

Investigating the role of adsorption and biodegradation in the removal of organic micropollutants during biological activated carbon filtration of treated wastewater

M. Rattier, J. Reungoat, W. Gernjak, A. Joss and J. Keller

ABSTRACT

Municipal water recycling may expose humans and the environment to trace organic contaminants. We assessed biological activated carbon (BAC) filtration for removal of organic micropollutants (MPs). Adsorption experiments were carried out in batch reactors containing secondary effluent and new granular activated carbon (GAC) and preloaded BAC media. Results show that BAC has good potential for removal of dissolved organic carbon (40%) and MPs (60–95%). The primary objective was to better understand removal mechanisms of representative MPs at environmentally relevant concentrations. Adsorption and biodegradation of 20 compounds of varying physico-chemical properties were investigated by inhibiting the biomass with azide. Average removal of compounds by adsorption on GAC was $88 \pm 5\%$ with no influence of azide. Average BAC removal was $72 \pm 15\%$, reduced to $59 \pm 20\%$ after azide addition, showing that biological activity is important for MP removal. Comparison of MP removal by BAC and BAC + azide showed a more important impact of the inhibition on the removal of negatively charged compounds. Sustained removal of recalcitrant compounds showed that BAC maintained sorption capacity. These results highlight the advantage of a combination of adsorption and biodegradation as compared to other biofiltration techniques for the long-term attenuation of MPs.

Key words | adsorption, biodegradation, microbial activity, organic micropollutants, wastewater reuse

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INTRODUCTION

Over the past decade, organic contaminants such as endocrine disrupting compounds (EDCs) and pharmaceutically active compounds have been detected in drinking water at very low levels (ng L^{-1} to $\mu\text{g L}^{-1}$) (Kümmerer 2009), suggesting that these compounds resisted removal through wastewater treatment processes. The removal of organic compounds during conventional activated sludge treatment and membrane bioreactors has been shown to be highly variable (Petrovic *et al.* 2009), with many of these micropollutants (MPs) reaching the aquatic environment. Under recharge conditions, polar pharmaceuticals, such as clofibrac acid, carbamazepine, primidone, diclofenac and iodinated contrast agents, can leach through the subsoil

and have also been measured in groundwater samples (Heberer 2002). Whether long-term exposure to trace concentrations is of any public health significance is currently unknown. Nevertheless, the simple fact that they have been observed and reported is a cause of scientific and public concern. The presence of antibiotics in wastewater and their persistence through wastewater treatment processes may contribute to the prevalence of resistance to antibiotics in bacterial species (Baquero *et al.* 2008; Richardson 2009). Reusing treated effluents for non-potable or potable purposes increases the range of human and environmental exposure scenarios to bacteria potentially harbouring antibiotic resistance. The question of mixture

toxicity has also recently gained more and more interest and additive effects are to be expected from mixtures of EDCs (Kümmerer 2009).

Wastewater treatment plants (WWTPs) have been designed to deal primarily with organic matter and nutrients (nitrogen and phosphorous). Residual effluent organic matter (EfOM) is present at concentrations 10^3 – 10^6 times higher than the MPs. Depending mainly on their sorption behaviour and biodegradability, chemicals bind to matter or can be transformed or mineralized. Currently available tertiary treatment aiming at further removal of MPs from wastewater is based on membrane filtration, activated carbon, oxidation, managed aquifer recharge and slow sand filtration. Activated carbon adsorption is very effective for MP removal, but is limited by its adsorption capacity and has to be renewed regularly or dosed continuously. Equilibrium and kinetic studies have shown that background organic matter (expressed in terms of dissolved organic carbon, DOC) present in ground and surface waters can reduce adsorption capacity and kinetics of MPs on granular activated carbon (GAC) (Ternes *et al.* 2002; Yu *et al.* 2009) shortening adsorbent bed service life (Knappe *et al.* 1999; Yu *et al.* 2009). For example, it was reported that when filtering a source of drinking water (3.5–5 mg_{DOC}/L) with GAC filters, the adsorption capacity of DOC was exhausted after filtering 5,000 bed volumes (approximately 6 months) (Simpson 2008; Yapsakli & Çeçen 2010). When a layer of dissolved organic matter (DOM) has been adsorbed on the carbon, the carbon becomes negatively charged due to organic matter coverage, with increasing negative charge at higher natural organic matter loading (Newcombe 1994). This may promote electrostatic repulsion and reduce adsorption of natural organic matter and anionic MPs (de Ridder *et al.* 2011). The *n*-octanol–water partition coefficients (K_{ow}) are commonly used to characterize relative lipophilicity and hydrophilicity of a compound. However for ionizable species, the pH dependent *n*-octanol–water distribution coefficient (D_{ow}) is generally considered a more relevant parameter (Katayama *et al.* 2010).

