



## Biodegradation of pharmaceuticals in hospital wastewater by a hybrid biofilm and activated sludge system (Hybas)

Escola Casas, Monica; Chhetri, Ravi Kumar; Ooi, Gordon Tze Hoong; Kaarsholm, Kamilla Marie Speht; Litty, Klaus; Christensson, Magnus; Kragelund, Caroline; Andersen, Henrik Rasmus; Bester, Kai

*Published in:*

Science of the Total Environment

*Link to article, DOI:*

[10.1016/j.scitotenv.2015.05.099](https://doi.org/10.1016/j.scitotenv.2015.05.099)

*Publication date:*

2015

*Document Version*

Peer reviewed version

[Link back to DTU Orbit](#)

*Citation (APA):*

Escola Casas, M., Chhetri, R. K., Ooi, G. T. H., Hansen, K. M. S., Litty, K., Christensson, M., ... Bester, K. (2015). Biodegradation of pharmaceuticals in hospital wastewater by a hybrid biofilm and activated sludge system (Hybas). *Science of the Total Environment*, 530-531, 383–392. DOI: 10.1016/j.scitotenv.2015.05.099

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10 **Biodegradation of pharmaceuticals in hospital**  
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13 **wastewater by a hybrid biofilm and activated sludge**  
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17 **system (Hybas)**  
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25 **Mònica Escolà Casas<sup>1</sup>, Ravi Kumar Chhetri<sup>2</sup>, Gordon Ooi<sup>1</sup>, Kamilla M. S. Hansen<sup>2</sup>, Klaus**  
26 **Litty<sup>3</sup>, Magnus Christensson<sup>4</sup>, Caroline Kragelund<sup>3</sup>, Henrik R. Andersen<sup>2</sup> and Kai Bester<sup>1\*</sup>**  
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32 1) Environmental Science, Aarhus University, Frederiksborgsvej 399, 4000 Roskilde, Denmark  
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34 2) Department of Environmental Engineering, Technical University of Denmark, Miljøvej 113,  
35  
36 2800 Kgs. Lyngby, Denmark  
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38  
39 3) Department of Chemistry and Biotechnology, Danish Technological Institute, Kongsvang Allé  
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41 29, 8000 Aarhus C, Denmark  
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43  
44 4) AnoxKaldnes, Klosterängsvägen 11A, 226 47 Lund, Sweden  
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## Abstract

Hospital wastewater contributes a significant input of pharmaceuticals into municipal wastewater. The combination of suspended activated sludge and biofilm processes, as stand-alone or as hybrid process (Hybrid biofilm and activated sludge system (Hybas<sup>TM</sup>)) has been suggested as a possible solution for hospital wastewater treatment. To investigate the potential of such a hybrid system for the removal of pharmaceuticals in hospital wastewater a pilot plant consisting of a series of one activated sludge reactor, two Hybas<sup>TM</sup> reactors and one moving bed biofilm reactor (MBBR) has been established and adapted during 10 months of continuous operation. After this adaption phase batch and continuous experiments were performed for the determination of degradation of pharmaceuticals. Removal of organic matter and nitrification mainly occurred in the first reactor. Most pharmaceuticals were removed significantly. The removal of pharmaceuticals (including x-ray contrast media,  $\beta$ -blockers, analgesics and antibiotics) were fitted to a single first-order kinetics degradation function, giving degradation rate constants from 0 to  $1.49 \text{ h}^{-1}$ , from 0 to  $7.78 \times 10^{-1} \text{ h}^{-1}$ , from 0 to  $7.86 \times 10^{-1} \text{ h}^{-1}$  and from 0 to  $1.07 \times 10^{-1} \text{ h}^{-1}$  for first, second, third and fourth reactor respectively. Generally, the highest removal rate constants were found in the first and third reactor while the lowest were found in the second one. When the removal rate constants were normalized to biomass amount, the last reactor (biofilm only) appeared to have the most effective biomass in respect of removing pharmaceuticals. In the batch experiment, out of 26 compounds, 16 were assessed to degrade more than 20% of the respective pharmaceutical within the Hybas<sup>TM</sup> train. In the continuous flow experiments, the measured removals were similar to those estimated from the batch experiments, but the concentrations of a few pharmaceuticals appeared to increase during the first treatment step. Such increase could be attributed to de-conjugation or formation from other metabolites.

## Keywords

Hospital wastewater, MBBR, X-ray contrast media, Pharmaceuticals, Removal rate constants

## 1. Introduction

Hospitals represent a substantial point source for pharmaceuticals discharged to the municipal sewer system and the wastewater from hospitals is usually co-treated together with municipal wastewater (Santos et al., 2013; Verlicchi et al., 2010; Thomas et al., 2007). Earlier studies have shown, that conventional activated sludge treatment in WWTPs is inefficient for full removal of pharmaceuticals (Ternes et al., 2004; Joss et al., 2006). Thus, source-treatment of hospital wastewater has been proposed in industrialized and developing countries to decrease the quantity of pharmaceuticals discharged to municipal WWTPs (Verlicchi et al.; 2010; 2015; Pauwels and Verstraete; 2006). This source-treatment proposal gets more important when it comes to big hospital sizes.

It is mainly due to pathogen-spreading concerns that countries like China and Japan are currently treating hospital wastewater based on conventional activated sludge process or MBR (Membrane Bioreactor) (Pauwels and Verstraete, 2006; Liu et al., 2010). There are only a few studies on hospital wastewater where the removal of pharmaceuticals is described: Marienhospital Gelsenkirchen and Waldbröl (Germany), Isala clinics in Zwolle (The Netherlands), Cantonal Hospital of Baden (Switzerland) (Pills, 2012), Ioannina hospital (Greece) (Kosma et al., 2010) and Herlev hospital (Denmark) (Nielsen et al., 2013). Processes based on activated sludge are, however, not sufficient to ensure high removal of pharmaceuticals, which means that additional post-treatment is required. Numerous post-treatment processes like activated carbon, ultraviolet photolysis, advanced oxidation processes (AOPs), reverse osmosis or nanofiltration can remove pharmaceuticals from wastewater (Pauwels and Verstraete, 2006; Joss et al., 2008). In some treatments based on membrane bioreactors with sludge (MBR), the systems were accompanied with AOPs or ultraviolet photolysis to be effective (Nielsen et al., 2013; Köhler et al., 2012; Kovalova et

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5 al., 2013; Kovalova et al., 2012). Fungal fluidized-bed bioreactors were also tested for  
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7 pharmaceuticals removal in hospital wastewater (Cruz-Morató et al., 2014), and presented better  
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9 efficacy than the activated sludge systems. Finally, suspended biofilm systems such as MBBRs  
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11 (Moving Bed Biofilm Reactors) have appeared to directly remove pharmaceuticals from hospital  
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13 wastewater (Escolà Casas et al., 2015).  
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18 Different biofilm technologies have recently appeared as an effective tool to remove micro-  
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20 pollutants with the main focus being on porous media biofilm processes i.e., slow sand filter e.g.  
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22 (Bester and Schafer, 2009; Escolà Casas and Bester, 2015; Heberer et al., 2004; Hijosa-Valsero et  
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24 al., 2011; Janzen et al., 2009; Li et al., 2014; Reungoat et al., 2011; Zearley and Summers, 2012).  
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26 Along with such biofilm technologies, MBBR also seemed to be a promising solution to remove  
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28 micro-pollutants, which cannot be removed with activated sludge treatment (Hapeshi et al. 2013;  
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30 Zupanc et al. 2013; Falås et al. 2012; Falås et al. 2013; Escolà Casas and Bester 2015).  
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36 Hybas<sup>TM</sup> is a hybrid process, based on the integrated fixed-film activated sludge technology, where  
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38 polyethylene carriers for biofilm growth are suspended within activated sludge in one reactor  
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40 (Christensson and Welander, 2004; Ødegaard et al., 2014). Such carriers are already in operation for  
41  
42 nitrification and denitrification processes. In this way, Hybas<sup>TM</sup> contains two separate biomasses:  
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44 one with low sludge age (activated sludge flocks), and one with high-sludge age (attached biofilm  
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46 on MBBR-carriers). This fact allows fast growing biomass to be in the form of activated-sludge  
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48 flocks, while the slow-growing biomass develops on the MBBR carriers. The presence of attached  
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50 biofilm in wastewater treatment systems have shown to improve the activated sludge performance  
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52 for nutrient removal (Debabrata, 2010, Randall and Sen, 1996). Falås et al., 2013 demonstrated that  
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5 attached biofilm contributes significantly to the overall removal of micro-pollutants in a Hybas™  
6 system.  
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10 According to the literature, Hybas™ treatment seems to be a promising option to treat hospital  
11 wastewater. Therefore, this study tested and evaluated for the first time the performance of a pilot  
12 Hybas™ plant for the removal of pharmaceuticals from mechanically pre-treated hospital  
13 wastewater.  
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## 19 20 21 **2. Materials and Methods** 22

