Micropollutant removal from black water and grey water sludge in a UASB-GAC reactor
A. Butkovskyi, L. Sevenou, R. J. W. Meulepas, L. Hernandez Leal, G. Zeeman and H. H. M. Rijnaarts

ABSTRACT
The effect of granular activated carbon (GAC) addition on the removal of diclofenac, ibuprofen, metoprolol, galaxolide and triclosan in a up-flow anaerobic sludge blanket (UASB) reactor was studied. Prior to the reactor studies, batch experiments indicated that addition of activated carbon to UASB sludge can decrease micropollutant concentrations in both liquid phase and sludge. In continuous experiments, two UASB reactors were operated for 260 days at an HRT of 20 days, using a mixture of source separated black water and sludge from aerobic grey water treatment as influent. GAC (5.7 g per liter of reactor volume) was added to one of the reactors on day 138. No significant difference in COD removal and biogas production between reactors with and without GAC addition was observed. In the presence of GAC, fewer micropollutants were washed out with the effluent and a lower accumulation of micropollutants in sludge and particulate organic matter occurred, which is an advantage in micropollutant emission reduction from wastewater. However, the removal of micropollutants by adding GAC to a UASB reactor would require more activated carbon compared to effluent post-treatment. Additional research is needed to estimate the effect of bioregeneration on the lifetime of activated carbon in a UASB-GAC reactor.

INTRODUCTION
Source separated sanitation allows for maximal recovery and reuse of resources that are present in domestic wastewater, including organic matter, nutrients and fresh water. Resource recovery is possible through separate collection and treatment of more concentrated toilet wastewater (black water) and the rest of the domestic wastewater (grey water) (Otterpohl 2002; Zeeman & Kujaawa-Roeleveld 2011). Black water contains a significant portion of organic matter (38% based on COD) and nutrients (88% of nitrogen and 68% of phosphorus) in a relatively small volume, which facilitates recovery of energy and fertilizers (Kujiawa-Roeleveld et al. 2006). Grey water can be reused after aerobic biological treatment (Ghunmi et al. 2011).

The core technology behind the concept is an up-flow anaerobic sludge blanket (UASB) reactor, used for energy recovery from organic matter of concentrated black water (Zeeman et al. 2008). Sludge from an aerobic bioflocculation system treating grey water can be added to the UASB reactor, thus increasing the overall energy efficiency of the concept (Hernández-Leal et al. 2011b; Tervahauta et al. 2014). A number of persistent organic micropollutants, including pharmaceuticals, estrogens and personal care products (PPCPs) are hardly biodegradable in the reactor under anaerobic conditions (Hernández-Leal et al. 2010; de Graaff et al. 2011; Alvarino et al. 2014). They have a negative impact on the environment, accumulate in tissues of living organisms, cause feminization of male organisms, are carcinogenic, etc. (Boxall et al. 2012) Thus, post-treatment of UASB reactor effluent and appropriate handling of UASB sludge are required prior to their discharge, disposal or reuse.

Sorption to activated carbon is often used as a post-treatment step to remove a wide range of micropollutants, including PPCPs, from effluents of sewage treatment plants (Snyder et al. 2003; Çeçen & Aktaş 2011). Application of powdered activated carbon (PAC) and granular activated carbon (GAC) within source separated sanitation was investigated by Hernandez Leal et al. (2010), who found it suitable
for removal of personal care and household products from aerobically treated grey water (Hernández-Leal et al. 2013).

Direct addition of activated carbon to the reactor is an alternative to the effluent post-treatment. It results in improved removal of refractory organics and lower effluent toxicity (Sublette et al. 1982; Kuai et al. 1998; Tsuno et al. 2006; Gonzalez-Gutierrez & Escamilla-Silva 2009). Activated carbon, added to a bioreactor, not only adsorbs refractory organic compounds present in the influent but has been suggested that carbon stimulates biological activity by concentrating substrates and nutrients within sludge flocs and removing inhibitory substances (Sublette et al. 1982). Removal of micropollutants by activated carbon is highly dependent on background concentrations of dissolved organic carbon, which can significantly reduce the carbon performance, especially in wastewater treatment (Snyder et al. 2007). On the other hand, the carbon surface in biological systems is regenerated through biodegradation of organic substances by microorganisms, increasing carbon lifetime (Aktas & Çeçen 2007).