The development of a robust, highly competitive process, with high performance and efficiency, based on the ability of biological materials to accumulate and eventually to transform molecules in effluents by physico-chemical or metabolic reactions, is strongly recommended. The

biological activated carbon (BAC) process would include a sorption step followed by a biodegradation step, depending on the microorganisms' ability to degrade the pollutants (Quintelas *et al.* 2010). Nonetheless, microorganisms colonize macropores and crevices of the carbon grains causing a layer of microbial slime (biofilm) to add an additional mass transfer resistance layer which reduces film diffusion. Biofilms growing in BAC are also a diverse microbial community (Zhang *et al.* 2011) that can participate in the treatment by removing biodegradable dissolved organic carbon (BDOC) (Dussert & Van Stone 1994). Bacterial and fungal cells have no special structures with which to acquire nutrients from their exterior environment and cytoplasmic membranes of bacterial and fungal cells are covered with cell walls. Uptake mechanisms of nutrients and other chemicals by organisms must occur through the cell walls and then through the cell membranes. Thus a concentration gradient between the outside and inside of the cell is the driving force for uptake. The biological membrane of cells is semi-permeable and has pores with diameters much smaller than 4 nm, thus water and dissolved molecules with molecular weights <100 Da (e.g. nitrogen gas) can pass through these pores (Katayama *et al.* 2010). Also, transport into the cell can be carried out by facilitated transport or energy-dependent transport whereby some highly polar compounds can be bound to membrane transport proteins and then transported into the cell (Katayama *et al.* 2010). Previous research demonstrated that the removal of non-diffusible organics in a biofilm reactor required hydrolysis by extracellular enzymes in the bulk water of the reactor (Rohold & Harremoes 1993). Still, passive diffusion is the most important mechanism by which chemicals enter cells (Nikaido 1993). The hydrophobicity of the central layer of cytoplasmic membranes constitutes a strong barrier to ionic, but not non-ionic compounds. Small compounds that are hydrophobic and weakly polar such as alcohols, fatty acids, benzene and non-ionic pesticides can penetrate and dissolve in the membrane's lipid phase (Katayama *et al.* 2010). Removal of MPs by biodegradation was reported previously during sand filtration of secondary effluent (Hollender *et al.* 2009; Reungoat *et al.* 2011), thus it is expected to occur during BAC filtration. Of great practical value would be a classification of important trace organic pollutants by removability during BAC filtration. A previous study on

the removal of cyanotoxins by BAC provided evidence that biodegradation is an efficient removal mechanism once it commences and that the rate of biodegradation was dependent upon temperature and initial bacterial concentration. The active biofilm present on the surface of the media also hindered adsorption of microcystin compared with a sterile GAC with no active biofilm. Up to 70% removal of microcystin-LR was still observed after 6 months of operation of the sterile GAC column, indicating that adsorption still played a vital role in the removal of this toxin (Wang *et al.* 2007). During biological filtration, sorption to the solid phase is the result of various physical, chemical and biological interactions between the sorbate and sorbent (Liu *et al.* 2007). The capability of diverse biological systems to degrade certain MPs has also been well described in WWTPs' biological processes (Onesios *et al.* 2009; Tadkaew *et al.* 2011), and most common biofiltration technologies (Petrovic *et al.* 2009; Rauch-Williams *et al.* 2010; Baumgarten *et al.* 2011).

Only a few studies have been published specifically on the MP removal capability of engineered BAC filters (Kim *et al.* 1997; Gerrity *et al.* 2011; Reungoat *et al.* 2011). Previous pilot scale BAC filters showed high performance in term of DOC and MPs (28–68% and >90% respectively) (Reungoat *et al.* 2011). Study of these filters showed that DOC removal had reached a steady state 2 years prior the study (Reungoat *et al.* 2011) indicating that adsorption process is under equilibrium conditions and suggesting that biodegradation is responsible for the removal observed. Clear evidence on the mechanism by which biological activity enhances MPs removal by GAC is currently lacking. Biodegradation and sorption are complementary mechanisms that extend the life of the GAC and delay organic breakthrough (Levine *et al.* 2000; Scharf *et al.* 2010). In this paper, the removal by BAC media of 20 MPs with a broad range of physico-chemical properties (hydrophobicity, charge and molecular weight) is investigated. In addition to biodegradation, the contribution of adsorption to MP removal was evaluated. For better understanding of the removal of EDCs and pharmaceutically active compounds during BAC treatment, the role of adsorption, biodegradation and the role of physico-chemical properties of the compounds with respect to their behaviour during BAC filtration are discussed.

