of the MLSS (7.5 ± 0.1), effluent conductivity (559 ± 19 $\mu\text{S}/\text{cm}$) were also
203 relatively stable during the entire experiment. In addition, no abnormal transmembrane pressure
204 increase was observed following the introduction of the trace contaminants to the feed solution
205 (data not shown).

206 Stable performance of the MBR system could also be observed with respect to the removal of
207 trace organic contaminants (Figure 1). It is noted that the error bars shown in Figure 1 represent
208 the standard deviations of eight influent and effluent samples, regularly collected in replicate
209 throughout the experiment. It is also notable that the removal efficiencies of the 40 compounds
210 investigated in this study vary significantly ranging from negligible removal (e.g.: atrazine,
211 carbamazepine, dilatin, and trimethoprim) to removal to below the analytical detection limit
212 (e.g.: 17 β -estradiol, testosterone, and triclocarban), indicating a removal of at least 98%. The
213 observed significant variation in the removal efficiency of the trace organic contaminants by
214 MBR treatment indicates that improved understanding of the key factors that govern the
215 elimination of specific chemicals is required to enable prediction of MBR treatment performance
216 for any particular chemical or class of chemicals.

217 [FIGURE 1]

218 3.2. *Removal of trace organic contaminants*

219 A logical approach to qualitatively predict the effectiveness of MBR treatment for the removal of
220 a wide range of trace organic contaminants is to evaluate their removal efficiency according to
221 the intended applications or origins of these compounds. Accordingly, Table 1 summarises the
222 removal efficiencies of the 40 compounds selected in this study. Data previously reported in
223 other studies, whenever available, are also included for comparison purposes. With caffeine
224 being the only noteworthy exception, results reported here are in good agreement with the
225 literature data. The mean removal efficiency of caffeine observed in our study is 49.6 %, which
226 is substantially lower than the previously reported values (Kim et al., 2007; Snyder et al., 2007).
227 In a recent study, Santos et al., (2009) examined the performance of four CAS wastewater
228 treatment plants in Seville city (Spain). They reported a highly variable caffeine removal
229 efficiency among these four treatment plants with the mean value ranging from as low as 44% up
230 to 75% (Santos et al., 2009). Given the similarity between MBR and CAS treatment, it is
231 possible that this discrepancy can be explained by the differences in operating conditions. [The](#)
232 [literature data presented in Table 1 are from a range of sources with different operating](#)
233 [conditions and system arrangements. The reported experimental results confirm that the MBR](#)
234 [system used in this study behaved well within the range of typical performance data from other](#)
235 [systems. Therefore, the results presented in this study and the conclusion drawn from them](#)

236 would be broadly applicable and generalisable to most typical MBR systems. In fact, data
237 presented in Table 1 suggest that some generalisation can be made about certain groups of
238 compounds.

239 **[TABLE 1]**

240 All the three pesticides investigated in this study showed very low removal efficiencies.
241 Atrazine, a chloro-triazine herbicide, was removed at a rate of less than 5%. It has been reported
242 to be poorly removed both in CAS and MBR (Bernhard et al., 2006) and that a major removal
243 mechanism was sorption onto withdrawn sludge (Bouju et al., 2008). Linuron is a dichloro-
244 phenylurea herbicide. Despite being a widely used herbicide, no reports on the removal of
245 Linuron in CAS or MBR could be found. However, its slow natural attenuation rate in various
246 soils and the evolution of more toxic and persistent chloroaniline intermediates in the process
247 have been reported (Dejonghe et al., 2003). A mean removal of 21% of linuron as achieved in
248 our MBR, therefore, appears to be consistent with the reported recalcitrance of this compound.
249 DEET is a toluamide compound and is the most common active ingredient in insect repellants. In
250 this study, a mean removal of 4.6% of DEET was recorded during MBR treatment. This removal
251 efficiency is at the lower end of range reported in other published studies. Bernhard et al., (2006)
252 reported nil to over 50% removal of DEET by MBR treatment and suggested that DEET removal
253 efficiency was dependent on the sludge retention time. Kim et al. (Kim et al., 2007) reported no
254 removal of DEET in their study; however, no information about the SRT was provided. The
255 highest removal efficiency of DEET of 78% was reported by Snyder et al., calculated from a one
256 off sampling event at a pilot scale treatment facility (Snyder et al., 2007).

257 Near complete removal or removal to below the analytical limit of all eight steroid hormones and
258 three other EDCs selected for investigation (bisphenol A, nonylphenol, and t-octylphenol) were
259 observed in this study. These results are consistent with other published studies (Table 1). It is
260 noteworthy that all of these compounds possess significant hydrophobicity and bear a similar
261 molecular backbone structure; which may, in part, explain the similarities of their removal
262 efficiencies.

263 No generalisation can be inferred for any of the six therapeutic classes of pharmaceuticals
264 investigated in this study (Table 1). Their removal efficiencies by MBR treatment vary widely
265 even within the same class of compounds. The removal efficiencies of the five non-steroidal

266 anti-inflammatory drugs (NSAIDs) differ remarkably from one another. For example, ibuprofen
267 registers a removal efficiency of 97% whereas the removal efficiency of diclofenac is only 17%.
268 Unlike the other NSAIDs, diclofenac is a chlorinated compound, which can possibly explain its
269 recalcitrant behavior in MBR treatment. Significant variation in the removal efficiency can also
270 be observed among compounds used as anti-depressants and mood stabilizers. Dilantin,
271 primidone and carbamazepine were poorly removed, whereas the removal efficiencies of
272 clozapine, risperidone, and amitriptyline were 85% and higher. Given the considerable
273 dissimilarity in the molecular structure among these anti-depressants and mood stabilizers,
274 differences in their removal efficiencies can be expected. Further analysis of the molecular
275 structures of these compounds is presented in section 3.3.2. Significant variation in removal
276 efficiency was observed among the other pharmaceutical groups (cardiovascular and other drugs)
277 and can again be attributed to their diverse molecular structures (Table 1 and supplementary data
278 1). Among the hypolipidemic agents (lipid lowering drugs) investigated in this study, simvastatin
279 is a hydrophobic compound with Log D (at pH 8) of 4.41 and the compound registers a removal
280 efficiency of 98% (Table 1). Simvastatin hydroxyacid) shares the same molecular backbone
281 structure with that of simvastatin. However, the 3, 5-dihydroxy-heptanoic acid functional group
282 of simvastatin hydroxyacid renders the compound much more hydrophilic (Log D at pH 8 of
283 0.64). Consequently, simvastatin hydroxyacid shows a much lower removal efficiency of 60% in
284 comparison to that of the related compound simvastatin.

285 Results reported in Table 1 suggest that the classification of trace organics according to their
286 intended use or origin can only be used to qualitatively predict the removal efficiencies of
287 compounds of similar molecular structure, having similar molecular features or physicochemical
288 properties. In fact, certain molecular features and physicochemical properties of the trace organic
289 contaminants appear to be the underlying factors governing their rate of removal during MBR
290 treatment.