### 23 24 25 **2.1 Treatment system** 26

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28 A lab-scale treatment line was established to treat a portion of the wastewater produced at the  
29 oncology section of Aarhus University Hospital. The line consisted of four three liter reactors in  
30 series (H1, H2, H3 and P). H1 contained only activated sludge, H2 and H3 contained activated  
31 sludge and biofilm carriers and P was a polishing step containing only biofilm carriers. Each reactor  
32 (H2, H3 and H4) contained 500 AnoxKaldnes™ K5 carriers (AnoxKaldnes, Lund, Sweden) and  
33 resulted in a filling ratio of 50%. The set-up is drawn in Figure 1. The mixing was performed using  
34 aeration and the wastewater was pumped at 0.95 L h<sup>-1</sup> flow into the treatment line. Sludge recycling  
35 rate from the settler to H1 was 100%.  
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47 The raw hospital wastewater was passed through an 80 µm filter from Amiat Water Systems  
48 (Bochum, Germany) using a monopump (Pump 1) from Seepex (Hillerød, Denmark) into the  
49 mixing tank (100 L). This equalization tank maintained a temperature between 15°C and 18°C and  
50 its function was to level out the flow and dynamics in the loading over the day as there was  
51 regularly low flow during nights in the sewage pipe. Using a peristaltic pump (Pump 2) from  
52 Watson-Marlow (Ringsted, Denmark) the water was pumped from H0 to H1. The main parameters  
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5 of the wastewater (pH, oxygen, temperature, COD, DOC, NH<sub>4</sub>-N, NO<sub>2</sub>-N and NO<sub>3</sub>-N) were  
6 measured regularly.  
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10 It was intended to perform BOD removal and nitrification in H1 and have biofilms operating on low  
11 BOD loading in H2, H3 and P being more adapted to degrade complex and difficult to degrade  
12 organic matter that the sludge cannot degrade. It was hypothesised that this might induce an ability  
13 to degrade pharmaceuticals that are recalcitrant to sludge treatment.  
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## 21 **2.2 Experimental design**

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24 The study used two different methodologies to experimentally investigate the system's capacity to  
25 degrade pharmaceuticals from hospital wastewater. First, a batch experiment was conducted to test  
26 which of the spiked compounds could be potentially degraded in the treatment train and to study  
27 their degradation kinetics. Then, a continuous flow experiment was performed to mimic the real  
28 performance of the system  
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### 38 **2.2.1 Batch experiment**

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41 To work with each reactor of the system as a unit, the flow was stopped by turning off the two  
42 pumps (See figure 1). The connecting tubes were also blocked with clamps. As not all compounds  
43 are used in a hospital at all times of the each reactor was spiked with a stock solution containing all  
44 the target compounds, which achieved nominal concentrations of 14 µg L<sup>-1</sup> for the pharmaceuticals  
45 and 200 µg L<sup>-1</sup> for the X-ray contrast media to make sure in all reactors was enough for kinetic  
46 assessment. After the spiking, from 1 minute to 24 h, 10 mL samples were taken with a glass pipette  
47 from each reactor. The concentrations of pharmaceuticals were analysed by HPLC-MS and the  
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5 determined concentrations (resulting from the original plus spiked) were used for further  
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7 assessment).

### 11 12 **2.2.2 Continuous flow experiment**

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15 A 'volume of water' was monitored through the stages of the system considering the hydraulic  
16 retention time. To exclude concentration variations, the inflow of water from the sewer into the  
17 equalization tank H0 was stopped (Pump 1; see figure 1) and the reactors received the water from  
18 H0. The system worked with native concentrations of compounds (as they occurred in the hospital  
19 wastewater). No additional pharmaceuticals were added. H0 and H1 were sampled three times  
20 during the first 3 hours of the experiment. For the following stages, the hydraulic flow was pursued  
21 by sampling in accordance to the hydraulic retention time of the system. Sampling times can be  
22 seen in Figure 3.  
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### 34 35 36 **2.3 Chemicals**

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39 The compounds used for calibration as well as for spiking in the batch experiments were obtained  
40 from diverse suppliers as presented in the supplementary material (S1) along with structures and  
41 CAS numbers. The selection was based on a Danish prioritisation list of pharmaceuticals of concern  
42 adopted to be run all in one chromatographic method to gain best insights into reactor performance.  
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5 The following compounds were analysed:  
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7 Antibiotics: Azithromycin, Ciprofloxacin, Clarithromycin, Clindamycin, Erythromycin,  
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9 Sulfadiazine, Sulfamethizole, Sulfamethoxazole, Trimethoprim  
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11 Blood pressure regulators: Atenolol, Metoprolol, Propranolol, Sotalol  
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13 Diverse: Carbamazepine, Diclofenac, Ibuprofen, Phenazone, Tramadol, Citalopram, Venlafaxine  
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15 X-ray contrast media: Diatrizoic acid, Iohexol, Iopromide, Iomeprol, Iopamidol  
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18 And the metabolite Acetyl-Sulfadiazine  
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23 Formic acid and gradient grade methanol were from Merck (Darmstadt, Germany); water was  
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25 obtained from an in-house Millipore apparatus.  
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## 30 **2.4 Quantification of general parameters** 31 32

33 The main parameters were measured frequently in the treatment train. Samples for Dissolved  
34 Organic Carbon (DOC), Chemical Oxygen Demand (COD), ammonium nitrogen ( $\text{NH}_4^+\text{-N}$ ), nitrite  
35 nitrogen ( $\text{NO}_2^-\text{N}$ ) and nitrate nitrogen ( $\text{NO}_3^-\text{N}$ ) were collected and analysed for all reactors.  
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37 Subsamples of approximately 50mL were filtered with a 0.45 $\mu\text{m}$  filter. Samples for DOC were  
38 preserved in the freezer until analysis, while samples for COD,  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_2^-\text{N}$  and  $\text{NO}_3^-\text{N}$   
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40 were analysed the same day.  
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47 COD,  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_2^-\text{N}$  and  $\text{NO}_3^-\text{N}$  concentrations [mg/L] were analysed on a Hach Lange robot  
48  
49 Rohasys, AP 3800 Multi with the application of Hach Lange cuvette tests for COD (LCK 414),  
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51  $\text{NH}_4^+\text{-N}$  (LCK 303/304),  $\text{NO}_2^-\text{N}$  (LCK 341) and  $\text{NO}_3^-\text{N}$  (LCK 339) following the manufacturer's  
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53 instructions (HACH LANGE GMBH, Düsseldorf, Germany).  
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## 2.5 Quantification of biomass in suspended sludge and biofilm on carriers

### 2.5.1 Suspended Sludge

Sludge from Viby municipal WWTP was used for seeding of the Hybas™ plant. The concentration of suspended solids was kept between 3.0- 3.5 g/L and fresh sludge was added every two weeks to compensate for losses. The suspended solids were determined by filtering through a paper filter with 0.45 µm pore width succeeded by drying and weight determination. The reactors were operated with 100% sludge recycle thus giving in reality a sludge age of around 20 weeks/140d.

### 2.5.2 Biofilm on carriers

Ten carriers from H2, H3 and P reactors were placed on an aluminum-foil cup, dried overnight at 105°C, and weighed. The carriers were then washed in tepid 2M NaOH and cleaned with de-ionized water. After washing, the carriers were dried again at 105 °C overnight and the aluminium cup was weighed with and without carriers. Content of biomass on the carriers was calculated as the weight difference before and after cleaning of carriers. The biomass content per area was calculated knowing that each carrier has a protective surface area of 0.00242 m<sup>2</sup>.

## 2.6 Quantification of pharmaceuticals

### 2.6.1 Sample preparation and analysis

After each sampling, the samples were stored in a fridge at 4°C. When the experiment was finished, all samples were transported under cooled conditions to the laboratory. Once there, 3.5 mL of methanol were added to each 10 mL sample and put to -20°C while waiting for analysis. When

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5 analysing, the samples were left to reach room temperature and homogenized. 1.5 mL subsamples  
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7 of each sample were transferred to HPLC vials and centrifuged for 10 minutes at 6000 rpm. 900  $\mu$ L  
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9 of the aqueous phase were taken with a syringe and transferred to a new HPLC vial. 100  $\mu$ L of  
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11 internal standard were added to each sample with a glass syringe. Finally, the samples were  
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13 analysed by means of HPLC-MS/MS. Injections of 10 and 50  $\mu$ L were used for the batch and  
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15 concentration profile experiments correspondingly.  
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## 21 22 **2.6.2 HPLC-MS/MS**

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25 The concentrations of the pharmaceuticals in the hospital wastewater and the treatment reactors  
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27 were quantified by means of HPLC-MS/MS. The HPLC had a dual low-pressure mixing ternary-  
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29 gradient system Ultimate 3000 from Dionex. The system operated with a pump of the 3000 series  
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31 (DGP-3600 M), a 3000 TSL autosampler (WPS 3000 TSL) and a column oven and degasser also  
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33 from Dionex 3000. The mass spectrometer model was an API 4000 (ABSciex, Framingham, MA,  
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35 USA). HPLC and MS/MS conditions are stated in the supplementary information (S2).  
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## 40 41 **2.6.3 Data treatment**

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44 The concentrations of all the compounds over time in the wastewater were plotted. For each  
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46 experiment, the data was treated in a different way as described in the following lines.  
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### 50 51 **2.6.3.1 Batch experiment**

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54 Concentrations were plotted for each reactor and compound. Figure 2 shows nine illustrative  
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56 compounds while the remaining compounds are shown in the supplementary material (Figure S1).  
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58 First-order degradation equation (Equation 1) was fitted using GraphPad Prism, with no weighting:  
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7 Equation 1:  $C = (C_0) \cdot e^{-kt}$   
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12  $C$  expresses initial and concentration,  $k$  is the removal rate constant,  $t$  stands for time.