The aim of this study was to investigate the potential of GAC for enhancing micropollutant removal in a UASB reactor treating source-separated black and grey water sludge from an aerobic biofloculation system. Batch experiments in 100 ml vessels were performed to assess sorption of micropollutants from liquid and solid phases of UASB sludge to GAC. Continuous experiments in a laboratory-scale UASB reactor with GAC addition were also performed to assess behavior of micropollutants in the UASB-GAC system on a long-term (122 days) basis, and the impact of GAC addition on the reactor performance. Addition of GAC to the UASB reactor was compared to post-treatment of the UASB effluent by GAC in terms of micropollutant removal. Micropollutants selected for the study include widely used pharmaceuticals (diclofenac, ibuprofen, metoprolol) and personal care and household products (galaxolide and triclosan).

MATERIALS AND METHODS

Chemicals and GAC

Ibuprofen, diclofenac, metoprolol-tartrate, fenoprofen and tonalide-d3 were purchased from Sigma Aldrich (Germany), triclosan from Fluka (Germany), and galaxolide from SAFC (Germany). Standard solutions of micropollutants were prepared in methanol (99.9%, VWR, Belgium) and stored at −20 °C. Spiking and calibration solutions were prepared by mixing single compound standard solutions and diluting them with methanol. Na₂CO₃ and NaCl were purchased from Sigma-Aldrich (Germany), acetic anhydride from Fluka (Germany), acetonitrile and sodium acetate from VWR (USA).

GAC type C Gran was obtained from Norit (The Netherlands). Norit C Gran has an open meso- and macroporous structure, high surface area (1,400 m²/g) and low apparent density (230 kg·m⁻³). It is activated with phosphoric acid, resulting in a weakly negatively charged acidic surface (point of zero charge, PZC = 4.2) with numerous oxygen groups (Baker et al. 2005; Carabineiro et al. 2012). These properties make Norit C Gran effective for adsorbing high molecular weight organic compounds (Schouten et al. 2007). Low apparent density (230 kg·m⁻³) stimulates distribution of GAC within the sludge blanket.

Batch experiments

The sludge was collected from a laboratory-scale UASB reactor (V = 50 l) operated at 25 °C and an HRT of 8.7 days, and fed with a mixture of black water and sludge from an aerobic grey water treatment system. Six Erlenmeyer flasks (V = 150 ml) were filled with 100 ml of sludge, closed with stoppers, punched with needles to control the gas pressure inside and incubated on a mixing plate with magnetic stir bars rotating at 350 rpm. 125 mg of sodium acetate was added to each bottle twice per week to maintain microbial activity. Diclofenac, ibuprofen, triclosan and galaxolide were spiked to the flasks after 3 or 7 weeks of incubation, 20 hours before the experiment was terminated. The duration of incubation periods in the batch experiment were chosen based on the preliminary tests. GAC (1 g) was added at the beginning of the experiment (Incubated carbon) or simultaneously with the micropollutants (Fresh carbon) (Figure S1 in the supplement, available with the online version of this paper).

Control batches were run in duplicate, and contained UASB sludge spiked with micropollutants and incubated for 20 h without the addition of GAC (Figure S1). Samples for micropollutant analysis were taken from all batches directly after spiking and at the end of incubation, and stored at −20 °C until being analyzed.

Continuous experiments

Two identical UASB reactors made of glass (V reaction = 4.7 l, h = 0.6 m, d inner = 0.1 m) with a cooling jacket for temperature control were operated during 260 days. The temperature inside the reactors was maintained at 25 °C.
by water recirculation from a water bath. The influent was supplied by peristaltic pumps (Masterflex L/S 7525–70). The even distribution of the influent through the reactor was achieved by a layer of glass beads placed at the bottom of the reactor. The flow of produced biogas was measured by gas flow meters (Ritter MGC-1).

The reactors were inoculated with flocculent sludge from a laboratory-scale UASB reactor (V = 50 L) operated at 25 °C and an HRT of 8.7 days. The reactors were fed with a 5:1 COD-based mixture of vacuum-collected black water and sludge from the adsorption stage (HRT = 1 h) of an adsorption/bio-oxidation (AB) grey water treatment system (Tervahauta et al. 2014). The mixture ratio was equal to the actual mixture ratio at the DeSaR (Decentralized Sanitation and Reuse) demonstration site (Sneek, The Netherlands), collecting black and grey water from 32 houses (Tervahauta et al. 2014). Black water was collected from the buffer tank, and grey water sludge from the settler of the adsorption stage of the AB treatment system at the same demonstration site. Both black water and grey water sludge were transported in 10 L plastic jerry cans and stored at 4 °C.