291 *3.3. Role of molecular features*

292 Attempts to fit the removal efficiency data obtained in our study and the corresponding available
293 biodegradability scores from BIOWIN model did not result in any meaningful correlations (data
294 not shown). Although this result is somewhat surprising, it does not necessarily invalidate the
295 model. BIOWIN is essentially a statistical model and the discrepancies may have arisen to some

296 extent due to the fact that the BIOWIN scores were derived from batch tests, which cannot
297 effectively replicate the biological conditions of an MBR. It is also noteworthy that only three
298 out of 40 compounds investigated in this study were included in the database which has been
299 used for the development of BIOWIN. Furthermore, BIOWIN would not account for the
300 adsorption of trace organics to biosolids which can be an important removal mechanism along
301 with biodegradation. Given the poor correlation between the removal efficiencies experimentally
302 obtained in this study and the BIOWIN biodegradability scores, it is necessary to further
303 examine the key physicochemical properties and molecular features that can govern the removal
304 efficiency of trace organic compounds.

305 *3.3.1 Effects of hydrophobicity*

306 The removal of trace organic contaminants by an activated sludge treatment process is a complex
307 function of both sorption and biological degradation. In a CAS treatment process, the sludge-
308 bound contaminants can be subsequently removed via sludge withdrawal. In addition, sorption of
309 trace organic contaminants to biosolids results in a longer residence time in the reactor, which
310 may lead to further removal via biodegradation. Because the MLSS content and sludge retention
311 time of typical MBR processes are much higher than those of CAS treatment, sorption has been
312 suggested as a major removal mechanism for the removal of trace organic contaminants by MBR
313 treatment. In a systematic survey of the literature data, Wells suggested that the sorption of a
314 trace organic contaminant to the activated sludge could be assessed by considering the Log D
315 value of the compound at a given pH (Wells, 2006). Experimental results presented in Figure 2
316 indicate that this finding can be extended to MBR treatment. There appears to be a ‘removal
317 envelop’ that can be defined by the hydrophobicity of the trace organic contaminants (Figure 2).
318 Removal of the very hydrophobic (Log D > 3.2) compounds is probably dominated by sorption
319 to the activated sludge facilitating enhanced biological degradation in some cases. Therefore,
320 these compounds consistently showed high removal efficiency (above 85%). As the Log D value
321 of the compounds decreased to below 3.2, sorption of these trace organic contaminants onto the
322 activated sludge was no longer a dominating removal mechanism and the removal efficiency of
323 these compounds is much more strongly influenced by their intrinsic biodegradability. As a
324 result, the removal efficiency of trace organics with low Log D values (at pH 8) varies
325 significantly from less than 20% to removal to below the analytical detection limit
326 (corresponding to a removal of at least 98%). Of particular note in Figure 2 is a cluster of five

327 compounds that show very low removal efficiencies despite their moderately high
328 hydrophobicity (Log D in the range from 2 to 3.2). It is also noteworthy that all five compounds
329 possess one or several electron withdrawing functional groups, such as a chlorine atom or amide
330 group. Results reported here suggest that individual molecular features can also be an important
331 factor governing the removal efficiency of trace organics during MBR treatment.

332 [FIGURE 2]

333 3.3.2 *Effects of molecular weight*

334 The molecular weights of the trace organics studied here ranged from 151 g/mol (paracetamol) to
335 455 g/mol (verapamil). There appears to be a weak but nevertheless discernible correlation
336 between the removal efficiency of these trace organics and their molecular weights (Figure 3).
337 Compounds with molecular weight of more than 300 g/mol were relatively well removed
338 (>60%), while the removal efficiencies those with molecular weight of less than 300 g/mol
339 varied from almost no removal to more than 98% (removal beyond the analytical detection
340 limit). A plausible explanation for this observation could be the relative hydrophobicity (log D at
341 pH 8 in the range from 2.03 to 5.74, see supplementary data) of the compounds having molecular
342 weight of more than 300 g/mol. In addition, in this study, removal efficiency does not necessarily
343 represent a complete mineralisation of the compound. Compounds with higher molecular weight
344 may have more branches, which would offer more opportunities for the microbes to selectively
345 cleave a certain target site and initiate degradation.

346 [FIGURE 3]

347 3.3.3 *Effect of chemical structure*

348 Experimental results obtained in this study confirm the possible role of molecular functional
349 groups in governing the removal of moderately hydrophobic and hydrophilic trace organic
350 compounds by MBR treatment. The 40 trace organic compounds investigated in this study can
351 be systematically categorized into three groups. Group A consists of compounds with Log D at
352 pH 8 of above 3.2. As discussed above, sorption was a dominant removal mechanism for these
353 hydrophobic compounds and the removal efficiencies of all compounds of group A were above
354 85% (Figure 2). To further elucidate the role of different molecular features, the rest of the
355 compounds can be categorised in terms of ring structure (heterocyclic/ non-heterocyclic, mono
356 or polynuclear) and functional groups (electron withdrawing/donating moieties). Figure 4 shows

357 the removal efficiency as a function of ring structure, whereas Figure 5 presents the compounds
358 under three distinct categories (B, C and C*) based on the presence and types of electron
359 withdrawing or donating functional groups.

360 **[FIGURE 4]**

361 **[FIGURE 5]**

362 No clear distinction between heterocyclic or non-heterocyclic compounds removal could be
363 observed in this study (Figure 4). Similarly, no discernible trend in terms of mononuclear or
364 polynuclear compound could be observed. It is generally considered that simple aliphatic and
365 monocyclic aromatic compounds are readily degradable, while polycyclic structures may be
366 more persistent (Jones et al., 2005). However, irrespective of the mono or polynuclear structure,
367 degradation can initiate by the mere cleavage of a side chain structure and then further
368 mineralisation may depend on the complexity of the nucleus. In this study, removal indicates the
369 loss of the parent structure, and not complete mineralisation. Therefore, the absence of any
370 discernible correlation between the removal efficiency and ring structure is not entirely
371 unexpected.

372 As shown in Figure 4, the compounds containing strong electron withdrawing groups (B)
373 consistently showed very low (<20%) removal efficiency. According to Knackmuss
374 (Knackmuss, 1996), the initial electrophilic attack by oxygenases of aerobic bacteria is often a
375 rate-limiting step and the first of a chain of reactions responsible for the biodegradation of many
376 organic compounds. As a result, the presence of electron withdrawing functional groups
377 generates an electron deficiency and thus renders the compounds less susceptible to oxidative
378 catabolism. Electron donating functional groups, on the other hand, render the molecules more
379 prone to electrophilic attack by oxygenases of aerobic bacteria. Consequently, the removal
380 efficiencies of organic compounds bearing strong electron donating functional groups were, in
381 most cases, much higher than those of group B in Figure 4. The removal of the compounds
382 containing both electron withdrawing and donating groups however showing less than 70%
383 removal have been placed in group C*.