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14 The  $k$  values of each reactor were used to calculate a removal (%) of each of the compounds within  
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16 the treatment train by using Equation 2:  
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18 Equation 2:  
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$$\frac{C_P}{C_{H0}} = \frac{v_i}{(1 + k_P \cdot \tau_P) \cdot ((1 + k_{H1} \cdot \tau_{H1}) \cdot (1 + k_{H2} \cdot \tau_{H2}) \cdot (1 + k_{H3} \cdot \tau_{H3}) \cdot (v_i + v_r) - v_r)}$$
  
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27  $C_P$  and  $C_{H0}$  express the concentrations in the reactors H0 and P [ $\mu\text{g/L}$ ] correspondingly,  $k_i$  express  
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29 the removal rate constants [ $\text{h}^{-1}$ ] in the H1, H2, H3 and P reactors.  $\tau_i$  refers to the hydraulic residence  
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31 times [h] of H1, H2, H3 and P reactors while  $v_i$  and  $v_r$  describe the inflow and recirculation flows  
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33 [ $\text{L/h}$ ] respectively.  
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39 As expressed on Equation 3, the hydraulic residence time for H1, H2 and H3 was the same and can  
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41 be found as the tank volume divided with the sum of inflow and recirculation flows. For the last  
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43 reactor (P), the hydraulic residence time was different than H1-H3 and can be calculated as tank  
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45 volume divided with inflow.  
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50 Equation 3:  
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$$\tau_{H1} = \frac{\text{Tank volume}}{v_i + v_r} = \tau_{H2} = \tau_{H3} \neq \tau_P; \tau_P = \frac{\text{Tank volume}}{v_i}$$
  
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5 Compounds giving estimated removals over 20% during the treatment system were considered to be  
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7 degradable.  
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### 10 11 **2.6.3.2 Continuous flow experiment** 12

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14 The concentrations measured during 3 hours in each reactor were represented in a plot. Figure 3  
15 shows four representative compounds. The removal (%) was calculated by comparing  
16 concentrations from H0 to H1, H2, H3 and P according to Equation 4:  
17  
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21 Equation 4:  
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$$23 \quad \text{Removal (\%)} = \left( 1 - \frac{\text{Concentration last tank (P)}}{\text{Concentration initial tank (H0)}} \right) \times 100$$

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30 For the degradation evaluation, a compound was considered degradable when the removal (%) after  
31 the treatment train would be over 20%. This assessment was based on an expanded uncertainty  
32 (measurements and experimental) of  $\pm 10\%$ .  
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## 41 **3. Results and discussion** 42

### 43 44 **3.1 General Parameters for the treatment system** 45

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47 The system had operated continuously for 10 months before the experiments were performed. All  
48 flows and set points of the system were kept constant for the last two months after which the  
49 experiments were performed. The parameters that characterise the general water treatment  
50 performance in this period are summed up in Table 1.  
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5 Oxygen was never below 2.5 mg/L in H1 (sludge only system) and 4.5 mg/L in H2-H4 (biofilm and  
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Oxygen was never below 2.5 mg/L in H1 (sludge only system) and 4.5 mg/L in H2-H4 (biofilm and sludge) thus oxygen concentrations were high enough to support nitrification and aerobic biodegradation at the substrate concentrations in the reactors. In the the two-month period before the experiments the pH was stable between 7 and 8 except in the last reactor (MBBR).

Nitrification reached 80-90 % already in H1 that only had sludge, and was complete in H2. This shows a high nitrification capacity in the sludge. Hybas<sup>TM</sup> systems are often designed with higher loading so the sludge mainly degrades organic material (BOD) but have too low sludge age to maintain a high nitrification capacity. Instead biofilm have high nitrifiers density as these bacteria are not washed out with the sludge growth. It can be concluded that the system was very low-loaded and likely a much higher ammonia load could have been treated.

In the experimental period the removal of most of the DOC and COD occurred in H1 which is consistent with the functional design of the system. In the next stages (H2 to P) a further reduction in both parameters is observed (See Table 1). From the COD in and out of the sludge tank H0 an operational BOD concentration  $BOD_{op}$  can be assessed as  $BOD_{op} = COD_{in} - COD_{out} = 244$  mg O/L.

### 3.2 Removal of Pharmaceuticals in the Hybas reactor

The found removal rate constants of the pharmaceuticals were highest in the sludge reactor (H1) during the batch experiment. In contrast, for the continuous flow experiments, the same reactor (H1) showed negative removals (production) for some compounds. Removals between 0 and 71% were observed for reactors H2, H3 and P (Figure 4).

### 3.2.1 Batch experiment

Single phase first-order degradation kinetics (Equation 1) was fitted for each pharmaceutical in the respective reactors. However, for propranolol, a two-phase first order kinetics approach seemed to be a more appropriate fitting (Figure 2) than single phase first-order kinetics. Such fitting is better explained in the supplementary information S5. Figure 2 shows nine illustrative compounds while the degradation kinetics of the rest of the investigated compounds is shown in the supplementary material (S3).

From these kinetic data, removal rate constants were calculated from the batch experiments (Table 2).

### 3.2.2 Continuous flow experiment

The concentrations of pharmaceuticals during the continuous flow experiment are shown as the average concentrations in each reactor. Figure 3 shows nine illustrative compounds. The rest of the compounds are presented in the supplementary information (S4). Considering the 17 detected compounds in H0, 20% of each of 13 compounds was removed during the treatment.

During the continuous flow experiment most pharmaceuticals were removed. However, some pharmaceuticals presented low or negative removals (i.e. they were produced) (Figures 3 and 4). This is a phenomenon that can occur with compounds that are eliminated via urine or faeces in a conjugate form. Such conjugates are usually formed during phase II of human metabolism by sulfation, acetylation and glucuronidation to increase solubility and to facilitate excretion (Timbrell 2009). These excreted conjugates can undergo de-conjugation in the course of the sewer or during the wastewater treatment by bacterial enzymes. In this way effluent concentrations of the parent compound can be bigger than those in the influent. Another study also proposed that the increase of

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5 a parent compound could happen via transformation of metabolites from other parent compounds  
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7 (Kovalova et al. 2012).  
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10 The rise of the amount of the parent pharmaceuticals in the effluent or at an intermittent treatment  
11 stage has formerly been observed in WWTPs (Ternes 1998, Onesios et al. 2009, Falås et al. 2012)  
12 and in hospital wastewater treatment plants (Kovalova et al. 2012, Cruz-Morató et al. 2014). This  
13 effect is expected to be even stronger in source treatment systems as hospital wastewater because  
14 the travel time of conjugates between the source and the treatment is much shorter than in the  
15 sewers that lead to municipal WWTPs. In Figure 4 the removal observed during the continuous  
16 flow experiment are compared to the calculated ones from the batch experiments. This comparison  
17 can be used to discern whether compounds could be potentially biodegraded and to understand each  
18 of the steps (reactors) in the treatment system.  
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30 The removals found in the batch experiment were comparable to the ones obtained in the  
31 continuous flow experiment. The difference in removal percentage between batch and experiment  
32 data corresponded to a standard deviation of  $\pm 33\%$ . This fact indicates that such systems could be  
33 modelled with batch experimental data. On the other side, this study also pointed out how  
34 measuring inlet and outlet concentrations from treatment systems could hide the actual  
35 biodegradation process of compounds that are excreted as conjugates as in the case of the first  
36 reactor (H1), where many compound removals were negative.  
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46 According to Equation 2 the removal over the series of reactors was assessed considering the batch  
47 reactors and compared to the removals found in the train during continuous flow. It was thus  
48 predicted that 16 out of 26 compounds were removed with more than 20% within the treatment  
49 system (Figure 4). From the  $k_i$  values for the system,  $k_{H1}$  or  $k_{H3}$  were in general the highest removal  
50 rate constants (Table 2). The fact that H3 had a high removal rate constant was probably related to a  
51 fairly efficient biomass, even if total biomass in this reactor was less than in the other ones. As H1  
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5 was the first reactor in the treatment train, most of the total removal was occurring there (Figure 4).  
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7 When correcting the  $k_i$  values with the amount of biomass per reactor volume ( $k_{bio}$ ) the most  
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9 efficient reactor appeared to be the polishing MBBR (P). This is an indication that P was actually  
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11 highly adapted to remove micro-pollutants.  
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### 16 **3.3 Pharmaceuticals Removal: Trends**

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19 Different trends were observed through the different compounds in terms of removal and  
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21 degradation kinetics.  
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#### 26 **3.3.1 Beta-blockers**

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29 Beta-blockers (atenolol, metoprolol, propranolol and sotalol) could be degraded in the batch  
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31 experiment. For atenolol (Supplementary information S2 and S 3, numerical data in Table 2) a  
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33 removal close to 100% was determined while it was a bit over 20% for the other beta-blockers.  
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35 During the continuous flow experiment atenolol, metoprolol and propranolol were detected in H0  
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37 and degraded later during the treatment train (Metoprolol and Propranolol in Figure 3). The  
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39 degradation rates were fairly matching the estimated ones from the batch experiment.  
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47 Propranolol concentrations were higher in H1 in comparison to H0, such formation of propranolol  
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49 was compensated later by the following reactors removal. This gave an apparent no-removal of  
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51 propranolol when comparing inlet (H0) and outlet (P) concentrations during the continuous flow  
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53 experiment (Figure 3). Propranolol is known to be the only beta-blocker forming conjugates  
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55 (Documed 2014) and, furthermore it was degraded in the batch experiment. Therefore, this apparent  
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57 no-removal was most likely the result of propranolol being formed by de-conjugation at a similar  
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5 rate than it was being degraded. This finding would also be in agreement with a previous MBR  
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7 study (Kovalova et al., 2012) that also observed negative removals of propranolol in hospital  
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9 wastewater treatments operating at similar hydraulic retention times.  
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14 Remarkably, atenolol was removed better than the other three beta-blockers. Such high removals  
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16 might be explained by co-metabolism. Unlike metoprolol and sotalol, the biodegradation of atenolol  
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18 has been previously observed and linked to the activity of ammonia-oxidizing bacteria and  
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20 heterotrophs (Sathyamoorthy et al., 2013). This hypothesis of co-metabolism fits the present study  
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22 because, again, unlike metoprolol and sotalol, the degradation of atenolol occurs in parallel to  
23  
24 ammonia oxidation (Table 2). The removal of atenolol from wastewater has also shown to be higher  
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26 than the removal for other beta-blockers in previous studies on hospital wastewater treatment  
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28 (Nielsen et al., 2013).  
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34 The degradation of propranolol, metoprolol and sotalol occurred in different tanks than the  
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36 nitrification. This points out that nitrification and degradation of beta-blockers (except perhaps  
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38 atenolol) are independent processes. On the other side, the the removal of atenolol, coinciding with  
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40 nitrification.  
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### 44 45 46 **3.3.2 Iodinated X-ray Contrast Media (ICM)** 47