The HRT was decreased from 34 days during the first 32 days of operation to 24 days during days 32–69 and finally to 20 days during days 69–260 in order to prevent accumulation of volatile fatty acids (VFA) during the start-up phase. The HRT of 20 days was chosen to compensate for possible fluctuations in the influent load, as advised by Wendland et al. 2007. The effluent was sampled from the effluent collection tank, and sludge was sampled at the middle of the sludge bed by a plastic tube connected to the tap at the bottom of the reactors.

GAC was added to one of the reactors on day 138 of operation. To introduce GAC to the reactor, its upper part was dismantled just above the sludge bed. The carbon was added from the top and gently mixed with the sludge bed manually. The quantity of added GAC (5.7 grams per liter of the reactor volume) was equal to the monthly influent load of dissolved COD. The influent of both reactors was spiked with micropollutants (ibuprofen, diclofenac, metoprolol, galaxolide, triclosan) daily from the addition of GAC to one of the reactors. Metoprolol was spiked in the continuous experiments, because it was found that the compound is present at high concentrations in the black water and is poorly removed in the full-scale source separated sanitation system (Butkovský et al. 2015). The properties of the spiked micropollutants and their average concentrations in the influent after spiking are presented in Table 1 (Toxnet Hazardous Substances Data Bank (HSDB); Joss et al. 2006; de Graaff et al. 2011). Each compound was spiked at 100 μg/l (upper range of the relevant concentrations found in black water and grey water sludge). Higher concentrations displayed in Table 1 are explained by the background concentrations of the micropollutants in black water and grey water sludge. The COD removal and specific methane production in the reactors with and without GAC addition were compared by one-way ANOVA statistical analysis with a significance level (α) of 0.05.

Influent, effluent and sludge samples were collected weekly. Total COD, dissolved COD, TSS, VFA and pH were analyzed directly after sampling. Samples for micropollutant analysis were taken on days 138, 150, 159, 173, 194, 217, 245 and 260 of reactor operation and stored at −20 °C until being analyzed.

**Sorption isotherms**

To assess micropollutant removal when GAC would be applied for effluent post treatment, the relation between carbon saturation and effluent micropollutant concentrations was determined using effluent from the UASB reactor without GAC that was used in this study. The UASB reactor was fed with a mixture of black water and sludge from the aerobic grey water treatment system spiked with micropollutants (ibuprofen, diclofenac, metoprolol, galaxolide and triclosan; 100 μg/l of each compound spiked). No additional spiking of the UASB reactor effluent was done prior to the determination of sorption isotherms. The effluent was collected during days 246–260, stored at −4 °C and fully mixed prior to the experiment. The isotherms were obtained according to the ASTM D 3860–98 standard method (ASTM 2008). The applied carbon weight ranged from 0 to 0.5 g and the contact time was 20 h.

**Analyses**

Total COD was analyzed with a cuvette test (Dr. Lange LCK014). Dissolved COD was analyzed in the influent with a cuvette test (Dr. Lange LCK514) after filtration of the samples through the 0.45 μm PTFE filter. TSS were determined according to standard methods (APHA 1998). pH was determined in the influent, effluent and inside the reactor with a pH meter (WTW 340i). VFA (acetic, propionic and butyric acids) were analyzed by ion chromatography (Metrohm 761 Compact IC) in the effluent samples filtered through 0.45 μm membrane filters and expressed as the sum of corresponding COD values. Biogas composition was analyzed by gas chromatography (Varian CP-4900 Micro-GC).
Diclofenac, ibuprofen and metoprolol were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) with in-line SPE, consisting of Phenomenex Kinetex Phenyl-Hexyl column (h = 100 mm, d = 2.1 mm, 100 Å pore size) and an Agilent 6410 triple quadruple mass spectrometer with electrospray ion source (Table S1). Galaxolide and triclosan were analyzed by gas chromatography-tandem mass spectrometry (GC-MS/MS) consisting of an Agilent 6890N gas chromatograph and Agilent 5975XL mass spectrometer modified with a Chromtech. Evolution triple quadruple (Butkovskyi et al. 2014). The mass spectrometers were operated in Selective Reaction Monitoring mode. Retention times and QqQ transitions for all analyzed compounds are presented in Table S2 and Table S3. (Tables S1–S3 are available with the online version of this paper.)