384 The elucidation of the overall influence of these functional groups and particularly their
385 opposing effects on the biodegradability of trace organic compounds is a complex task and
386 would generally require extensive exercise involving simultaneous application of quantitative

387 structure activity relationship and biochemical interpretation, as demonstrated for a particular
388 compound class (N-heterocycles) by Philipp et al. (2007) . Because a large number of diverse
389 compound classes were studied here, such a rigorous approach falls beyond the scope of this
390 paper. Nevertheless some general inference, can be drawn from the results in the light of
391 metabolic pathway information retrieved from the sparse literature and also from biodegradation
392 prediction tools such as UM-BBD PPS (Wackett and Ellis, 1999).

393 The biodegradation of amide-only compounds needs to proceed from conversion of the amide
394 group to primary amine (Hart and Orr, 1975). As suggested by the low removal of
395 carbamazepine and dilantin, this pathway appears to be extraordinarily recalcitrant. All the tested
396 compounds possessing only methyl (weak electron donor) and amide (strong electron
397 withdrawing) groups including primidone, DEET and meprobamate were very poorly removed.
398 The presence of methyl groups means that the degradation could initiate from conversion of the
399 methyl group to alcohol (Shaw and Harayama, 1992), bypassing the recalcitrant amide
400 conversion. However, methyl and other aliphatic groups have very weak electron donating
401 capacity, and thus in presence of a strong electron withdrawing group they may have limited
402 activating effect.

403 All three compounds (i.e. atenolol, enalapril and caffeine) containing both the amine (strong
404 electron donating) and the amide (strong electron withdrawing) functional groups were quite
405 well removed (50-97%). Degradation of compounds with amine group may proceed by
406 converting the existing amine to a less substituted form of amine and aldehyde/ketone (Hakil et
407 al., 1998). Comparing the performance of these three compounds (containing amide and amine
408 groups) with that of primidone, DEET and meprobamate (containing amide and methyl groups),
409 it appears that the co-existence of the amine, and not the methyl group, with the amide group
410 may make these compounds more amenable to biodegradation. The excellent removal of another
411 amide-containing compound paracetamol can be attributed to the presence of the hydroxyl group
412 which is also a strong electron donating functional group. In this context, it is noteworthy that the
413 entire set of hydroxyl group-containing compounds tested in this study showed high removal.
414 Such positive impact of hydroxyl group on biodegradation is in line with the literature reports
415 (Tunkel et al., 2000).

416 Halogenated organics comprise a superset which has many antimicrobial as well as human toxic
417 and carcinogenic industrial chemicals as members (Hägglom and Bossert, 2004). Linuron
418 contains both halogen and amide groups and accordingly demonstrated low removal.
419 Interestingly, of the halogenated compounds with amine group, risperidone (containing amine,
420 methyl and amide) and hydroxyzine (containing amine and hydroxyl) showed good removal
421 while diclofenac (containing amine and carboxylic) and atrazine (containing amine and methyl)
422 showed poor removal. Literature review regarding the metabolic pathways of these compounds
423 provided further insights but could not resolve the paradox. It is suggested in the literature that
424 the metabolism of hydroxyzine can proceed simultaneously through the conversion of amine to
425 aldehyde/ketone or through oxidation of the alcohol moiety to a carboxylic acid (Campoli-
426 Richards et al., 1990). In case of risperidone the degradation may initiate via 9-hydroxylation
427 and/or via N-dealkylation at the piperadine nitrogen (Mannens et al., 1993). Diclofenac has been
428 suggested to be degraded by hydroxylation of the 1-amino-2-unsubstituted aromatic fragment
429 (Marco-Urrea et al., 2010). The degradation of atrazine, on the other hand, has been reported to
430 be initiated through N-monodealkylation, hydroxylation of the isopropyl or tert-butyl moiety
431 (Lang et al., 1996) or in the rare case via oxidation of the s-triazine ring to hydroxy-s-triazine
432 (De Souza et al., 1995). While it is certain that the aerobic oxidation of the halogenated
433 compounds initiate from the co-existing electron withdrawing groups and not via
434 dehalogentaion, it is not clear why despite seemingly similar metabolic pathways (e.g.,
435 hydroxylation, dealkylation) the compounds exhibit different extents of recalcitrance.

436 Notably hydroxylation of the vicinal unsubstituted aromatic fragment and the mono-carbon-
437 substituted benzenoid are the predominant initial degradation pathways (Quintana et al., 2005)
438 for the well removed compounds ibuprofen (97%) and ketoprofen (70%), respectively. It is,
439 however, not clear why despite possessing the similar metabolic pathway as ketoprofen,
440 triamterene registered a rather low removal of 28%. The absence of any literature data regarding
441 triamterene removal by CAS or MBR restricts further clarification regarding this matter. The
442 only possible distinction that can be offered at this stage is that triamterene is a heterocyclic
443 compound.

444 It is known that the degradation of compounds with an aromatic-aliphatic ether fragment can
445 proceed by ether cleavage, producing phenol derivative and aldehyde (Bernhardt et al., 1988). Of
446 the tested compounds that fit into this category gemfibrozil (98%) and verapamil (87%) were

447 well removed while omeprazol (62%) and naproxen (40%) demonstrated moderate removal, and
448 trimethoprim was poorly removed (16%). The predominant biodegradation route of naproxen
449 and trimethoprim appears to be via ether cleavage (Quintana et al., 2005); however, the
450 degradation can potentially proceed via conversion of tertiary/secondary aliphatic to
451 corresponding alcohol. On the other hand, in addition to the ether cleavage verapamil may be
452 degraded by N-demethylation (Unadkat et al., 2008). The degradation of omeprazole can also
453 initiate from the conversion of di-[C,O]-substituted sulfoxide to sulfone (Kanazawa et al., 2003),
454 and gemfibrozil can be degraded also through conversion of aromatic methyl to primary alcohol
455 (Hermening et al., 2000). The discrepancy in the removal rates of these compounds may,
456 therefore, be attributed to the distinct alternate routes of biodegradation, which may govern the
457 overall removal.

458 The combined effect of functional groups and hydrophobicity on the removal of trace organic
459 compounds by the MBR is shown in Figure 6. It is evident from the above discussion that all the
460 aspects of chemical structure i.e., aromatic moiety, ring composition, substituent groups, side
461 chain and associated metabolic pathway need to be taken into account in conjunction with
462 physical parameters namely hydrophobicity and molecular weight to explain observed
463 variabilities in trace organic removal by MBR.

464 As noted earlier, in an MBR, adsorption and biodegradation may simultaneously play important
465 roles. However, for the compounds with low hydrophobicity, properties such as molecular
466 weight, ring structure and functional groups may influence the biodegradability and consequently
467 govern the overall removal. Although some similarities can be expected, the purpose of this
468 section is clearly not to describe the biodegradability of trace organics in biological wastewater
469 treatment in general. The comprehensive discussion on biodegradability and metabolic pathway
470 as furnished here serves the important purpose of explaining the removal of compounds with low
471 hydrophobicity in the MBR.