EXPERIMENTAL METHOD

GAC and BAC media

The BAC media used in this study was sampled from a pilot scale BAC filter installed in December 2006 at the South Caboolture Water Reclamation Plant. The pilot plant was described previously (van Leeuwen *et al.* 2003; Reungoat *et al.* 2010). The reclamation plant receives water from a 40,000 equivalent people using a sequencing batch reactor process which achieves partial nutrient removal. The treatment process before filtration on the BAC filter sampled for this study incorporates a biological nutrient removal WWTP, biological denitrification, pre-ozonation, coagulation/flocculation and dissolved air flotation-sand filtration. The BAC media sample was taken from the top 10 cm of a column filtering bed (Acticarb BAC GA1000N supplied by Activated Carbon Technologies Pty Ltd, Australia) with an empty bed contact time of 1 hour and a flow rate of 30 L h⁻¹. The top of the columns are filled with water and compressed oxygen is injected above the filtering bed to ensure a high level of dissolved oxygen (DO) and support biological activity throughout the filtering bed. Over more than 4 years of operation, the bed has filtered a total of approximately 35,000 bed volumes of treated effluent. Observation of the performance of the column was completed during the 9 months preceding the sampling of the biofilter. The DOC levels in the influent of the sampled column were between 5 and 9 mg_{DOC} L⁻¹. Removal of DOC (37 ± 7%) by the biofilter was accompanied by a remarkable DO consumption of 7.3 ± 3 mg_{DO} L⁻¹. At this stage in the BAC filtration process (>35,000 bed volumes filtered), DOC removal efficiencies of 15–45% have been reported previously (Servais *et al.* 1994; Simpson 2008). The influent of the column was refrigerated within the hour following collection and used in batch experiments within the next 24 h. The BAC grains sampled at the same time were kept at ambient temperature in a 1.5 L bottle containing column influent. Characteristics of the fresh GAC media were obtained from the supplier or determined at The University of Queensland and are given in Table 1.

The Acticarb BAC GA1000N is an anthracite-based granular activated carbon and has been steam activated under controlled atmosphere at 850 °C. It has a meso- and micro-pore structure comparable to other coal-based granular

Table 1 | Properties of granular activated carbon packed in the pilot-scale column sampled for this study (Reungoat *et al.* 2011)

Filter	Acticarb BAC GA1000N
Origin	Coal – steam activated
Bulk density (kg m^{-3})	564
Mean 2D equivalent diameter (mm)	1.60
Particle effective size (mm)	0.6
Uniformity coefficient	<1.8
Iodine number (mg g^{-1})	1,050
BET surface area; based on Argon adsorption ($\text{m}^2 \text{g}^{-1}$)	1,146
Total porous volume ($\text{cm}^3 \text{g}^{-1}$)	0.476
Microporous volume < 2 nm ($\text{cm}^3 \text{g}^{-1}$)	0.360
Mesoporous volume 2–50 nm ($\text{cm}^3 \text{g}^{-1}$)	0.116

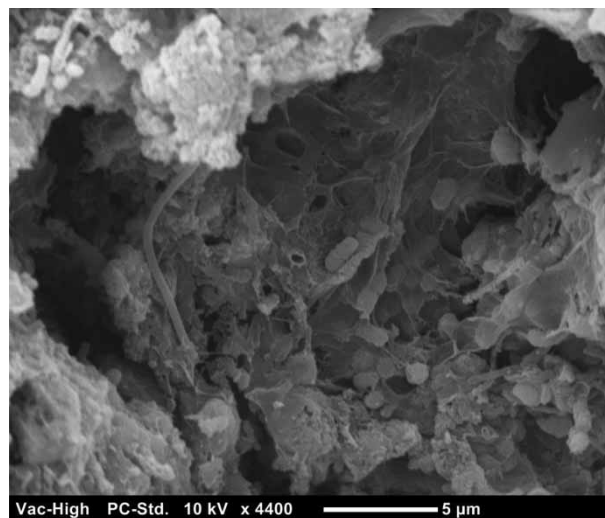
BET: Brunauer–Emmett–Teller.

activated carbon, produced by heat activation (Kalkan *et al.* 2011). The influent MP and DOC concentrations, contact time with the GAC and type of GAC are major parameters influencing the adsorption. Given the amount of bed volumes filtered and consequent preloading, adsorption properties as given in Table 1 are expected to be different for the media used in the batch experiments. Indeed, as the GAC media particles slowly became exhausted, it was observed that the rough porous surface of fixed bed GAC granular media was amenable to microbial colonization (Figure 1).

Particles contain pores of different sizes, many of which are smaller than microorganisms. Therefore, bacteria will be physically excluded from most of the intraparticle pores of these grains (Liu *et al.* 2007) and are assumed to be distributed on the outer surface. Although bacteria themselves are not capable of entering the meso-pores, their ‘exo-enzymes’ can (Scholz & Martin 1997).

Adsorption and biodegradation batch experiments

Batch experiments were conducted in four parallel amber glass reactors by adding 11.5 g of BAC media and 2 L of feed water: $8.25 \text{ mg}_{\text{DOC}} \text{L}^{-1}$ and MP concentrations given in Table 2 and Figures 3 and 4. Reactors were kept for 5 days at room temperature ($23 \pm 1^\circ \text{C}$) and hand shaken twice a day. Hand shaking was chosen over stirring in order to limit the release of attached bacteria and extracellular materials from

**Figure 1** | Scanning electron microscopy of the pore entry of a BAC particle exposed to 35,000 bed volumes of tertiary treated wastewater.