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49 Iopamidol, iohexol and iomeprol could be degraded following first-order kinetics (Figure 2,  
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51 supplementary material, S2 and S3, numerical data in Table 2), with both, estimated and real  
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53 removal between 60 and 80% (Figure 4). Iopromid and diatrizoic acid were not removed. ICM are  
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55 not metabolized or conjugated in humans (Bourin et al., 1997). Therefore the fact that the data for  
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5 ICMs from the batch experiment matches the continuous flow results better than most other groups  
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7 of pharmaceuticals investigated might be due to the lack of de-conjugation processes.  
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10 Slow sand-filtration biofilm reactors were able to degrade ICM (Escolà Casas and Bester 2015).  
11  
12 Also, MBBR were shown to degrade iohexol and diatrizoic acid from wastewater (Hapeshi et al.,  
13  
14 2013). As the hydraulic retention time in the present study was similar to Kovalova et al., (2012), it  
15  
16 seems that the key to degrade ICM is more likely to rely on the biomass characteristics. In this case  
17  
18 the determining factor appears to be the presence of different types of biofilm in the treatment  
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20 system that are the common factor (Escolà Casas and Bester 2015; Hapeshi et al., 2013; this study).  
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22 In this study biodegradation was detected in the sludge-only reactor (H1). This result is supporting  
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24 the theory that degradation of ICM is not necessarily only occurring in biofilms but can possibly  
25  
26 also be performed by sludge organisms. However, selection of microorganisms that grow up in the  
27  
28 biofilm and seed the sludge might also be of importance. The fact that the system is very low loaded  
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30 in BOD gives a low sludge production and thus the possibility for slow growing microorganisms to  
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32 survive in the sludge without being washed out.  
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37 Iohexol, iomeprol and iopamidol presented the highest  $k_{bio}$  in H1 ( $k_{H1bio}$  in table 2), which contained  
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39 only sludge, and the lowest  $k_{bio}$  in P, which contained only MBBR. The  $k_{bio}$  values in H2 and  
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41 H3 ( $k_{H2bio}$  and  $k_{H3bio}$  in table 2) were lower but close to the H1 value. Since the main part of biomass  
42  
43 in these two tanks was mainly consisting of recirculated sludge from H3 to H1, it makes sense that  
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45 such values do not differ so much from each other (Tables 1 and 2). According to the  $k_{bio}$  values, it  
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47 seems that in this set-up, the sludge biomass community was more efficient in removing ICM than  
48  
49 the MBBR community.  
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53 ICM are usually persistent to conventional WWTP treatment as well as realistic ozone treatment  
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55 (Kovalova et al., 2012, Hapeshi et al., 2013). MBR treatment showed negligible removals of ICM  
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57 from hospital wastewater, except for iopromide which was removed up to 31% (Kovalova et al.,  
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5 2012). Also other hospital wastewater-treatment studies employing MBR (Nielsen et al., 2013) or  
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7 fungal fluidized-bed bioreactor in non-sterile conditions (Cruz-Morató et al., 2014) found low  
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9 removal (ranging from 0 and 44%) for ICM. The direct degradation of the three X-ray contrast  
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11 media by the present treatment train can position Hybas<sup>TM</sup> technology as a solution to remove this  
12  
13 kind of compounds.  
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### 16 17 18 **3.3.3 Sulfonamides** 19 20

21  
22 Sulfadiazine, sulfamethoxazole and sulfamethizole as well as the sulfadiazine metabolite (acetyl-  
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24 sulfadiazine) are a group related structures (supplementary information, S1) that behaved differently  
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26 during the wastewater treatment.  
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29 During the batch experiment, the concentration of sulfadiazine decreased in H1, H2 and H3  
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31 reactors, while it remained stabled in reactor P. Acetyl-sulfadiazine, its main metabolite, seemed to  
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33 follow the same pattern, degrading fairly fast also in H1, H2 and H3 but not in P. Sulfadiazine is  
34  
35 mainly conjugated to acetyl-sulfadiazine in humans and excreted as such (Vree et al., 1995). In the  
36  
37 batch experiments, sulfadiazine and acetyl-sulfadiazine were added, so the de-conjugation of  
38  
39 sulfadiazine and formation of acetyl-sulfadiazine could not be observed. Instead, it was observed  
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41 that both compounds can be degraded in the Hybas<sup>TM</sup> set-up.  
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47 In the continuous flow experiment sulfadiazine concentrations remained stable, while the  
48  
49 concentration of acetyl-sulfadiazine increased by 0.6µg L<sup>-1</sup> (Figure 3). Thus, the expectations that  
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51 acetyl-sulfadiazine would de-conjugate to form sulfadiazine were not fulfilled. This suggests that  
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53 other interactions might occur between sulfadiazine and sulfadiazine metabolites.  
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5 The batch experiment showed that both, sulfamethoxazole and sulfamethizole could be degraded by  
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7 the treatment system (Figure 2 and supplementary information S3). In humans, sulfamethoxazole is  
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9 usually also excreted in acetylated form while sulfamethizole is only acetylated in small amounts  
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11 (3%) (Hekster and Vree 1982). When operating the system at continuous flow, 25% of  
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13 sulfamethizole was removed while the other three sulfonamide concentrations appeared to increase  
14  
15 or stay stable. This is comparable to the results obtained with an MBR by Kovalova et al., (2012)  
16  
17 but different from Nielsen et al., (2013) who found almost complete removal of sulfonamides by an  
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19 MBR treatment.  
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25 Therefore an explanation for stable concentrations of sulfamethoxazole during the continuous flow  
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27 experiment (Figure 3) could be due to the fact that de-conjugation of acetyl-sulfamethoxazole and  
28  
29 degradation of sulfamethoxazole would happen at similar rate constants. De-conjugation of acetyl-  
30  
31 sulfamethoxazole has already been observed in previous studies (, Köhler et al., 2012, Kovalova et  
32  
33 al., 2012) as well as during studies on municipal WWTPs (Onesios et al., 2009).  
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39 Finally, sulfamethizole concentrations diminished by circa  $0.7\mu\text{g L}^{-1}$ . The calculated removal of  
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41 sulfamethizole matched well the experimental removal during the continuous flow experiment. This  
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43 was expected as this compound is not excreted as an acetylated conjugate.  
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#### 48 **3.3.4 Analgesics**

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51 During the batch experiment, phenazone concentrations decreased following first order kinetics but  
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53 the overall removal was under 20%.. In the present study the pure MBBR reactor (P) showed to be  
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55 the one with the lowest removal. This finding might indicate that the degradation of phenazone is a  
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57 co-metabolic process. Phenazone was not detected during the continuous flow experiment.  
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7 In the batch experiments the concentrations of tramadol were decreasing but the removal calculated  
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10 with the resulting reaction rate constants was below 20% (not significant). The removal determined  
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12 during the continuous flow experiment agreed to this calculation. However, during the batch  
13  
14 experiment the removal rate constant of tramadol was the lowest in reactor P while during the  
15  
16 continuous flow experiment reactor P was more effective in removing tramadol. In the previous  
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18 study, on pure MBBR operations, tramadol could not be removed. Also Kovalova et al., (2012)  
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20 found that tramadol was not degraded in an MBR.  
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25 Diclofenac did not degrade in the batch experiment. The degradation data of diclofenac is  
26  
27 inconsistent, showing degradation in some systems and to be recalcitrant in others (Onesios et al.,  
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29 2009). Further studies regarding the degradation conditions for diclofenac should be conducted in  
30  
31 the future.  
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### 3.3.5 Antidepressants/Antiepileptic

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36 For batch and continuous flow experiments, venlafaxine removal was under 20%. Previously  
37  
38 described treatment systems (Nielsen et al., 2013, Kovalova et al., 2012) and data from municipal  
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40 WWTPs (Metcalf et al., 2010) also described poor removal for this compound.  
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45 The removal of carbamazepine was calculated to be close to 20% during the batch. This  
46  
47 concentration decrease only occurred in the P reactor during the continuous flow experiment. A  
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49 previous project operating MBBR reactors in series showed similar results for carbamazepine. The  
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51 fact that carbamazepine could be degraded by this MBBR is a small success as carbamazepine is  
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53 usually a very recalcitrant compound in activated sludge WWTP and it has not been degraded in  
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5 other hospital wastewater-treatment projects (Nielsen et al., 2013, Kovalova et al., 2012, Falås et  
6 al., 2013, Onesios et al., 2009).  
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11 In the batch experiment citalopram was removed up to a bit less than 20% while during the  
12 continuous flow experiment the concentration of this compound increased during the H1 treatment,  
13 stayed stable through H2 and H3 and then decreased during its pass through the P reactor. In a  
14 previous experiment using MBBR reactors in series citalopram was degraded during the batch  
15 experiment but it remained stable during the continuous flow experiment. No removal of citalopram  
16 was also observed in similar projects and WWTPs (Nielsen et al., 2013, Metcalfe et al., 2010). The  
17 increase of citalopram concentrations in H1 might be due to de-conjugation or formation from other  
18 metabolites. Such citalopram concentrations were attenuated during through the passage of the P  
19 reactor.  
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### 37 **3.3.6 Macrolides and other antibiotics**