The influent, the sludge samples and the samples from the batch experiment were centrifuged at 3750 rpm prior to analysis. The liquid phase and sludge were separated from GAC by centrifugation as shown in Figure S2 (available with the online version of this paper). Supernatants of the centrifuged influent samples, samples from the batch experiments and the non-centrifuged effluent samples were filtrated through a 0.45 μm PTFE filter. The filter was subsequently washed with acetonitrile. 0.9 ml of the acetonitrile was evaporated under the nitrogen gas and reconstituted in 0.1 ml of methanol and 0.9 ml of the filtrated sample. The reconstituted sample was injected to the LC-MS/MS equipped with in-line SPE. Extraction of the personal care products from the supernatants was done by stir-bar sorptive extraction followed by thermal desorption in Gerstel TDU and injection to GC-MS/MS via Gerstel CIS4 injection system (Butkovskyi et al. 2014).

Extraction of micropollutants from centrifuged solids was done by ultrasonication in 7 ml of organic solvent (mixture of acetonitrile and methanol at 6:1 volumetric ratio) during 20 minutes. The extracts were treated as liquid

Table 1 | Chemical structures, properties and measured initial concentrations (C0) of the studied micropollutants in the influent of UASB-GAC reactor after spiking

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structural formula</th>
<th>logK&lt;sub&gt;ow&lt;/sub&gt;</th>
<th>pK&lt;sub&gt;a&lt;/sub&gt;</th>
<th>K&lt;sub&gt;SS&lt;/sub&gt;</th>
<th>C0, μg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diclofenac</td>
<td><img src="image" alt="Diclofenac" /></td>
<td>4.51</td>
<td>4.14</td>
<td>&lt;0.1</td>
<td>137 ± 43</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td><img src="image" alt="Ibuprofen" /></td>
<td>3.97</td>
<td>4.91</td>
<td>9–35</td>
<td>160 ± 30</td>
</tr>
<tr>
<td>Metoprolol</td>
<td><img src="image" alt="Metoprolol" /></td>
<td>1.69</td>
<td>9.68</td>
<td>n.d.&lt;sup&gt;c&lt;/sup&gt;</td>
<td>101 ± 12</td>
</tr>
<tr>
<td>Galaxolide</td>
<td><img src="image" alt="Galaxolide" /></td>
<td>5.90</td>
<td>n.d.</td>
<td>&lt;0.03</td>
<td>340 ± 39</td>
</tr>
<tr>
<td>Triclosan</td>
<td><img src="image" alt="Triclosan" /></td>
<td>4.76</td>
<td>7.9</td>
<td>n.d.</td>
<td>117 ± 15</td>
</tr>
</tbody>
</table>

<sup>a</sup>logK<sub>ow</sub> and pK<sub>a</sub> data from Toxnet Hazardous Substances Data Bank (HSDB) (http://toxnet.nlm.nih.gov/index.html) and de Graaff et al. (2011).

<sup>b</sup>K<sub>SS</sub> data from Joss et al. (2006).

<sup>c</sup>No data.

Diclofenac, ibuprofen and metoprolol were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) with in-line SPE, consisting of Phenomenex Kinetex Phenyl-Hexyl column (h = 100 mm, d = 2.1 mm, 100 Å pore size) and an Agilent 6410 triple quadruple mass spectrometer with electrospray ion source (Table S1). Galaxolide and triclosan were analyzed by gas chromatography-tandem mass spectrometry (GC-MS/MS) consisting of an Agilent 6890N gas chromatograph and Agilent 5975XL mass spectrometer modified with a Chromtech. Evolution triple quadruple (Butkovskyi et al. 2014). The mass spectrometers were operated in Selective Reaction Monitoring mode. Retention times and QqQ transitions for all analyzed compounds are presented in Table S2 and Table S3. (Tables S1–S3 are available with the online version of this paper.)

The influent, the sludge samples and the samples from the batch experiment were centrifuged at 3750 rpm prior to analysis. The liquid phase and sludge were separated from GAC by centrifugation as shown in Figure S2 (available with the online version of this paper). Supernatants of the centrifuged influent samples, samples from the batch experiments and the non-centrifuged effluent samples were filtrated through a 0.45 μm PTFE filter. The filter was subsequently washed with acetonitrile. 0.9 ml of the acetonitrile was evaporated under the nitrogen gas and reconstituted in 0.1 ml of methanol and 0.9 ml of the filtrated sample. The reconstituted sample was injected to the LC-MS/MS equipped with in-line SPE. Extraction of the personal care products from the supernatants was done by stir-bar sorptive extraction followed by thermal desorption in Gerstel TDU and injection to GC-MS/MS via Gerstel CIS4 injection system (Butkovskyi et al. 2014).