the media surface (Camper *et al.* 1986). Whole carbon grains were used, either new GAC or preloaded BAC. To discriminate between biodegradation and adsorption in BAC, the inhibition of the biomass activity was achieved by adding 300 mg L^{-1} sodium azide in the second reactor (BAC + azide). The azide anion blocks electron transfer and thereby energy production in the cells by inhibition of the synthesis of adenosine triphosphate (ATP), which will stop energy-dependent intracellular activities. It has been used previously in several studies at concentrations ranging between 0.04 and 400 mg L^{-1} (Nishijima & Speitel 2004; Uhl *et al.* 2006; Rossner *et al.* 2009; Yamamoto *et al.* 2009; Lin *et al.* 2010). The two other parallel reactors were set up in the exact same conditions with the exception of the adsorption media used. GAC instead of BAC was added to the reactors in order to verify that sodium azide did not influence adsorption. In order to ensure that conditions are kept aerobic during the batch experiment, the DO concentration was regularly measured in the biologically active batch (BAC reactor). When the DO concentration was below 4 mg L^{-1} in the BAC reactor, every batch was aerated until the liquid phase was saturated with oxygen. Batch experiments are often the first experimental tool to diagnose the nature of the removal phenomenon. However, solution in a batch may exhibit non-ideality, not only because of lateral interactions between adsorbed molecules but also because of non-ideality in the solution (Moreno-Castilla 2004). No MPs were added to the batch solution.

Table 2 | Concentration, and physico-chemical characteristics of the micropollutants found in the secondary effluent used in this study (octanol–water distribution coefficient D_{ow} in $L\ kg^{-1}$)

Compound	$C_{influent}$ ($ng\ L^{-1}$)	Class/action	Molecular weight ($g\ mol^{-1}$)	Charge (pH 7)	$\log D_{ow}$ (pH 7)
2,4-D	93	Herbicide	221.04	Negative	-1.14
Acetaminophen	253	Analgesic and antipyretic agent	151.16	Neutral	0.47
Atenolol	411	Beta-blocker	266.3	Positive	-2.02
Caffeine	110	Stimulant	194.19	Neutral	-0.63
Carbamazepine	660	Anticonvulsant	236.27	Neutral	1.89
Citalopram	105	Antidepressant	324.39	Positive	0.06
Diazinon	112	Organophosphorus insecticide	304.35	Neutral	3.77
Diclofenac	141	NSAID ^a	296.15	Negative	1.28
Doxylamine	158	Sedative	270.37	Positive	0.7
Erythromycin	197	Antibiotic (macrolide)	733.95	Positive	0.81
Gemfibrozil	70	Hypolipidemic agent	250.3	Negative	2.15
Hydrochlorothiazide	461	Diuretic	297.74	Negative	-0.02
Ibuprofen	75	NSAID	206.29	Negative	1.16
Ketoprofen	46	NSAID	254.28	Negative	0.19
Metoprolol	267	Beta-blocker	267.36	Positive	-0.33
Naproxen	157	NSAID	230.27	Negative	0.85
Roxithromycin	108	Antibiotic (macrolide)	837.1	Positive	1.75
Sulfamethoxazole	558	Antibiotic (sulfonamide)	253.28	Neutral	-0.22
Tramadol	1,256	Narcotic analgesic	263.4	Positive	0.03
Venlafaxine	1,100	Antidepressant	277.402	Positive	0.7

^aNon-steroidal anti-inflammatory drug.

Micropollutants studied

Twenty compounds (Table 2) were selected to be representative of trace organic contaminants based on physical properties that are known to affect sorption, molecular weight, ionic state and hydrophobicity. The targeted compounds were also selected from different contaminant classes.

Analytical methods

Samples from the reactors after 5 days of contact time were compared to the feed for performance evaluation. The method for the assessment of MP concentration consisted of solid phase extraction, elution, concentration and analysis by liquid chromatography coupled with tandem mass spectrometry (Reungoat *et al.* 2011). Two hundred millilitre samples were extracted on a Visiprep manifold system (SigmaAldrich, USA) using Oasis HLB cartridges (60 mg, 3 mL) from Waters Corporation

(USA), previously conditioned with 9 mL of methanol and 9 mL of deionized water (HPLC grade). Analyses of non-purgeable organic carbon (NPOC) were completed. The samples were filtered prior to all measurements using 0.45 mm filters, thus the determined NPOC is equivalent to DOC. The removal of a given compound in the experiment stage was reported only when its concentration was above the limit of quantification before and after the experiment.

RESULTS AND DISCUSSION

Dissolved organic carbon removal in GAC and BAC

The DOC removal achieved in different batch experiments is shown in Figure 2. The DOC measurement of one sample was repeated five times and the obtained relative error on the measurement was applied to other samples.

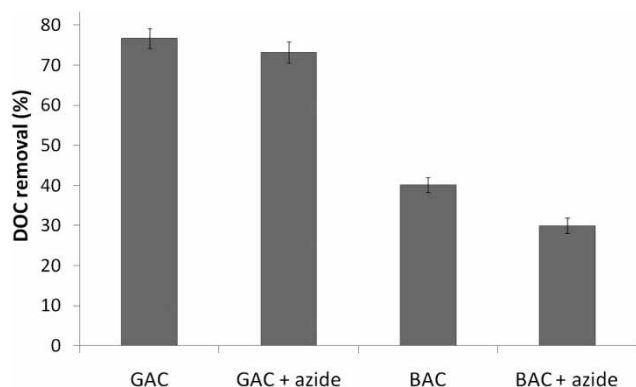


Figure 2 | Percentage dissolved organic carbon (DOC) removal. Bars are the value of a single measurement. Error bars represent the propagated error calculated for the removal value. (The relative variation of DOC measurement was obtained by repeating the sampling and measurement of a single sample five times.)