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40 The removals of the macrolides erythromycin, clindamycin and clarithromycin were estimated from  
41 the batch experiments and found to range from 30 to 55% while azithromycin did not degrade. For  
42 these compounds, the removals in continuous flow experiments were around 40% for erythromycin  
43 and close to 100% for clindamycin. This data is to some extent in agreement with the previous  
44 studies by Kovalova et al., (2012) who reported macrolides removals by an MBR between 20 to  
45 60%. Concerning clindamycin, in two prior studies, it was formed rather than eliminated (Nielsen et  
46 al., 2013, Kovalova et al., 2012). In contrast, in the present study, clindamycin degraded in both, the  
47 batch and continuous flow experiments. The key to understand the results discrepancy with  
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5 previous literature might rely on the sludge/biofilm characteristics of the system that, somehow,  
6 developed efficient clindamycin degraders.  
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11 Regarding other antibiotics, ciprofloxacin was not degraded as reported in Kovalova et al., (2012)  
12 and Nielsen et al., (2013), where ciprofloxacin degraded 50 and 36% respectively in the MBR. The  
13 estimated removal of trimethoprim from the first-order kinetics of batch experiments was a bit over  
14 20%, which agrees well with the removal found in the continuous flow experiment (Figure 2).  
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21 Trimethoprim was produced in H1 but degraded during the following steps of the treatment train.  
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## 24 25 26 **5. Conclusion** 27

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29 A train of four reactors combining sludge, Hybas<sup>TM</sup> and a polishing MBBR reached similar or better  
30 removal efficiencies of pharmaceuticals compared to other technologies (i.e activated sludge or  
31 MBR), particularly when considering specific compounds (like X-ray contrast media).  
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35 The degradation of organic matter and nitrification mainly occurred in the first treatment reactor  
36 indicating that the system was a low loaded one compared to the systems for treating general  
37 wastewater. In addition, Hybas<sup>TM</sup> also reached low organic matter values in the outlet through a  
38 consistent (but small) stepwise removal in the following treatment steps which maintains a low  
39 loaded biofilm adapted for growing on hardly degradable organic matter. This process results in an  
40 effluent which is low in DOC and thus more suitable for a cost effective polishing by ozonation  
41 (Antoniou, 2013).  
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52 The batch experiment helped to assess the potential biodegradation of pharmaceuticals in the  
53 systems whereas the continuous flow experiment reported actual performance values. Batch and  
54 continuous flow experimental results matched for several compounds, especially those that are  
55 excreted unchanged. This means that de-conjugation, bonding to particles or formations from other  
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5 metabolites are facts that have to be taken in account in such wastewater systems. – It is, however  
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7 expected that the reaction rate constants in full scale will not 100% match those determined in the  
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9 small pilot. For these reasons currently a large pilot with 1m<sup>3</sup> tanks is under construction that will  
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11 be able to treat wastewater for a whole hospital department before applying these principles to  
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13 fullscale plants.  
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16 This study also evaluated the effectiveness and role of each reactor. The first three reactors were  
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18 well designed for COD, nitrogen removal and for compounds that might degrade preferably through  
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20 sludge biomass (e.g. X-ray contrast media). On the other hand, the specific activity ( $k_{bio}$ ) of the  
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22 biomass for micro-pollutants removal was the highest in the last reactor in most cases. Therefore,  
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24 further designs such as up-scaling or addition of ozonation steps should envision a larger polishing  
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26 reactor in order to maximize the removal of recalcitrant pharmaceuticals. Another design option  
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28 could be to operate a high-load Hybas<sup>TM</sup> for BOD removal and a secondary Hybas<sup>TM</sup> for removal of  
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30 organic micro-pollutants.  
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34 The tested combination of sludge, Hybas<sup>TM</sup> and MBBR resulted to be a compact, robust and easy-  
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36 to-operate technology that showed to remove not only COD and nitrogen but also some recalcitrant  
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38 micro-pollutants. Therefore, this type of wastewater treatment can be seen as a clever solution for  
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40 hospital wastewater treatment.  
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## 43 44 45 46 **Acknowledgments**

47 The authors want to acknowledge the AUFF grant “Advanced water purification using bio-  
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49 inorganic nanocatalysts and soil filters” (<http://waterpurification.au.dk/>) and the MERMISS project  
50  
51 (Miljøeffektiv rensning af højpotente lægemiddelstoffer i hospitalsspildevand - Environmentally  
52  
53 effective removal of pharmaceuticals from hospital wastewater) with all its co-founding, active and  
54  
55 supporting partners. The authors are also grateful to Kim Sundmark and the rest of the operation  
56  
57 team for the support in operating the pilot plant.  
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## Tables and Figures

### Tables:

Table 1: General parameters  $\pm$  standard deviation for the Hybas™ system during a two months period including the sampling for both experiments.

Reactor	HRT	Biomass			pH	DOC	COD	NH <sub>4</sub> <sup>+</sup> -N	NO <sub>2</sub> <sup>-</sup> -N	NO <sub>3</sub> <sup>-</sup> -N
		Sludge [g/L]	Carriers [g/m <sup>2</sup> ]	[g/L]						
H0	-	0.17	-	-	7.6 $\pm$ 0.28	59 $\pm$ 27	299 $\pm$ 209	54 $\pm$ 16	0.12 $\pm$ 0.13	1.7 $\pm$ 1.3
H1	3	3.12	-	-	7.4 $\pm$ 0.24	15 $\pm$ 3	55 $\pm$ 3	7 $\pm$ 6	0.97 $\pm$ 1.32	50 $\pm$ 7
H2	3	3.12	6.0	2.4	7.3 $\pm$ 0.42	16 $\pm$ 1	50 $\pm$ 10	0.30 $\pm$ 0.16	0.06 $\pm$ 0.04	56 $\pm$ 5
H3	3	3.12	1.9	0.8	7.4 $\pm$ 0.52	14 $\pm$ 2	49 $\pm$ 8	0.06 $\pm$ 0.05	0.03 $\pm$ 0.02	60 $\pm$ 9
P	3	0.04	2.5	1.0	7.8 $\pm$ 0.48	12 $\pm$ 3	43 $\pm$ 12	0.60 $\pm$ 0.74	0.02 $\pm$ 0.01	49 $\pm$ 14

HRT= Hydraulic retention time [h]

Table 2: Parameters from curve fitting (Eq. 1). R2 relates to the fit for determining the rate constant  $k$ ,  $k_i$ : reactor removal rate constant,  $k_{bio}$ : removal rate constant per gram of biomass per liter (according to Table 1). Bold values: highest  $k_i$  or  $k_{bio}$  among the three reactors. na=mathematically not defined.