Extraction of micropollutants from centrifuged solids was done by ultrasonication in 7 ml of organic solvent (mixture of acetonitrile and methanol at 6:1 volumetric ratio) during 20 minutes. The extracts were treated as liquid
samples as described above. Despite fairly good separation of GAC from sludge by centrifugation, as shown in Figure S2, separated sludge occasionally contained GAC particles. However, extraction of micropollutants from GAC was not achievable, with recoveries of all analyzed compounds adsorbed to GAC in MilliQ being below 1% when extracted by ultrasonication. Thus, the fraction of micropollutants extracted from sludge containing GAC particles was equal to the fraction adsorbed to the sludge.

Fenoprofen and tonalide-d3 were spiked as internal standards to liquid and solid samples prior to extraction (Table S1). Recoveries and limits of quantification (LOQ) were determined for method validation separately for each analyzed matrix (Table S4 and Table S5, available with the online version of this paper). The LOQs were determined from the calibration curve as a signal/noise ratio 10. All samples were measured in triplicates and relative standard deviations were calculated.

RESULTS

Batch experiments

Removal of micropollutants from the liquid phase in the batch experiment exceeded 98% in all batches except for the control (Figure 1).

Micropollutant concentrations in sludge were lower for the batches where the GAC was only added together with the micropollutants compared to the batches where the GAC was added at the start of incubation. The longer the GAC was incubated together with sludge, the higher were the final micropollutant concentrations in sludge. Micropollutant removal was negligible in the control batches. Ibuprofen was mostly present in the liquid phase in the control batches after incubation (83% of the initial amount) whereas diclofenac, galaxolide and triclosan were mostly present in the solid phase (79%, 95% and 88% respectively).

Continuous experiments: reactor performance

The total influent COD was 17.1 ± 4.1 g/l. The average COD load for days 69–260 of reactor operation at HRT of 20 d was (0.84 ± 0.22) kg COD·m⁻³·d⁻¹ for the reactor without GAC addition (UASB) and (0.78 ± 0.28) kg COD·m⁻³·d⁻¹ for the reactor with GAC addition (UASB-GAC). The average COD removal during the same period was 81% ± 8% and 77% ± 9% for the UASB reactor without and with GAC addition (Figure S3, available with the online version of this paper), with effluent COD concentrations equal to (2.9 ± 0.9) g/l and (3.4 ± 1.1) g/l respectively. These values are comparable to the COD removal of 81% found by Tervahauta et al. (2014) for a laboratory-scale UASB reactor fed with the same mixture of black water and sludge from an
aerobic grey water treatment system. The drop in COD removal to 57% and even 52% were attributed to occasional sludge washout, which occurred several times during the first 120 days of reactor operation. The specific methane production was \(0.19 \pm 0.06\) \(\text{Nm}^3\text{kg}^{-1}\) \(\text{COD}_{\text{in}}\) and \(0.17 \pm 0.04\) \(\text{Nm}^3\text{kg}^{-1}\) \(\text{COD}_{\text{in}}\) in UASB and UASB-GAC reactors respectively (Figure S3). These values are comparable to the methane production of \(0.17 \pm 0.04\) \(\text{Nm}^3\text{kg}^{-1}\) \(\text{COD}_{\text{in}}\) in the laboratory-scale UASB reactor fed with the same influent (Tervahauta et al. 2014). According to the results of the ANOVA test, the COD removal and the specific methane production were not significantly different between the UASB and UASB-GAC reactors after addition of GAC on day 138, with \(F(3.23) < F_{\text{crit}}(4.17)\) and \(F(2.29) < F_{\text{crit}}(4.20)\) respectively.

The pH in both reactors was 8.0 ± 0.3. VFA concentrations reached 1.32 and 1.23 g COD/l in the UASB and UASB-GAC reactors on day 14, indicating an imbalance between acidification and methanogenic activity during the start-up phase. The concentrations gradually decreased till day 32, and remained stable afterwards at \(0.17 \pm 0.12\) g COD/l in UASB and \(0.16 \pm 0.09\) g COD/l in UASB-GAC.

**Continuous experiments: micropollutant removal**

The cumulative amounts of micropollutants discharged in the effluent of the UASB reactors with and without GAC addition, adsorbed to sludge and removed, are presented in Figure 2. The sum of the three fractions is the cumulative amount of micropollutants fed to the reactors. Volatilization plays a negligible role in the removal of the analyzed micropollutants due to their low Henry's law constant. Therefore in the case where the UASB reactor did not contain GAC, the removed fraction is most probably attributed to biological transformation. For the UASB-GAC reactor, the removal can be attributed to biological transformation and sorption to GAC.