As expected, performance achieved by adsorption or/and biodegradation by BAC reactors (30–40% DOC removal) was lower than by fresh GAC (75% DOC removal). This is due to the decreased adsorption capacity with EfOM load on adsorption sites of the carbon and potential resulting pore blockage (Newcombe & Drikas 1997; Knappe *et al.* 1999). To investigate the adsorption and biodegradation removal mechanisms in BAC, sodium azide was added to inhibit microbial activity. Respirometry was performed and showed that respiration in the inhibited batch reactor, if occurring at all, was less than 10% of the respiration in the non-inhibited reactor. A more accurate statement is impossible due to the level of precision of the respirometry. Removal of the DOC in the BAC reactors took place accompanied by a greater reduction of DO concentration as compared to the BAC + azide reactor. This strongly suggests that part of the DOC removal in the BAC system is due to biodegradation.

The removal of DOC decreased from 40 to 30% in the presence of inhibitor but it is not clear if adsorption or biodegradation was responsible for most of the removal. Indeed, the mechanisms could not be fully separated by the addition of sodium azide to the system which caused an incomplete blocking of the microbial activity. Sodium azide blocks certain kinds of microorganisms, while others possibly stimulate the alternative routes of energy supply, i.e. bypassing ATP synthesis. Still, the partial inhibition achieved decreased the performance of the BAC system by 10%. This clearly shows that biodegradation plays a role in

the removal of some of the EfOM. The observed advantage in term of DOC removal for the non-inhibited BAC compared to inhibited BAC at the end of the batch test was probably due to direct uptake of BDOC by the biomass.

Micropollutant removal in GAC and influence of sodium azide on adsorption

In the same test, analysed MPs were present in the feed at concentrations below 300 ng L⁻¹ with the exception of atenolol, carbamazepine, sulfamethoxazole, tramadol and venlafaxine (411, 660, 558, 1,256 and 1,100 ng L⁻¹ respectively). Activated carbon was confirmed to have a great capacity to bind compounds with a broad range of physico-chemical properties by adsorption and the removals found were above 80% for the tested compounds as shown in Figure 3.

Adsorption by fresh GAC proved to be an effective removal mechanism of organic MPs in the batch experiments. Practically, the efficiency of activated carbon will mainly rely on its accessible surface area where physical adsorption takes place, on the heteroatom content and the properties of the adsorbates. Adsorption from solution is essentially an exchange process, and hence molecules adsorb not only because they are attracted by solids but also because of their low affinity with the solvent (Moreno-Castilla 2004). Thus, the characteristics of the solution chemistry can also influence the adsorption process (Schreiber *et al.* 2005). It can be noted that the presence of sodium azide in the water did not influence the removal of organic MPs. Hydrophobic effects are the dominant mechanism in activated carbon adsorption of organic compounds (Yoon *et al.* 2003). Octanol–water distribution coefficient, log D_{ow} , is an important physico-chemical parameter that can be used for predicting the tendency of a chemical to partition between water and solids and/or solid organic matter. A general sense is that compounds with a higher log D_{ow} value should have higher sorption affinity on activated carbon. However, the results obtained in the present investigation at ng L⁻¹ concentrations confirmed that this may not be the case in very dilute solutions, as previously observed by Yu *et al.* (2009). The performance and removal mechanisms of activated carbon in natural water are also impacted by EfOM present in the