472 [FIGURE 6]

473 3.3.4 *A framework to predict removal efficiency*

474 Notwithstanding a few exceptions which will be subjected to further investigation, results
475 reported in this study indicate a clear link between molecular features and the removal of trace
476 organic compounds by MBR treatment. Figure 7, based on the data presented in this study,

477 outlines a qualitative and schematic framework for the prediction of the removal efficiency of
478 any given compound by an aerobic MBR treatment process. Given the similarities between CAS
479 and MBR treatment, the framework proposed here may also be applicable to CAS treatment
480 processes to some extent. However, differences in operational conditions between MBR and
481 CAS must be carefully considered. For example, because MBR usually operates at a much
482 longer sludge retention time and can offer complete retention of the biomass, hydrophobicity of
483 the trace organic compounds would have a more profound impact on their removal efficiency by
484 MBR than that by CAS. For the compounds with low hydrophobicity, where biodegradability is
485 likely to govern the overall removal, the performance of CAS operated under the same loading
486 and sludge retention time may be comparable to MBR (Clara et al., 2005). However it also needs
487 to be noted that MBR may facilitate growth and maintenance of special degrading microbes (Hai
488 et al., 2010) which may contribute to enhanced removal of compounds with low hydrophobicity.
489 To derive further insight into this matter long-term investigation with the same set of data
490 comparing the performance of CAS and MBR will need to be carried out. That, however, is
491 beyond the scope of this study. It is prudent to note that this proposed framework has been based
492 on a limited set of data of only 40 compounds. Nevertheless, this framework has the potential to
493 provide significant insights to the removal of trace organic contaminants by MBR treatment.
494 With ongoing scientific and dedicated efforts in this field, the framework can be a foundation for
495 a future quantitative model for the prediction of trace organic removal by MBR and CAS
496 treatment.

497 **[FIGURE 7]**

498 **4 Conclusion**

499 Results reported in this study indicate an apparent correlation between molecular features and the
500 removal of trace organic contaminants by a laboratory scale MBR system. The removal
501 efficiencies of all 14 very hydrophobic trace organic compounds ($\text{Log } D \text{ at pH } 8 > 3.2$) selected
502 in this study consistently showed removal efficiencies in the range between 85% to removal to
503 below the analytical detection limit, indicating a removal of at least 98%. The occurrence of
504 electron withdrawing or electron donating functional groups appears to be another important
505 factor governing their removal by MBR treatment. All hydrophilic and moderately hydrophobic
506 ($\text{Log } D < 3.2$) compounds possessing strong electron withdrawing functional groups consistently

507 showed removal efficiency of well below 20%. In contrast, high removal efficiency was
508 observed with most compounds bearing electron donating functional groups such as hydroxyl
509 groups and primary amine groups. Nevertheless, further analysis also revealed several exceptions
510 which remained unexplainable given the current lack of biochemical data about these compounds
511 of interest. Based on the reported data, a qualitative framework for the assessment of trace
512 organics removal by MBR treatment was presented.

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685 **Table 1:** Removal efficiencies of the selected trace organic contaminants (n=16) obtained in this
 686 investigation and corresponding values recorded in the literature.

Class	Compound	This study (%) (Average \pm Std)	Literature (%) (min – max)	References ^a
Pesticides	Atrazine	4.4 \pm 3.7	9 – 40	1,2
	Linuron	21.1 \pm 4.1	not available	
	DEET	4.6 \pm 2.4	0 – 78	1, 3, 4
Non-steroidal anti- inflammatory	Paracetamol	95.1 \pm 3.4	\geq 99	3, 5-7
	Ketoprofen	70.5 \pm 0.8	43.9 – 95	5-6, 8-9
	Naproxen	40.1 \pm 2.8	36 – 91.6	3, 5-7, 9-10
	Ibuprofen	96.7 \pm 0.7	\geq 90	1,3, 5-7, 9, 11-14
	Diclofenac	17.3 \pm 4.2	0 – 87.4	1,3, 5,6, 9, 12, 13, 15, 16
Anti- depressants & mood stabilizers	Clozapine	84.8 \pm 5.4	not available	
	Risperidone	95.8 \pm 2.2	not available	
	Primidone	12.4 \pm 4.3	not available	
	Carbamazepine	13.4 \pm 4.3	0 – 13	1,5, 7, 11, 13, 17
	Dilantin	5.4 \pm 3.6	0 – 12	4
	Amitriptyline	97.8 \pm 0.8	not available	
Antibiotic & antiseptic	Triclosan	>91.8	61 – 95	3, 4
	Triclocarban	>98.4	not available	
	Sulfamethoxazole	91.9 \pm 0.6	52 – 80.8	3,5,6, 11-13, 18
	Trimethoprim	16.6 \pm 3.7	0 – 90	3, 6, 11, 18
Hypolipidemic agents	Simvastatin	97.9 \pm 0.9	not available	
	Gemfibrozil	98.95 \pm 0.1	32.5 – 90	5, 6, 11
	Sim-hydroxyacid	59.6 \pm 2.8	not available	
Cardiovascular drugs	Atenolol	96.9 \pm 0.2	70	5, 9
	Verapamil	88.4 \pm 6.1	not available	
	Enalapril	97.1 \pm 0.1	not available	
Other drugs	Triamterene	27.9 \pm 6.3	not available	
	Hydroxyzine	>92.2	not available	
	Meprobamate	14.5 \pm 3.3	not available	
	Caffeine	49.6 \pm 4.1	98 – 99	3, 4
	Omeprazole	62.1 \pm 3.5	not available	
Steroid hormones	Estrone	98.0 \pm 0.2	96.3	13, 19
	17 β -estradiol	>99.4	100	13, 19
	Androstenedione	>99.5	not available	
	Estriol	98.2 \pm 1.9	>99	13
	Testosterone	>99.4	not available	
	Etiocholanolone	>99.4	not available	
	Androsterone	>99.3	not available	
	17 α -ethynylestradiol	93.5 \pm 1.2	81.9 – 93.6	19
Other EDCs	Bisphenol A	90.4 \pm 3.1	68.9 – 99.0	10, 12, 13, 19, 20
	Nonyphenol	99.3 \pm 0.2	0 – 88	12, 13, 21, 22
	t-octylphenol	94.5 \pm 1.1	44.9 – 99.0	13

687 ^a References: ¹(Bernhard et al., 2006); ²(Bouju et al., 2008); ³(Kim et al., 2007); ⁴(Snyder et al.,
688 2007); ⁵(Radjenovic et al., 2007); ⁶(Radjenovic et al., 2009); ⁷(Joss et al., 2005); ⁸(Kimura et al.,
689 2005); ⁹(Quintana et al., 2005); ¹⁰(Urase et al., 2005); ¹¹(Reif et al., 2008); ¹²(Kreuzinger, 2004);
690 ¹³(Clara et al., 2005); ¹⁴(Smook et al., 2008); ¹⁵(Gonzalez et al., 2006); ¹⁶(Abegglen et al., 2009);
691 ¹⁷(Clara et al., 2004); ¹⁸(Göbel et al., 2007); ¹⁹(Lyko et al., 2005); ²⁰(Chen et al., 2008); ²¹(Cirja
692 et al., 2006); ²²(Hu et al., 2007).
693

694 LIST OF CAPTIONS

695 **Figure 1:** Influent and effluent concentration of the selected trace organic contaminants.
696 Samples were collected twice a week and in duplicate for four weeks. Error bars represent the
697 standard deviation of 16 measurements.