The highest removal in the UASB reactor without GAC addition was observed for diclofenac (60%). The removal was enhanced only to 67% by addition of GAC. The rest of the influent fraction of diclofenac was mainly discharged with the effluent, with only 5% being adsorbed to sludge. Ibuprofen removal in the UASB reactor without GAC addition was 30%. With GAC addition, removal increased to 60%. The effluent load of ibuprofen was thus decreased by 42%. Ibuprofen was not adsorbed to sludge in both reactors. Metoprolol removal was below 10% in the UASB reactor without GAC addition. Addition of GAC increased metoprolol removal to 70%. The effluent load of metoprolol decreased by 76%. Similarly to the ibuprofen and diclofenac, metoprolol sorption to sludge was low (<10%) in both reactors.

Galaxolide and triclosan were mostly adsorbed to sludge in the UASB reactor without GAC addition. The removal of these compounds was 20% and 40% respectively without addition of GAC, but increased to 75% and 80% when GAC was added. The increase in the cumulative amount removed with GAC addition was accompanied by a decrease of the fraction adsorbed to UASB sludge by 70%. The cumulative effluent amount for galaxolide and triclosan was low in the UASB reactors with and without GAC addition.

**Isotherms**

The isotherms of diclofenac, ibuprofen and metoprolol sorption to GAC are presented in Figure 3. The isotherms were obtained using the effluent of the UASB reactor without GAC as the medium. Isotherms were not obtained for galaxolide and triclosan, because these compounds are effectively removed in the UASB reactor without GAC addition, with the effluent concentrations being close to or below LOQ.

The post-treatment of UASB effluent with activated carbon is an alternative for GAC addition to the UASB reactor. The amount of GAC that would be required to achieve the same decrease of effluent concentrations as in the UASB-GAC reactor was calculated. The carbon saturation at the micropollutant concentrations measured in the effluent of UASB with GAC addition on day 260 was obtained from the isotherms (Figure 3). The amount of GAC required to remove an accumulative amount of micropollutants, achieving equivalent effluent concentrations, was calculated from the carbon saturation, accumulative difference in micropollutant washout between the UASB and UASB-GAC reactors during the spiking period, and the total volume of the effluent produced during the spiking period (27.7l) (Table 2).

The amount of GAC required for removal of diclofenac, ibuprofen and metoprolol from the UASB effluent was respectively 6%, 56% and 70% lower when compared to the amount of GAC added to the reactor (0.98 g/l\(_{\text{effluent}}\)).

**DISCUSSION**

**Retention of GAC sorptive capacity**

Results of the batch experiments showed a positive impact for GAC addition to the UASB sludge on micropollutant...
removal. Moreover, incubation of GAC with the UASB sludge during 7 weeks of batch experiments does not show any negative effects on the adsorption of micropollutants by GAC from the liquid phase (Figure 1). The addition of GAC also significantly decreased the concentrations of micropollutants adsorbed to sludge.

Figure 2 | Accumulative absolute amounts of micropollutants washed out with the effluent (hatched), adsorbed to the sludge (black) and removed (dotted). The removed fraction comprises the biodegradation and GAC absorption fractions.
Retention of the carbon sorptive capacity over prolonged time in batch experiments, as well as in the continuously running UASB reactor, is possibly achieved due to carbon bioregeneration. The meso- and macroporous structure of Norit C Gran promotes diffusion of hydrolytic exoenzymes into the pores, increasing bioregeneration capacity (Sirotkin et al. 2001). Additionally, low molecular weight intermediates of anaerobic biodegradation pathways may enhance anaerobic biodegradation of micropollutants in biological activated carbon systems (Abromaitis et al. 2016).

**Mechanisms of micropollutant removal in a UASB-GAC reactor**

Addition of GAC to the UASB reactor treating black water and grey water sludge improved removal of four out of five studied micropollutants. Diclofenac and ibuprofen with pKa values of 4.1 and 4.9 respectively occur in anionic forms at pH values between 6.5 and 8.0. A pH of 8.0 was measured in both reactors. The anionic form dominates in these conditions and repulsion between the micropollutants and negatively charged sludge surface can be expected, despite quite high logKow of both compounds (diclofenac – 4.51, ibuprofen – 3.97). This explains the very low sorption of these compounds to the UASB sludge: only 7% of diclofenac is adsorbed, while ibuprofen is not adsorbed at all (Figure 2). These results are, however, contradictory to the batch experiments, where significant amount of ibuprofen and diclofenac was sorbed to sludge in the control batches without addition of activated carbon. Acidification of the medium due to incomplete degradation of substrate resulting in VFA production is the possible explanation of the results, observed in the batch tests (Vieno & Sillanpää 2014). Significant removal of ibuprofen (30%) and diclofenac (60%) in the continuous experiment also does not match the