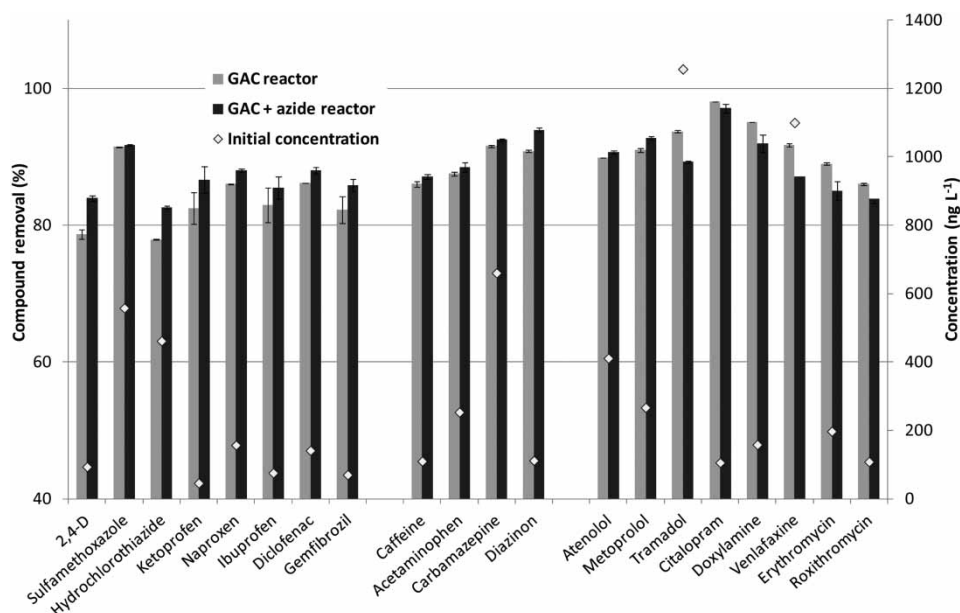


Figure 3 | Removal of micropollutants by GAC and GAC + 300 mg L⁻¹ sodium azide (arranged from left to right in groups of charge; anionic, neutral and cationic and arranged in order of increasing log D_{ow}). Bars represent the mean of duplicates. Error bars represent minimum and maximum removal calculated from duplicates.

water. Also, charge interactions are expected to be limited on fresh carbon (de Ridder *et al.* 2011). No correlation between removal and any of the reported physico-chemical parameters was found by the authors.

Micropollutant removal by BAC

The performance of GAC media is expected to decrease due to accumulation of EfOM and microbial colonization on the media subsequently termed BAC. Removal of target organic MPs by conventional BAC (BAC reactor) and inhibited BAC (BAC + azide reactor) are presented in Figure 4. As expected, loading of the media with organic matter and MPs not only reduced the removal of DOC but also the removal of MPs as compared to GAC. Indeed, limitation of diffusion by the biofilm, competition for adsorption sites with the EfOM and pore constriction by the EfOM and biomass are expected to decrease MP removal (Yu *et al.* 2009; Corwin & Summers 2011; de Ridder *et al.* 2011). Nevertheless, a reduction in the concentration of MPs was achieved by the BAC reactor, with most of the quantified compounds decreased in the liquid phase by more than 60%. Interestingly, compounds such as carbamazepine, citalopram, diclofenac and sulfamethoxazole manifesting resistance to

biological transformation previously (Suarez *et al.* 2010), and persistence through subsoil filtration (Heberer 2002) were removed to a great extent by the BAC.

Whereas the impact of preloading was expected to be more severe for hydrophilic (Snyder *et al.* 2007) or dissociated solutes (Yu *et al.* 2009), no clear correlation was found between log D_{ow} or the charge of different compounds and their removal in the BAC reactor. Average removals for anionic, neutral and cationic compounds in the BAC reactor were 69, 71 and 77%. No correlation between the other physico-chemical properties and the decrease in removal of the different compounds. Also, a diminished but substantial overall removal was achieved by the BAC + azide. Decrease in the removal of several compounds was attributed to the inhibition of biodegradation. For practical purposes, the interpretation of the results to better understand compound behaviour in BAC will be separated in two different groups.

Compounds showing a decrease in removal after inhibition

The compounds in this group are: 2,4-dichlorophenoxyacetic acid (2,4-D), sulfamethoxazole, hydrochlorothiazide,

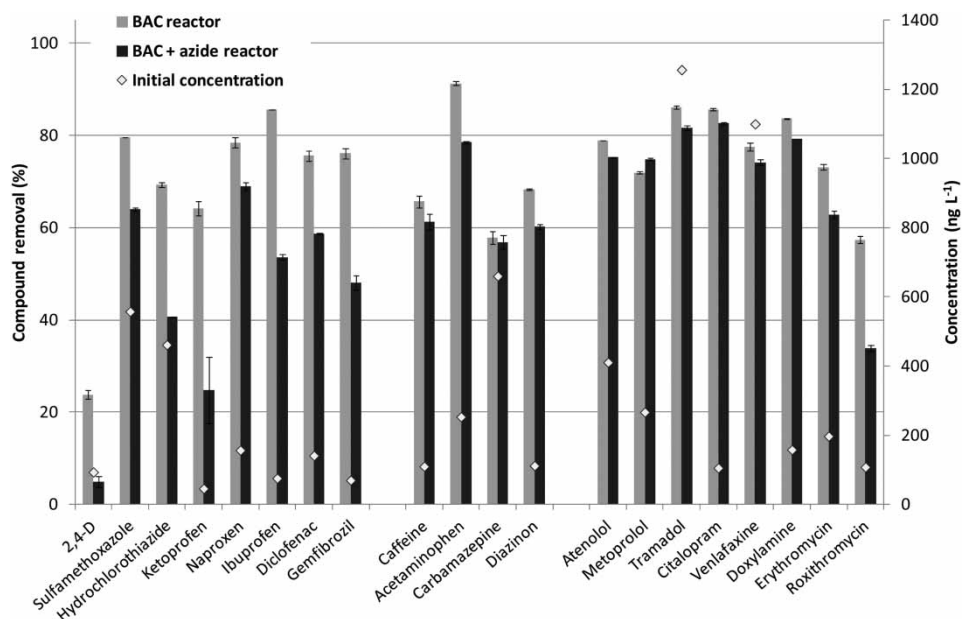


Figure 4 | Removal of micropollutants by BAC and BAC + 300 mg L⁻¹ sodium azide (arranged from left to right in groups of charge; anionic, neutral and cationic and arranged in order of increasing log D_{ow}). Bars represent the mean of duplicates. Error bars represent minimum and maximum removal as calculated from duplicate samples of the liquid phase before and after 5 days of contact time.

ketoprofen, naproxen, ibuprofen, diclofenac, gemfibrozil, acetaminophen, diazinon, erythromycin and roxithromycin.