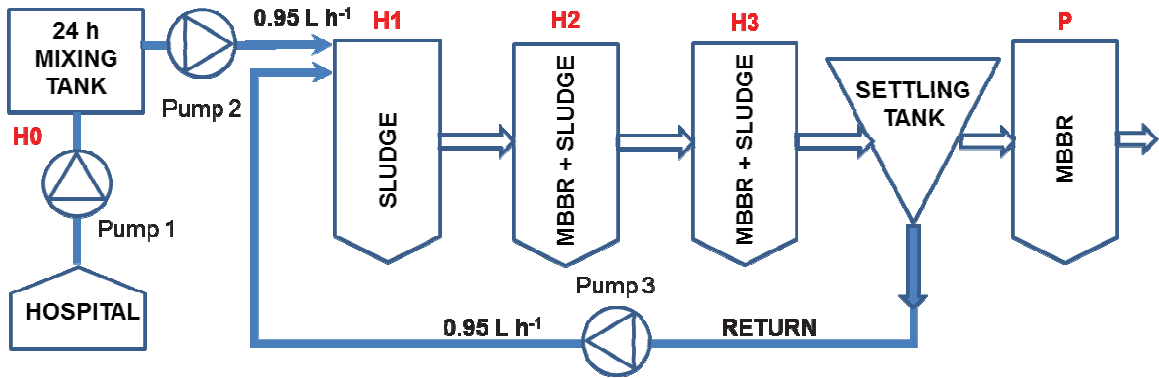
	Reactor H1			Reactor H2			Reactor H3			Reactor H4		
	$R^2$	$k_{H1}$	$k_{H1bio}$	$R^2$	$k_{H2}$	$k_{H2bio}$	$R^2$	$k_{H3}$	$k_{H3bio}$	$R^2$	$k_{H4}$	$k_{H4bio}$
Acetyl-sulfadiazine	0.93	<b>1.49×10<sup>0</sup></b>	<b>4.77×10<sup>-1</sup></b>	0.99	7.78×10 <sup>-1</sup>	1.41×10 <sup>-1</sup>	0.97	7.86×10 <sup>-1</sup>	2.01×10 <sup>-1</sup>	0.20	1.13×10 <sup>-2</sup>	1.09×10 <sup>-2</sup>
Atenolol	0.94	<b>1.14×10<sup>0</sup></b>	<b>3.66×10<sup>-1</sup></b>	0.97	6.82×10 <sup>-1</sup>	1.23×10 <sup>-1</sup>	0.98	7.37×10 <sup>-1</sup>	1.88×10 <sup>-1</sup>	0.90	5.19×10 <sup>-2</sup>	4.99×10 <sup>-2</sup>
Azitromycin	na	na	na	na	na	na	na	na	na	na	na	na
Carbamazepine	0.44	1.57×10 <sup>-2</sup>	5.03×10 <sup>-3</sup>	0.57	1.81×10 <sup>-2</sup>	3.28×10 <sup>-3</sup>	0.61	<b>1.88×10<sup>-2</sup></b>	4.81×10 <sup>-3</sup>	0.54	1.68×10 <sup>-2</sup>	<b>1.62×10<sup>-2</sup></b>
Clarithromycin	0.86	<b>9.10×10<sup>-2</sup></b>	2.92×10 <sup>-2</sup>	0.90	7.62×10 <sup>-2</sup>	1.38×10 <sup>-2</sup>	0.93	8.23×10 <sup>-2</sup>	2.10×10 <sup>-2</sup>	0.75	3.59×10 <sup>-2</sup>	<b>3.45×10<sup>-2</sup></b>
Ciprofloxacin	na	na	na	na	na	na	na	na	na	na	n.a	na
Citalopram	0.57	1.66×10 <sup>-2</sup>	5.32×10 <sup>-3</sup>	0.71	1.50×10 <sup>-2</sup>	2.72×10 <sup>-3</sup>	0.59	1.52×10 <sup>-2</sup>	3.87×10 <sup>-3</sup>	0.85	<b>2.14×10<sup>-2</sup></b>	<b>2.06×10<sup>-2</sup></b>
Clindamycin	0.81	<b>5.04×10<sup>-2</sup></b>	1.62×10 <sup>-2</sup>	0.92	4.80×10 <sup>-2</sup>	8.69×10 <sup>-3</sup>	0.90	3.45×10 <sup>-2</sup>	8.80×10 <sup>-3</sup>	0.68	1.87×10 <sup>-2</sup>	<b>1.80×10<sup>-2</sup></b>
Diatrizoic acid	0.03	4.61×10 <sup>-3</sup>	1.48×10 <sup>-3</sup>	0.07	5.05×10 <sup>-3</sup>	9.14×10 <sup>-4</sup>	0.09	<b>5.57×10<sup>-3</sup></b>	1.42×10 <sup>-3</sup>	0.09	5.41×10 <sup>-3</sup>	<b>5.20×10<sup>-3</sup></b>
Diclofenac	0.23	6.67×10 <sup>-3</sup>	2.14×10 <sup>-3</sup>	0.76	1.09×10 <sup>-2</sup>	1.97×10 <sup>-3</sup>	0.69	<b>1.33×10<sup>-2</sup></b>	<b>3.39×10<sup>-3</sup></b>	0.13	3.48×10 <sup>-3</sup>	3.34×10 <sup>-3</sup>
Erythromycin	0.68	3.28×10 <sup>-2</sup>	1.05×10 <sup>-2</sup>	0.84	2.88×10 <sup>-2</sup>	5.22×10 <sup>-3</sup>	0.93	<b>3.32×10<sup>-2</sup></b>	8.47×10 <sup>-3</sup>	0.71	2.09×10 <sup>-2</sup>	<b>2.01×10<sup>-2</sup></b>
Iohexol	0.94	<b>2.40×10<sup>-1</sup></b>	<b>7.68×10<sup>-2</sup></b>	0.99	1.96×10 <sup>-1</sup>	3.55×10 <sup>-2</sup>	0.99	1.97×10 <sup>-1</sup>	5.03×10 <sup>-2</sup>	0.39	1.29×10 <sup>-2</sup>	1.24×10 <sup>-2</sup>
lomeprol	0.91	<b>1.48×10<sup>-1</sup></b>	<b>4.73×10<sup>-2</sup></b>	0.93	1.34×10 <sup>-1</sup>	2.42×10 <sup>-2</sup>	0.95	1.31×10 <sup>-1</sup>	3.33×10 <sup>-2</sup>	0.32	1.13×10 <sup>-2</sup>	1.08×10 <sup>-2</sup>
lopamidol	0.89	2.03×10 <sup>-1</sup>	<b>6.51×10<sup>-2</sup></b>	0.96	1.79×10 <sup>-1</sup>	3.24×10 <sup>-2</sup>	0.97	1.72×10 <sup>-1</sup>	4.39×10 <sup>-2</sup>	0.58	<b>2.22×10<sup>-2</sup></b>	2.14×10 <sup>-2</sup>

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Iopromide	0.20	<b>1.16×10<sup>-2</sup></b>	3.73×10 <sup>-3</sup>	0.10	7.87×10 <sup>-3</sup>	1.43×10 <sup>-3</sup>	0.15	7.52×10 <sup>-3</sup>	1.92×10 <sup>-3</sup>	0.15	6.94×10 <sup>-3</sup>	<b>6.68×10<sup>-3</sup></b>
Metoprolol	0.89	<b>4.04×10<sup>-2</sup></b>	<b>1.29×10<sup>-2</sup></b>	0.98	3.66×10 <sup>-2</sup>	6.64×10 <sup>-3</sup>	0.99	3.79×10 <sup>-2</sup>	9.67×10 <sup>-3</sup>	0.88	9.95×10 <sup>-3</sup>	9.57×10 <sup>-3</sup>
Phenazone	0.67	1.75×10 <sup>-2</sup>	5.62×10 <sup>-3</sup>	0.93	1.70×10 <sup>-2</sup>	3.08×10 <sup>-3</sup>	0.96	<b>1.80×10<sup>-2</sup></b>	4.59×10 <sup>-3</sup>	0.73	6.10×10 <sup>-3</sup>	<b>5.86×10<sup>-3</sup></b>
Propranolol	0.76	<b>2.46×10<sup>-2</sup></b>	7.89×10 <sup>-3</sup>	0.76	2.25×10 <sup>-2</sup>	4.08×10 <sup>-3</sup>	0.73	2.22×10 <sup>-2</sup>	5.66×10 <sup>-3</sup>	0.88	2.15×10 <sup>-2</sup>	<b>2.07×10<sup>-2</sup></b>
Sotalol	0.76	<b>2.29×10<sup>-2</sup></b>	7.34×10 <sup>-3</sup>	0.96	2.20×10 <sup>-2</sup>	3.99×10 <sup>-3</sup>	0.98	2.19×10 <sup>-2</sup>	5.59×10 <sup>-3</sup>	0.88	1.01×10 <sup>-2</sup>	<b>9.72×10<sup>-3</sup></b>
Sulfadiazine	0.71	<b>3.68×10<sup>-2</sup></b>	<b>1.18×10<sup>-2</sup></b>	0.76	3.40×10 <sup>-2</sup>	6.16×10 <sup>-3</sup>	0.64	2.80×10 <sup>-2</sup>	7.14×10 <sup>-3</sup>	0.00	1.42×10 <sup>-3</sup>	1.36×10 <sup>-3</sup>
Sulfamethizole	0.72	3.05×10 <sup>-2</sup>	9.78×10 <sup>-3</sup>	0.88	3.39×10 <sup>-2</sup>	6.14×10 <sup>-3</sup>	0.94	<b>3.93×10<sup>-2</sup></b>	<b>1.00×10<sup>-2</sup></b>	0.56	8.95×10 <sup>-3</sup>	8.61×10 <sup>-3</sup>
Sulfamethoxazole	0.81	3.12×10 <sup>-2</sup>	<b>9.99×10<sup>-3</sup></b>	0.93	3.17×10 <sup>-2</sup>	5.75×10 <sup>-3</sup>	0.95	<b>2.99×10<sup>-2</sup></b>	7.62×10 <sup>-3</sup>	0.48	3.75×10 <sup>-3</sup>	3.60×10 <sup>-3</sup>
Trimethoprim	0.74	2.40×10 <sup>-2</sup>	7.68×10 <sup>-3</sup>	0.91	2.98×10 <sup>-2</sup>	5.39×10 <sup>-3</sup>	0.88	<b>3.02×10<sup>-2</sup></b>	7.70×10 <sup>-3</sup>	0.57	1.17×10 <sup>-2</sup>	<b>1.12×10<sup>-2</sup></b>
Tramadol	0.67	1.75×10 <sup>-2</sup>	5.62×10 <sup>-3</sup>	0.93	1.70×10 <sup>-2</sup>	3.08×10 <sup>-3</sup>	0.96	<b>1.80×10<sup>-2</sup></b>	4.59×10 <sup>-3</sup>	0.73	6.10×10 <sup>-3</sup>	<b>5.86×10<sup>-3</sup></b>
Venlafaxine	0.34	<b>7.87×10<sup>-3</sup></b>	2.52×10 <sup>-3</sup>	0.93	6.87×10 <sup>-3</sup>	1.25×10 <sup>-3</sup>	0.86	7.69×10 <sup>-3</sup>	1.96×10 <sup>-3</sup>	0.82	6.48×10 <sup>-3</sup>	<b>6.23×10<sup>-3</sup></b>



Figure 1: Overview of the pilot plant in Aarhus University Hospital.



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Figure 2: Concentrations of selected pharmaceuticals during the batch experiment in each reactor (H1, H2, H3 and P). Filled lines correspond to a first-order kinetics fitting (Equation 1).