698 **Figure 2:** The relationship of removal of trace organic compounds with effective hydrophobicity
699 (Log D). The MLSS pH during the experiment was 7.5 ± 0.1 . Log D values were obtained from
700 the SciFinder Scholar (ACS) database. Error bars represent the standard deviation of 16
701 measurements.

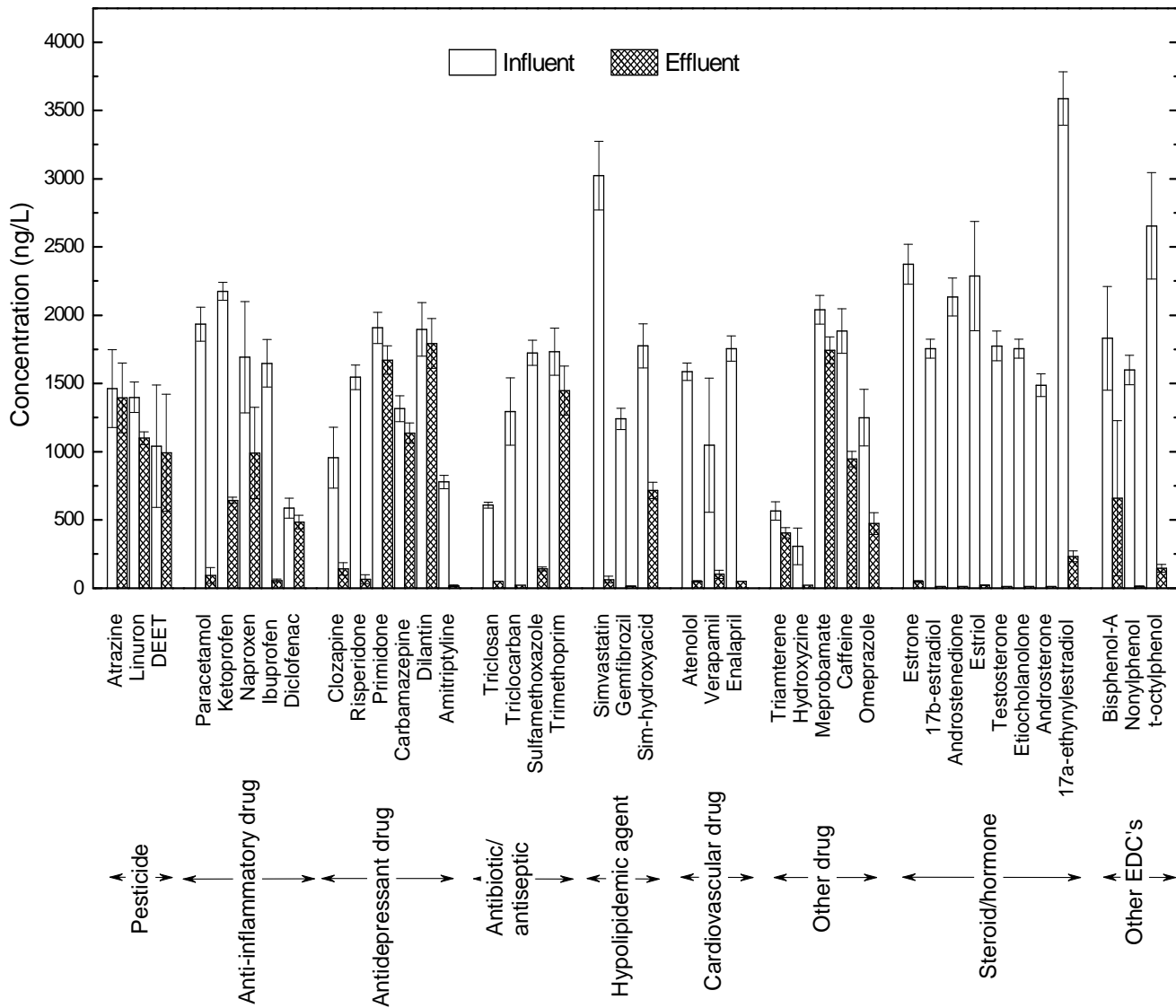
702 **Figure 3:** Removal efficiency of trace organic compounds as a function of their molecular
703 weight. Error bars represent the standard deviation of 16 measurements.

704 **Figure 4:** Removal efficiency as a function of ring structure. Error bars represent the standard
705 deviation of 16 measurements.

706 **Figure 5:** Compound classification according to the presence of electron donating or
707 withdrawing functional groups.

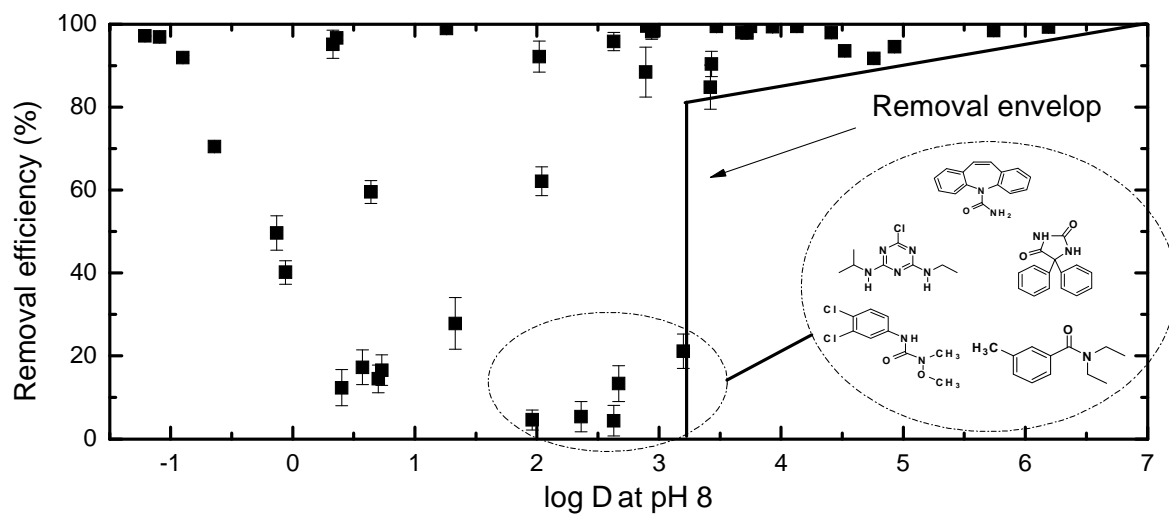
708 **Figure 6:** The combined effects of functional group and hydrophobicity on the removal of trace
709 organic compounds by the MBR. Error bars represent the standard deviation of 16
710 measurements. Group A: all compounds with $\text{Log D} > 3.2$ (at pH 8). Groups B, C, and C* are
711 defined in Figure 5.