The herbicide 2,4-D is considered biodegradable since a wide range of soil microorganisms were found to be capable of transforming it (Gaultier *et al.* 2008). Removal by BAC was relatively low and inhibition decreased the removal further to less than 5%. Sorption of 2,4-D by GAC was among the lowest (~80%) compared to other compounds and it is also the negative compound with the lowest log D_{ow} . Microbial degradation of diazinon and other organophosphorus insecticides has been previously observed in the environment (Cycon *et al.* 2009). Both diazinon and 2,4-D removal decreased by 8 and 20% respectively upon biomass inhibition. Diazinon was less impacted as a consequence of a better adsorbability. Indeed, diazinon is neutral and has a high log D_{ow} so it is well adsorbed by solids loaded with organic matter. Similar observation can be made for acetaminophen which was easily degraded and removed by the WWTPs (Heberer 2002) and sand filtration (Reungoat *et al.* 2011). The removal of gemfibrozil, often considered to be very well biodegradable (Drewes *et al.* 2002; Bendz *et al.* 2005; Rauch-Williams *et al.* 2010; Tadkaew *et al.* 2011)

was found to be negatively impacted by the inhibition of the biomass of the tested BAC. Adaptation of the microbial community to the compound's degradation was shown when Lin *et al.* (2006) found that with subsequent additions of ibuprofen and gemfibrozil to river microcosms, degradation of these compounds proceeded more rapidly. It is also interesting to note that removal of acetaminophen and ibuprofen by GAC and BAC were similar in this experiment indicating the important role of biodegradation for these compounds. The removal of roxithromycin was also expected to be lowered by the inhibition of BAC since the compound can be considered biodegradable as it was reduced by 54% during biofiltration on sand filters (Reungoat *et al.* 2011). A number of pilot-scale and field studies have shown significant removals of ibuprofen, ketoprofen and naproxen during BAC filtration (Ray *et al.* 2003; Reungoat *et al.* 2011) or soil passage (Ray *et al.* 2003; Kagle *et al.* 2009; Maeng *et al.* 2011). With the exception of ketoprofen (log D_{ow} = 0.19), these non-steroidal anti-inflammatory drugs have moderately high octanol-water partition coefficients (log D_{ow} > 0.8), and sorption would likely be the main mechanism of their removal by activated carbon. Results of

this study showed that removal of ibuprofen, ketoprofen and naproxen decreased after biomass inhibition, demonstrating potential for biodegradation removal of these compounds. Several studies also demonstrated the biodegradation potential of these compounds in laboratory-scale experiments (Buser *et al.* 1999; Kagle *et al.* 2009). Hydrochlorothiazide was considered recalcitrant to biodegradation since it showed a very low removal (5%) during biological sand filtration (Reungoat *et al.* 2011), but microbial communities can differ between sand and BAC (Yapsakli & Çeçen 2010). Limited removal of hydrochlorothiazide (<44%) by full-scale WWTP was previously observed (Castiglioni *et al.* 2006). Low removal (Maeng *et al.* 2011) and no transformation of diclofenac (Quintana *et al.* 2005) were observed in membrane bioreactors. The poor biodegradation of diclofenac was previously attributed to the presence of chlorine (Kimura *et al.* 2005) and other electron-withdrawing moieties such as a carboxyl group in its molecular structure (Tadkaew *et al.* 2011). The removal of diclofenac, hydrochlorothiazide and sulfamethoxazole decreased significantly after the addition of the inhibitor suggesting biodegradation as a mechanism potentially participating in the removal of these recalcitrant compounds. The porous structure of activated carbon may facilitate growth and maintenance of special degrading microbes which may contribute to enhanced removal of particular compounds. The longer biomass retention time has been suggested to allow for the enrichment of slower growing bacterial species and therefore, to provide greater diversity of enzymes, some of which are capable of breaking down pharmaceutical compounds (Jones *et al.* 2005, 2007). The characteristics of the DOC also need to be considered in the case of biodegradation of relatively recalcitrant compounds. Indeed, Lim *et al.* (2008) observed that contaminants such as sulfamethoxazole were less difficult to remove in BDOC derived from aquatic plants, compared to BDOC derived from wastewater effluent dominated surface water. The variation in biotransformation rates of test compounds in biofilms receiving different sources of BDOC was attributed to the differences in microbial diversity and functionality among the microcosms. This implies that compounds being termed recalcitrant in secondary treatment could be amenable to degradation by the microorganisms

growing on BAC. Co-metabolism of sulfamethoxazole has been previously observed and as much as 20% could be removed (Gauthier *et al.* 2010).