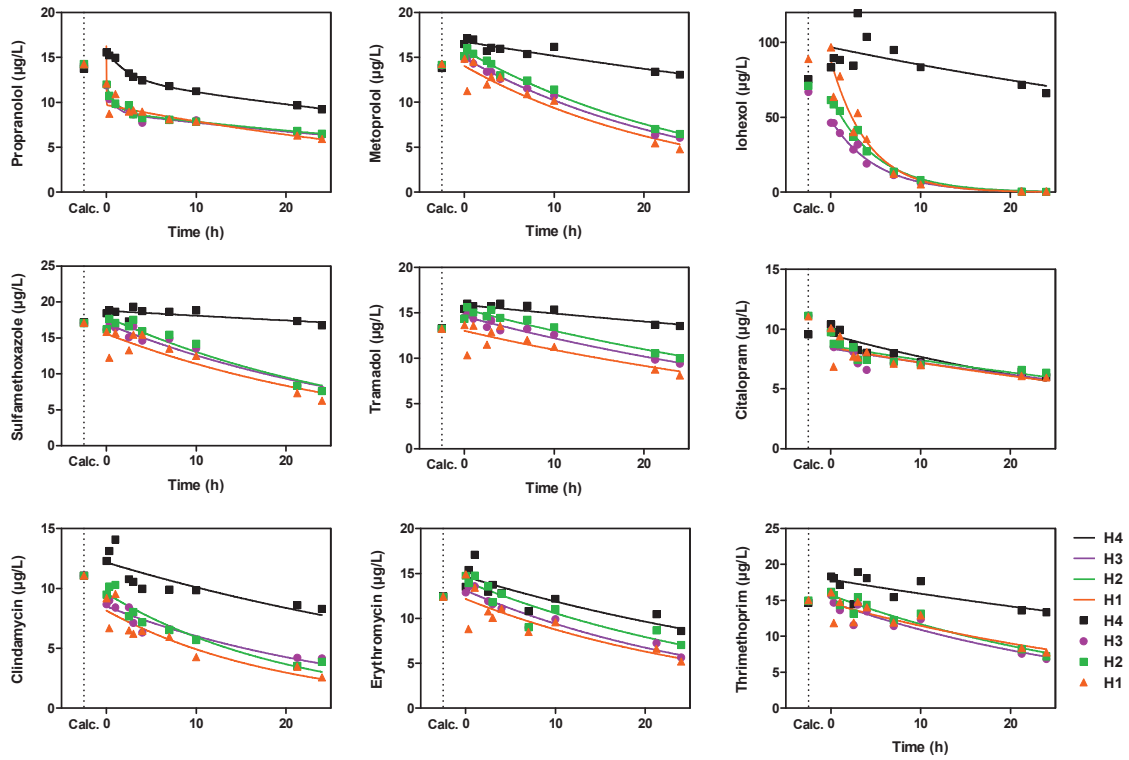


Figure 3: Average concentrations for selected compounds in the reactors during the continuous flow experiment. The error bars show the SD (n=2 samples for H0 and n=4 for the rest, each sample was analysed twice. SD was then derived from 4 measurements in H0 and 8 measurements in the rest of the tanks).

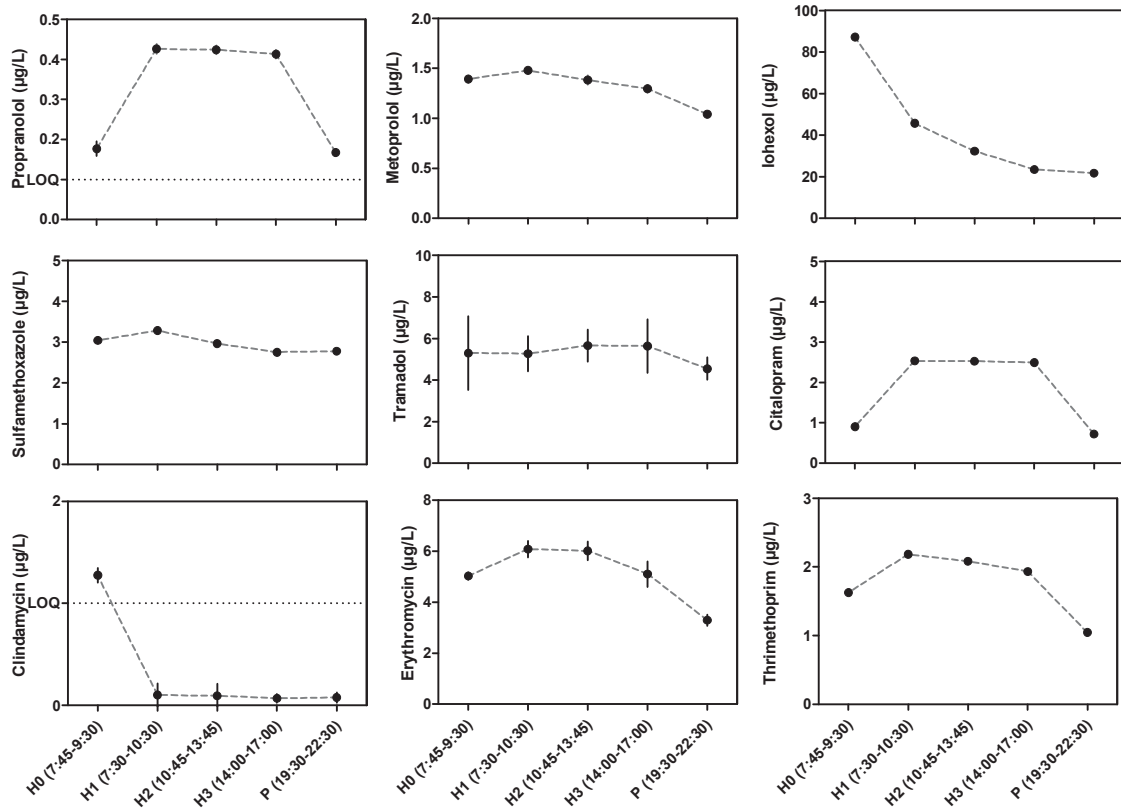
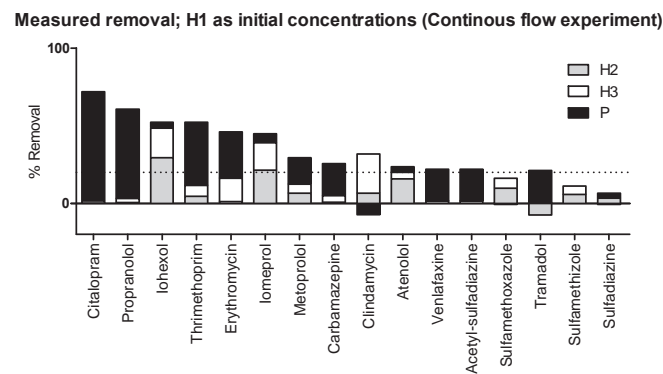
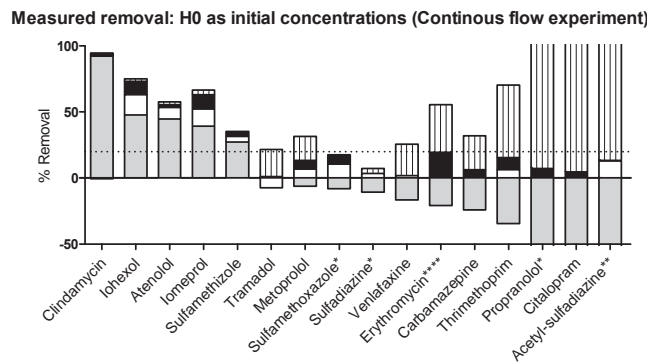
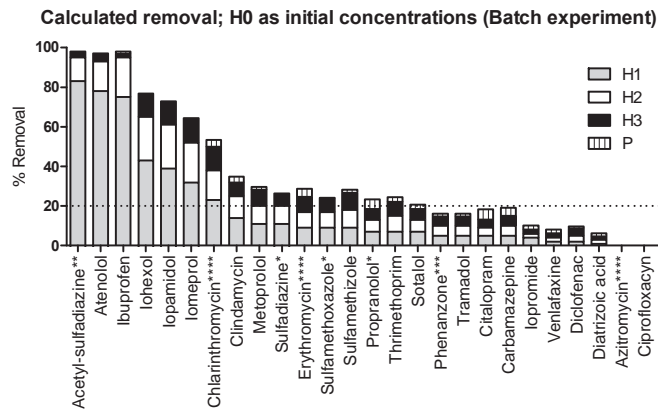


Figure 4: Removal estimated from the batch experiment (Equation 2) compared to the measured removal in the continuous flow experiment by applying Equation 4. \*Known to be excreted as conjugates, \*\*known metabolites \*\*\*suggested to be formed from metabolites of other compounds (Kovalova et al. 2012), \*\*\*\*suggested to be bound to faecal particles (Göbel et al. 2007).



**Table 1: General parameters  $\pm$  standard deviation for the Hybas<sup>TM</sup> system during a two months period including the sampling for both experiments.**

Reactor	HRT	Biomass			pH	DOC	COD	NH <sub>4</sub> <sup>+</sup> -N	NO <sub>2</sub> <sup>-</sup> -N	NO <sub>3</sub> <sup>-</sup> -N
		Sludge	Carriers			[mgC/L]	[mgO/L]	[mgN/L]	[mgN/L]	[mgN/L]
		[g/L]	[g/m <sup>2</sup> ]	[g/L]						
H0	-	0.17	-	-	7.6 $\pm$ 0.28	59 $\pm$ 27	299 $\pm$ 209	54 $\pm$ 16	0.12 $\pm$ 0.13	1.7 $\pm$ 1.3
H1	3	3.12	-	-	7.4 $\pm$ 0.24	15 $\pm$ 3	55 $\pm$ 3	7 $\pm$ 6	0.97 $\pm$ 1.32	50 $\pm$ 7
H2	3	3.12	6.0	2.4	7.3 $\pm$ 0.42	16 $\pm$ 1	50 $\pm$ 10	0.30 $\pm$ 0.16	0.06 $\pm$ 0.04	56 $\pm$ 5
H3	3	3.12	1.9	0.8	7.4 $\pm$ 0.52	14 $\pm$ 2	49 $\pm$ 8	0.06 $\pm$ 0.05	0.03 $\pm$ 0.02	60 $\pm$ 9
P	3	0.04	2.5	1.0	7.8 $\pm$ 0.48	12 $\pm$ 3	43 $\pm$ 12	0.60 $\pm$ 0.74	0.02 $\pm$ 0.01	49 $\pm$ 14

HRT= Hydraulic retention time [h]

Table 2: Parameters from curve fitting (Eq. 1). R2 relates to the fit for determining the rate constant  $k$ ,  $k_i$ : reactor removal rate constant,  $k_{bioMI}$ : removal rate constant per gram of biomass per liter (according to Table 1). Bold values: highest  $k_i$  or  $k_{bio}$  among the three reactors. na=mathematically not defined.