712 **Figure 7:** A qualitative framework for the prediction of trace organic removal by MBR
713 treatment.



714

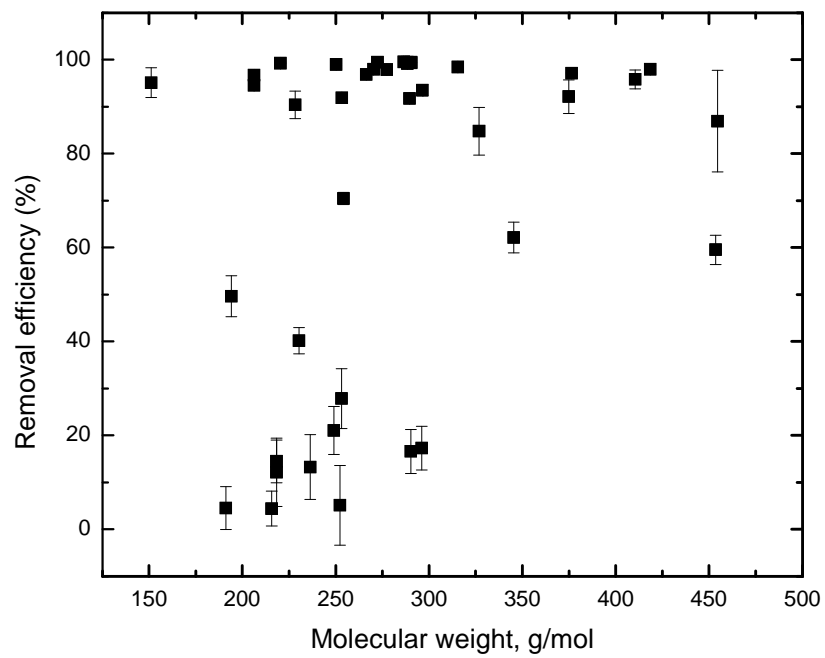
715 **Figure 1**



716

717 **Figure 2**

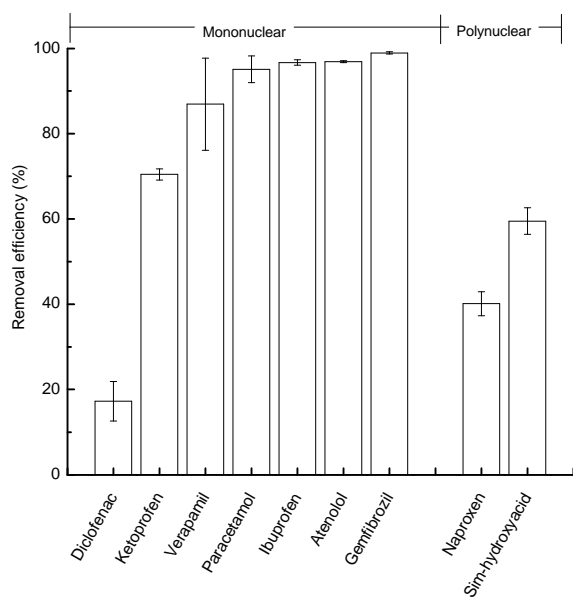
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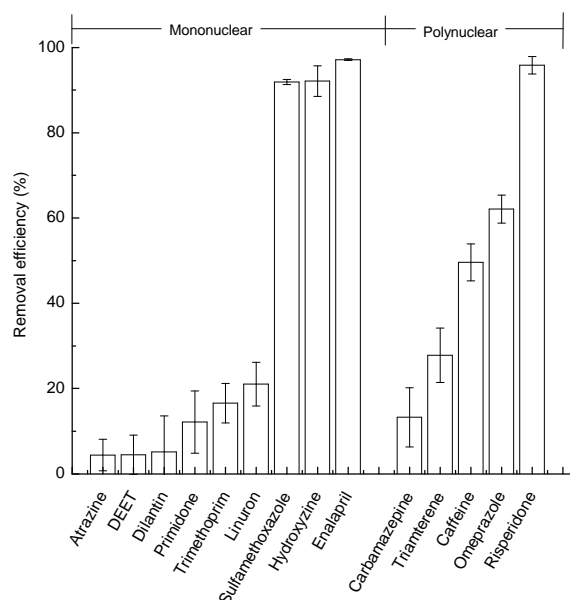
719

720 **Figure 3**

721



Non-heterocyclic compounds



Heterocyclic compounds

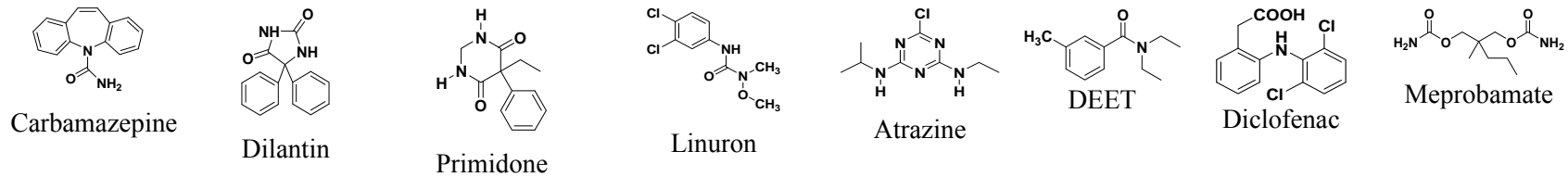
722
723 **Figure 4**

Electron withdrawing groups (EWG)

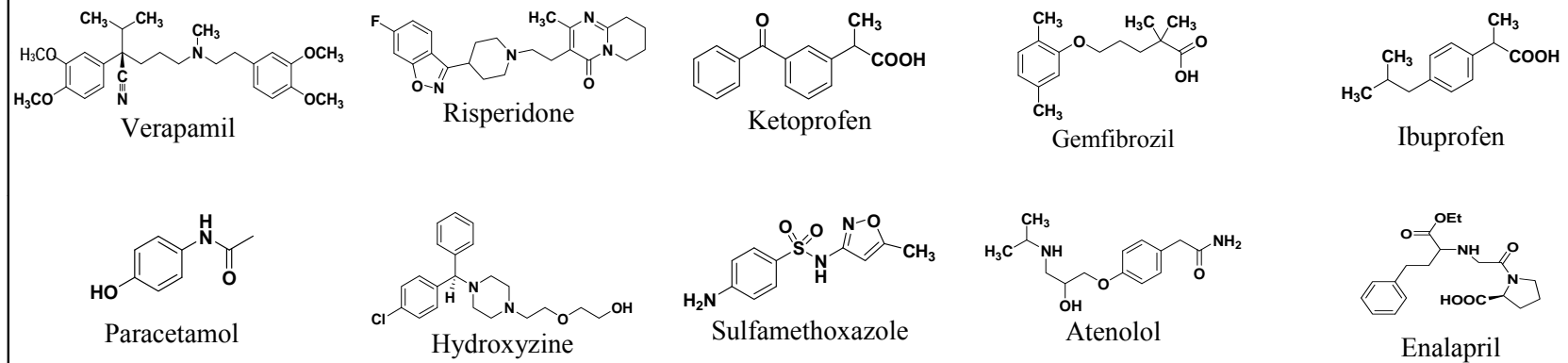
Electron donating groups (EDG)