![Freundlich isotherms for metoprolol (a), ibuprofen (b) and diclofenac (c) adsorption to GAC (Norit C GRAN) in the effluent of the UASB reactor without GAC.](image)

![Carbon saturation, μg/g](image)

**Figure 3** | Freundlich isotherms for metoprolol (a), ibuprofen (b) and diclofenac (c) adsorption to GAC (Norit C GRAN) in the effluent of the UASB reactor without GAC. x/m – carbon saturation, cᵋ – remaining concentration of the micropollutant.

**Table 2** | Amount of GAC required for micropollutant removal from UASB effluent at given carbon saturation values and accumulative difference in micropollutant washout between the UASB and UASB with GAC

<table>
<thead>
<tr>
<th>Compound</th>
<th>Micropollutant concentrations in the effluent of the UASB with GAC addition, μg/l*</th>
<th>Carbon saturation, μg/g</th>
<th>Accumulative difference in micropollutant washout between the UASB and UASB with GAC, μg/l*</th>
<th>GAC required to remove the accumulative amount of micropollutants, g/lefluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diclofenac</td>
<td>69.26</td>
<td>109.72</td>
<td>2.8 × 10⁷</td>
<td>0.92</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>130.73</td>
<td>222.95</td>
<td>2.7 × 10⁷</td>
<td>0.43</td>
</tr>
<tr>
<td>Metoprolol</td>
<td>43.29</td>
<td>265.25</td>
<td>2.2 × 10⁵</td>
<td>0.31</td>
</tr>
</tbody>
</table>

*On day 260 of the continuous experiment.
results of the batch tests, where removal of these compounds was not observed. Short incubation time (20 h) along with the addition of sodium acetate as the main carbon source did not promote anaerobic biodegradation of the micropol- lutants in the batch experiments (Suárez et al. 2008).

Hydrophobic interaction is considered to contribute strongly to sorption of diclofenac and ibuprofen to activated carbon (Ternes et al. 2002; Moreno-Castilla 2004). However, addition of GAC to the reactor only slightly improved removal of diclofenac, whereas removal of ibuprofen was enhanced from 40% to 70%. The repulsion between negatively charged carbon surface (PZC = 4.2) and negatively ionized molecules of diclofenac and ibuprofen at pH 8.0 measured inside the reactor is a possible explanation for these observations. The high concentrations of dissolved organic matter, which is known to inhibit sorption of hydrophobic organic compounds more strongly than sorption of hydrophilic ones, can also prevent sorption of diclofenac and ibuprofen to GAC (Nam et al. 2014). Thus, Margot et al. (2015) reported lower sorption of diclofenac from sewage effluents to activated carbon, with 65% removal in PAC-ultrafiltration system (Margot et al. 2015). Notable is that total COD of the sewage effluent in the experiments of Margot et al. (2015) was (24.3 ± 12) mg/l, whereas total COD of the UASB influent in this research was (6686 ± 4250) mg/l, thus, three orders of magnitude higher than the values reported for the sewage effluent.

Metoprolol was efficiently removed by GAC despite its hydrophobic nature. The electrostatic interaction between the positively charged molecules of metoprolol (pKa 9.68) and negatively charged surface groups of activated carbon is a possible explanation for the observed removal (De Ridder et al. 2011).

Hydrophobicity played major role in the removal of triclosan and galaxolide by sorption to GAC, even though triclosan was present in ionized form at pH 8.0, measured inside the reactor (Nghiem & Coleman 2008). GAC addition to the UASB reactor also decreased sludge concentrations of strongly hydrophobic compounds, such as galaxolide and triclosan. The majority of the total influent load of these compounds was adsorbed to sludge in the control reactor without GAC addition, whereas the sludge of the reactor with GAC addition contained less than 25% of the total influent load 122 days after carbon addition. Activated carbon is known to significantly reduce concentrations of hydrophobic organic pollutants, including triclosan, in contaminated sediments and soils (Rakowska et al. 2012; Bair et al. 2016). A similar effect is shown for anaerobic sludge for the first time. Addition of GAC does not reverse sorption of organic micropollutants to sludge; however, 99% of the porewater concentrations of hydrophobic micropollutants can be achieved, which is a possible explanation of the observed phenomenon (Rakowska et al. 2012). Sorption of organic micropollutants to sludge also decreases as a result of GAC addition to the reactor, whereas organic micropollutants, already sorbed to sludge before amendment with carbon can be biologically degraded, resulting in their lower sludge concentrations.