Compounds showing no decrease in removal after inhibition

The compounds in this group are: caffeine, carbamazepine, atenolol, metoprolol, tramadol, citalopram, doxylamine and venlafaxin.

Removal was similar with and without inhibition, suggesting that biological transformation was slow or non-existent toward these compounds. Carbamazepine has previously been shown to be very persistent in the aquatic environment, and its poor biodegradability resulted in low removal (<10%) in most WWTPs (Maeng *et al.* 2011). Drewes *et al.* (2002) found that there was no change in carbamazepine concentrations during soil aquifer treatment for estimated travel times up to 6 years. As such, carbamazepine was expected not to show any variation in removal after adding sodium azide. This result also shows that adsorption still plays an important role in the performance of the studied BAC. Surprisingly, a well-biodegradable compound, caffeine, was also found in this category. Both caffeine and acetaminophen are neutral compounds known to be very well biodegradable but the impact of the inhibition on the removal was more important for acetaminophen. The lower impact of the inhibition of biomass on caffeine removal was attributed to the higher sorption of the compound on the media. Indeed, values of the sorption coefficient (K_d) of acetaminophen and caffeine on river sediments were 5 and 250 L kg⁻¹ respectively (Lin *et al.* 2010) even though hydrophobicity of acetaminophen (as measured by $\log D_{OW}$) is higher than caffeine. This confirms that parameters other than hydrophobicity are relevant to sorption mechanism. Results from the current study also showed no impact of the biomass inhibition on the removal of atenolol, metoprolol, tramadol, citalopram, doxylamine and venlafaxine. This was attributed to the fact that these positively charged compounds were well adsorbed on the biological media due to electrostatic interaction with the negatively charged biofilm. Erythromycin and roxithromycin are exceptions to this, which concurs with them being the least adsorbed positive compounds on

BAC. This might rather be related to their high molecular weight compared to the other MPs (700–800 Da vs 150–300 Da).

It can be seen that the removal was generally more impacted by the inhibition for negatively charged compounds than for neutral and positively charged compounds. Indeed, average removal of anionic, neutral and cationic compounds decreased by $20 \pm 10\%$, $5 \pm 5\%$ and $4 \pm 8\%$ respectively, when the biomass was inhibited. Sorption occurs rapidly compared to biodegradation and is greatly affected by the charge of the compounds. The removal of most positive compounds was similar with and without inhibition meaning that they were primarily removed from the liquid phase by sorption on the negatively charged biofilm and/or the media. The organic contaminants that have a greater sorption affinity especially due to electrostatic interaction with the BAC media are less likely to be biodegraded because the compounds are less likely to be present in the aqueous phase. This does not exclude biodegradation of positive compounds in the longer term, but the present study is unable to determine if biodegradation of positive compounds is occurring.

CONCLUSIONS

The analysis of performance mechanisms of BAC involves the need to determine which fraction of the carbon activity refers to biological processes and which to adsorption. To this end, the inhibitor substance sodium azide was added to the experimental system, but participation of both adsorption and biodegradation mechanisms could not be strictly separated. Removal of MPs by GAC was not influenced by molecular weight, hydrophobicity, charge of the compounds or the presence of sodium azide in the solution. When comparing performance by GAC and BAC + azide, 9–76% lower MP removal was observed for the inhibited BAC. This confirms that the presence of the EfOM preloading and biofilm found on BAC decreased adsorption capacity as compared to new GAC. Results of inhibition also showed that adsorption was still partly responsible for recalcitrant MPs removal in BAC after 4 years' filtering of treated secondary effluent (35,000 bed volumes). Indeed, removal of highly stable compounds such as carbamazepine, citalopram, diclofenac and sulfamethoxazole were not significantly impacted by the

inhibition but a substantial adsorption capacity remained and enabled removals higher than 60%. Also the removal of acetaminophen and ibuprofen by GAC and BAC were similar, confirming that BAC is a filter medium upon which beneficial microbes (biomass, biofilm) develop. The removal of compounds known as relatively more recalcitrant was attributable to either acclimatization (e.g. gemfibrozil) or adsorption (e.g. carbamazepine). These results also confirm the high potential of BAC filtration for the long-term removal of MPs during advanced treatment of WWTP effluents.

Inhibition of the biomass and therefore biological activity diminished the percentage of removal by BAC for these compounds evidencing the importance of biodegradation. In reactors containing BAC, the inhibition of the biodegradation was responsible for the reduction of the removal of DOC and 12 MPs by 10% and between 8 and 39% respectively showing that removal of DOC and MPs are significantly enhanced by the action of microbial biomass in BACs. This effect was observed to be stronger for compounds that are less adsorbed (e.g. 2,4-D) and anionic compounds and some very well degradable neutral compounds. Organic compounds may exist as free constituents in the BAC solution, may be degraded by biological processes or may be sorbed onto BAC grains. These three processes are determinant for the availability of MPs to microorganisms and potentially their biodegradation. However, the current experimental set-up is unable to assess whether biodegradation occurs or not for compounds that are well adsorbed (i.e. positive compounds and most neutral compounds).

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