Group B: Compounds containing strong EWG and showing low removal efficiency



Group C: Compounds containing EDG and showing high removal efficiency



Group C*: Compounds containing both EDG and EWG or only EDG but showing low removal efficiency

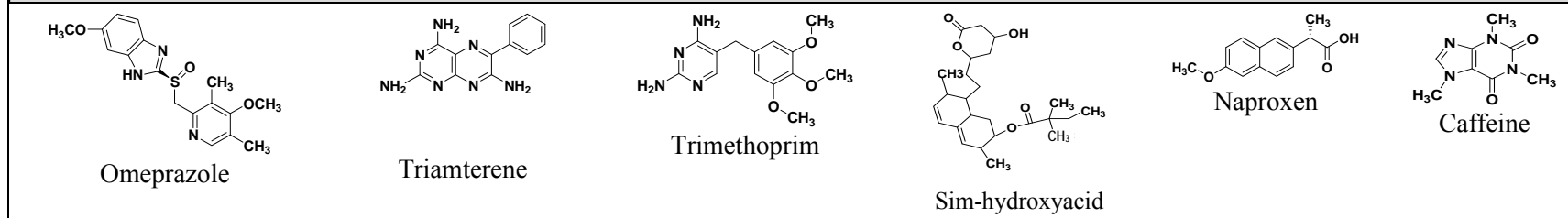


Figure 5

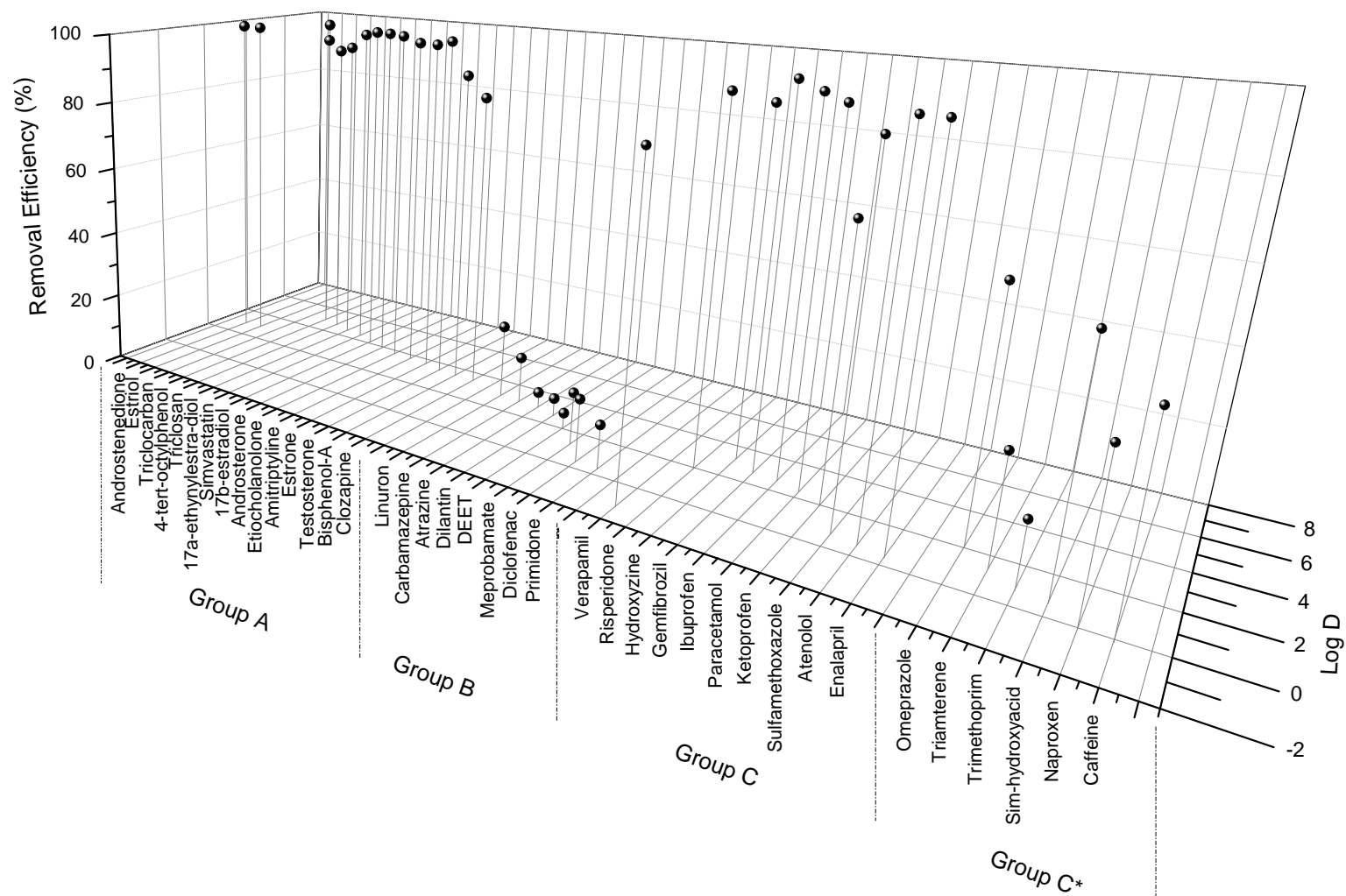


Figure 6

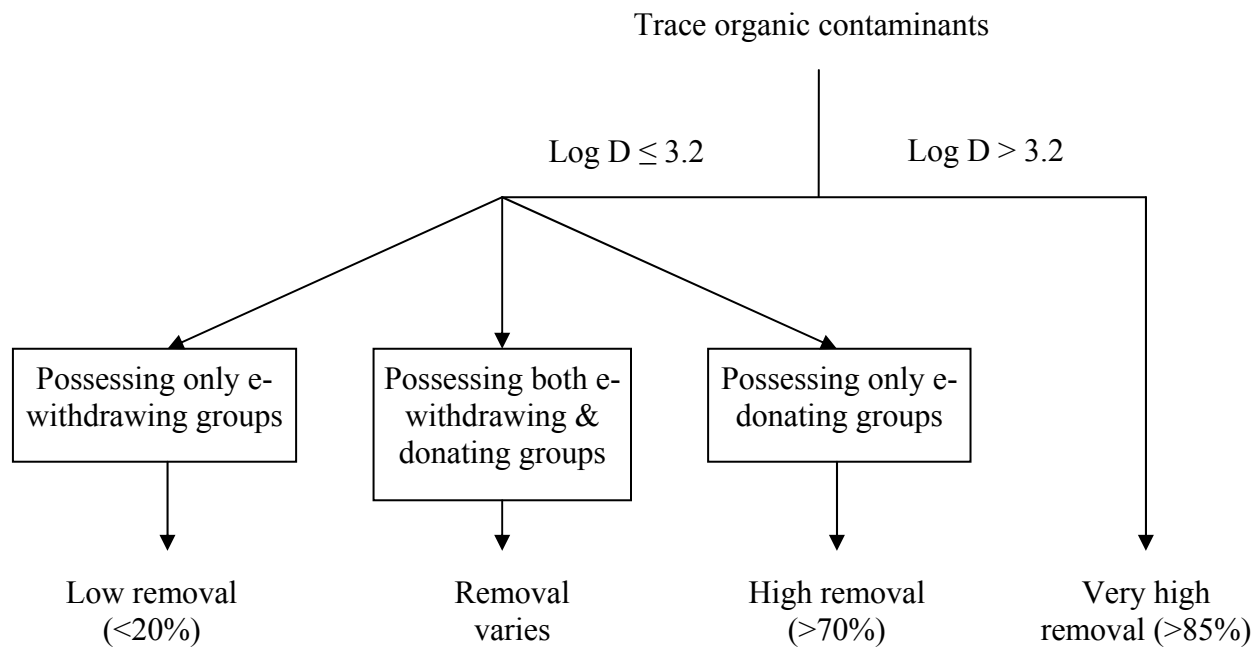


Figure 7

Removal of trace organics by MBR treatment: the role of molecular properties

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Supplementary data

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Supplementary data: Physicochemical properties of the selected trace organic contaminants.

Compound	CAS number	Formula	MW (g/mol)	Log K _{ow}	Log D (at pH 8)	pK _a
Paracetamol	103-90-2	C ₈ H ₉ NO ₂	151.2	0.33	0.33	9.86; 1.72
DEET	134-62-3	C ₁₂ H ₁₇ NO	191.3	1.95	1.96	-1.37
Caffeine	58-08-2	C ₈ H ₁₀ N ₄ O ₂	194.2	-0.13	-0.13	0.73
Ibuprofen	15687-27-1	C ₁₃ H ₁₈ O ₂	206.3	3.72	0.36	4.41
t-Octylphenol	140-66-9	C ₁₄ H ₂₂ O	206.3	4.93	4.93	10.15
Atrazine	1912-24-9	C ₈ H ₁₄ ClN ₅	215.7	2.63	2.63	2.35
Meprobamate	57-53-4	C ₉ H ₁₈ N ₂ O ₄	218.3	0.70	0.70	13.09; -1.09
Primidone	125-33-7	C ₁₂ H ₁₄ N ₂ O ₂	218.3	0.40	0.40	12.26; -1.07
Nonylphenol	104-40-5	C ₁₅ H ₂₄ O	220.4	6.19	6.19	10.14
Bisphenol-A	80-05-7	C ₁₅ H ₁₆ O ₂	228.3	3.43	3.43	9.73
Naproxen	22204-53-1	C ₁₄ H ₁₄ O ₃	230.3	3.00	-0.06	4.84
Carbamazepine	298-46-4	C ₁₅ H ₁₂ N ₂ O	236.3	2.67	2.67	13.94; -0.49
Linuron	330-55-2	C ₉ H ₁₀ Cl ₂ N ₂ O ₂	249.1	3.20	3.20	12.13; -1.04
Gemfibrozil	25812-30-0	C ₁₅ H ₂₂ O ₃	250.3	4.39	1.26	4.75
Dilantin	57-41-0	C ₁₅ H ₁₂ N ₂ O ₂	252.3	2.52	2.36	8.33; -2.81