The synergistic effect between the sorption and biological oxidation in the UASB systems exists due to stimulation of biological activity through addition of activated carbon and bioregeneration of carbon by bacteria present in the system (Sublette et al. 1982). While low anaerobic degradability was reported for the compounds included in this study, some authors showed that their anaerobic biodegradation can be enhanced with longer SRT applied (McAvoy et al. 2002; Queiroz et al. 2012; Alvarino et al. 2014). Addition of activated carbon to the UASB reactor can improve biodegradability of these compounds by increasing their retention in the reactor, concentrating them in the microhabitats of the microorganisms, responsible for their degradation (Sublette et al. 1982) and by reducing inhibitory effects of micropollutants at elevated concentrations.

Comparison of UASB-GAC reactor with GAC treatment of the UASB effluent

Two methods of metoprolol, ibuprofen and diclofenac removal with GAC were compared, namely GAC addition to the UASB reactor and GAC treatment of the UASB effluent. The latter option was found to require less GAC per volume of treated wastewater. However, positive effects of the first method also need consideration. According to Yu et al. (1999), addition of activated carbon improves granulation and increases organic loading rates during the start-up of a UASB reactor (Yu et al. 1999). Another positive effect of GAC addition is the decrease of micropollutant concentrations in the UASB sludge, as demonstrated in the present study for the first time. Post-treatment of the effluent by GAC requires an additional treatment unit, while direct addition of carbon to the reactor may decrease capital and operational costs of the treatment system. Finally, bioregeneration of activated carbon achieved in the UASB reactor can extend the GAC lifetime. Additional studies in batch systems with simultaneous adsorption and biodegradation are required for quantification of the carbon lifetime and extent of bioregeneration (Walker & Weatherley 1998).
Decrease of micropollutant concentrations in the UASB sludge by activated carbon can be used to improve the quality of sludge before its application as a soil amendment. However, ways of efficient separation of GAC from sludge have to be found if the latter is going to be used in agriculture. Gradient separation could be achieved by centrifugation, which was successfully applied in this study for separation of sludge from carbon, though a fraction of sludge still remained mixed with carbon when this approach was used. Fixation of activated carbon within the reactor or utilization of pre-fabricated magnetic activated carbon, which can be separated by an external magnetic field, are possible solutions for the recovery of spent activated carbon from sludge (Liu et al. 2014).

**CONCLUSIONS**

Removal of the pharmaceuticals ibuprofen and metoprolol, the biocide triclosan and the fragrance galaxolide was enhanced in the UASB reactor by direct addition of GAC (Norit C Gran). Ibuprofen and metoprolol were mainly discharged with the effluent from the UASB reactor without GAC addition, whereas addition of GAC decreased their effluent loads by 42% and 76% respectively. Galaxolide and triclosan were mainly adsorbed to sludge in the UASB reactor with GAC addition, whereas addition of GAC decreased their sludge loads by 70%. The removal of diclofenac was not enhanced in the UASB reactor with GAC addition because of electrostatic repulsion with GAC particles. The presence of GAC in the UASB reactor did not influence COD removal (78%) and methane production (0.17 Nm³ CH₄/kg COD).

Addition of GAC to the UASB reactor requires 70%, 56% and 6% more carbon to remove the equivalent cumulative amount of metoprolol, ibuprofen and diclofenac, when compared to the post-treatment of the effluent. However, addition of GAC to the UASB reactor also improves the quality of sludge by mitigation of hydrophobic micropollutants and extends carbon lifetime through bioregeneration.

Additional research is needed to estimate the lifetime of GAC, bioregeneration capacity of UASB-GAC reactor and the effect of the carbon PZC and surface chemistry on micropollutant removal in a UASB-GAC reactor.

**ACKNOWLEDGEMENT**

This work was performed in the cooperation framework of Wetsus, European Centre of Excellence for Sustainable Water Technology (www.wetsus.nl). Wetsus is co-funded by the Dutch Ministry of Economic Affairs and Ministry of Infrastructure and Environment, the European Union Regional Development Fund, the Province of Fryslân, and the Northern Netherlands Provinces. The authors would like to thank the participants of the research theme ‘Separation at Source’ for the fruitful discussions and their financial support. The authors would like to thank Ton van der Zande and Isabel Blotnik for their contribution to the experimental part of the study.

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First received 27 June 2017; accepted in revised form 13 December 2017. Available online 28 December 2017.