Triamterene	396-01-0	C ₁₂ H ₁₁ N ₇	253.3	1.34	1.33	6.30
Sulfamethoxazole	723-46-6	C ₁₀ H ₁₁ N ₃ O ₃ S	253.3	0.89	-0.9	5.81; 1.39
Ketoprofen	22071-15-4	C ₁₆ H ₁₄ O ₃	254.3	2.81	-0.64	4.23
Atenolol	29122-68-7	C ₁₄ H ₂₂ N ₂ O ₃	266.3	0.10	-1.09	13.88; 9.16
Estrone	53-16-7	C ₁₈ H ₂₂ O ₂	270.4	3.69	3.68	10.25
17β-estradiol	50-28-2	C ₁₈ H ₂₄ O ₂	272.4	4.13	4.13	10.27
Amtriptyline	50-48-6	C ₂₀ H ₂₃ N	277.4	4.92	3.72	9.18
Androstenedione	63-05-8	C ₁₉ H ₂₆ O ₂	286.4	2.90	2.90	8.78
Estriol	50-27-1	C ₁₈ H ₂₄ O ₃	288.4	2.94	2.94	10.25
Testosterone	58-22-0	C ₁₉ H ₂₈ O ₂	288.4	3.47	3.47	15.06
Triclosan	3380-34-5	C ₁₂ H ₇ Cl ₃ O ₂	289.5	5.17	4.76	7.80
Trimethoprim	738-70-5	C ₁₄ H ₁₈ N ₄ O ₃	290.3	0.79	0.73	7.20
Etiocholanolone	53-42-9	C ₁₉ H ₃₀ O ₂	290.4	3.75	3.75	15.13
Androsterone	53-41-8	C ₁₉ H ₃₀ O ₂	290.4	3.93	3.93	15.14
Diclofenac	15307-86-5	C ₁₄ H ₁₁ Cl ₂ NO ₂	296.2	4.06	0.57	4.18; -2.25
17α-ethynylestradiol	57-63-6	C ₂₀ H ₂₄ O ₂	296.4	4.52	4.52	10.24
Triclocarban	101-20-2	C ₁₃ H ₉ Cl ₃ N ₂ O	315.6	5.74	5.74	12.77; -0.34
Clozapine	5786-21-0	C ₁₈ H ₁₉ ClN ₄	326.8	3.48	3.42	7.14
Omeprazole	73590-58-6	C ₁₇ H ₁₉ N ₃ O ₃ S	345.4	2.17	2.04	8.46; 4.72
Hydroxyzine	68-88-2	C ₂₁ H ₂₇ ClN ₂ O ₂	374.9	2.03	2.02	14.41; 6.12
Enalapril	75847-73-3	C ₂₀ H ₂₈ N ₂ O ₅	376.5	2.43	-1.21	3.17; 5.43
Risperidone	106266-06-2	C ₂₃ H ₂₇ FN ₄ O ₂	410.5	2.88	2.63	7.89
Simvastatin	79902-63-9	C ₂₅ H ₃₈ O ₅	418.6	4.41	4.41	13.49
Sim-hydroxy acid	121009-77-6	C ₂₅ H ₄₀ O ₆	436.6	4.05	0.64	4.31
Verapamil	52-53-9	C ₂₇ H ₃₈ N ₂ O ₄	454.6	3.90	2.89	8.97

Source: SciFinder Scholar, data calculated using Advanced Chemistry Development (ACD/Labs) Software V8.14 for Solaris (1994-2007 ACD/Labs).