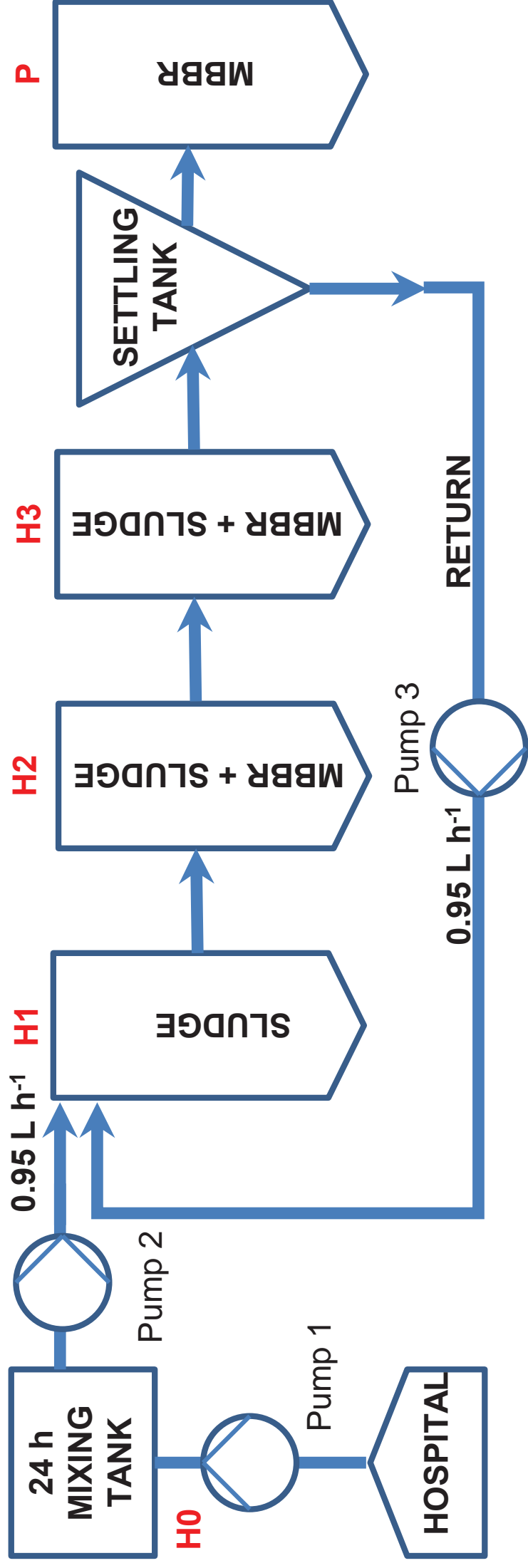
	Reactor H1			Reactor H2			Reactor H3			Reactor H4		
	$R^2$	$k_{H1}$	$k_{H1bio}$	$R^2$	$k_{H2}$	$k_{H2bio}$	$R^2$	$k_{H3}$	$k_{H3bio}$	$R^2$	$k_{H4}$	$k_{H4bio}$
Acetyl-sulfadiazine	0.93	<b>1.49×10<sup>0</sup></b>	<b>4.77×10<sup>-1</sup></b>	0.99	7.78×10 <sup>-1</sup>	1.41×10 <sup>-1</sup>	0.97	7.86×10 <sup>-1</sup>	2.01×10 <sup>-1</sup>	0.20	1.13×10 <sup>-2</sup>	1.09×10 <sup>-2</sup>
Atenolol	0.94	<b>1.14×10<sup>0</sup></b>	<b>3.66×10<sup>-1</sup></b>	0.97	6.82×10 <sup>-1</sup>	1.23×10 <sup>-1</sup>	0.98	7.37×10 <sup>-1</sup>	1.88×10 <sup>-1</sup>	0.90	5.19×10 <sup>-2</sup>	4.99×10 <sup>-2</sup>
Azithromycin	na	na	na	na	na	na	na	na	na	na	na	na
Carbamazepine	0.44	1.57×10 <sup>-2</sup>	5.03×10 <sup>-3</sup>	0.57	1.81×10 <sup>-2</sup>	3.28×10 <sup>-3</sup>	0.61	<b>1.88×10<sup>-2</sup></b>	4.81×10 <sup>-3</sup>	0.54	1.68×10 <sup>-2</sup>	<b>1.62×10<sup>-2</sup></b>
Clarithromycin	0.86	<b>9.10×10<sup>-2</sup></b>	2.92×10 <sup>-2</sup>	0.90	7.62×10 <sup>-2</sup>	1.38×10 <sup>-2</sup>	0.93	8.23×10 <sup>-2</sup>	2.10×10 <sup>-2</sup>	0.75	3.59×10 <sup>-2</sup>	<b>3.45×10<sup>-2</sup></b>
Ciprofloxacin	na	na	na	na	na	na	na	na	na	na	n.a	na
Citalopram	0.57	1.66×10 <sup>-2</sup>	5.32×10 <sup>-3</sup>	0.71	1.50×10 <sup>-2</sup>	2.72×10 <sup>-3</sup>	0.59	1.52×10 <sup>-2</sup>	3.87×10 <sup>-3</sup>	0.85	<b>2.14×10<sup>-2</sup></b>	<b>2.06×10<sup>-2</sup></b>
Clindamycin	0.81	<b>5.04×10<sup>-2</sup></b>	1.62×10 <sup>-2</sup>	0.92	4.80×10 <sup>-2</sup>	8.69×10 <sup>-3</sup>	0.90	3.45×10 <sup>-2</sup>	8.80×10 <sup>-3</sup>	0.68	1.87×10 <sup>-2</sup>	<b>1.80×10<sup>-2</sup></b>
Diatrizoic acid	0.03	4.61×10 <sup>-3</sup>	1.48×10 <sup>-3</sup>	0.07	5.05×10 <sup>-3</sup>	9.14×10 <sup>-4</sup>	0.09	<b>5.57×10<sup>-3</sup></b>	1.42×10 <sup>-3</sup>	0.09	5.41×10 <sup>-3</sup>	<b>5.20×10<sup>-3</sup></b>
Diclofenac	0.23	6.67×10 <sup>-3</sup>	2.14×10 <sup>-3</sup>	0.76	1.09×10 <sup>-2</sup>	1.97×10 <sup>-3</sup>	0.69	<b>1.33×10<sup>-2</sup></b>	<b>3.39×10<sup>-3</sup></b>	0.13	3.48×10 <sup>-3</sup>	3.34×10 <sup>-3</sup>
Erythromycin	0.68	3.28×10 <sup>-2</sup>	1.05×10 <sup>-2</sup>	0.84	2.88×10 <sup>-2</sup>	5.22×10 <sup>-3</sup>	0.93	<b>3.32×10<sup>-2</sup></b>	8.47×10 <sup>-3</sup>	0.71	2.09×10 <sup>-2</sup>	<b>2.01×10<sup>-2</sup></b>
Iohexol	0.94	<b>2.40×10<sup>-1</sup></b>	<b>7.68×10<sup>-2</sup></b>	0.99	1.96×10 <sup>-1</sup>	3.55×10 <sup>-2</sup>	0.99	1.97×10 <sup>-1</sup>	5.03×10 <sup>-2</sup>	0.39	1.29×10 <sup>-2</sup>	1.24×10 <sup>-2</sup>
lomeprol	0.91	<b>1.48×10<sup>-1</sup></b>	<b>4.73×10<sup>-2</sup></b>	0.93	1.34×10 <sup>-1</sup>	2.42×10 <sup>-2</sup>	0.95	1.31×10 <sup>-1</sup>	3.33×10 <sup>-2</sup>	0.32	1.13×10 <sup>-2</sup>	1.08×10 <sup>-2</sup>
lopamidol	0.89	2.03×10 <sup>-1</sup>	<b>6.51×10<sup>-2</sup></b>	0.96	1.79×10 <sup>-1</sup>	3.24×10 <sup>-2</sup>	0.97	1.72×10 <sup>-1</sup>	4.39×10 <sup>-2</sup>	0.58	<b>2.22×10<sup>-2</sup></b>	2.14×10 <sup>-2</sup>
Iopromide	0.20	<b>1.16×10<sup>-2</sup></b>	3.73×10 <sup>-3</sup>	0.10	7.87×10 <sup>-3</sup>	1.43×10 <sup>-3</sup>	0.15	7.52×10 <sup>-3</sup>	1.92×10 <sup>-3</sup>	0.15	6.94×10 <sup>-3</sup>	<b>6.68×10<sup>-3</sup></b>
Metoprolol	0.89	<b>4.04×10<sup>-2</sup></b>	<b>1.29×10<sup>-2</sup></b>	0.98	3.66×10 <sup>-2</sup>	6.64×10 <sup>-3</sup>	0.99	3.79×10 <sup>-2</sup>	9.67×10 <sup>-3</sup>	0.88	9.95×10 <sup>-3</sup>	9.57×10 <sup>-3</sup>

Phenazone	0.67	$1.75 \times 10^{-2}$	$5.62 \times 10^{-3}$	0.93	$1.70 \times 10^{-2}$	$3.08 \times 10^{-3}$	0.96	$1.80 \times 10^{-2}$	$4.59 \times 10^{-3}$	0.73	$6.10 \times 10^{-3}$	$5.86 \times 10^{-3}$
Propranolol	0.76	<b><math>2.46 \times 10^{-2}</math></b>	$7.89 \times 10^{-3}$	0.76	$2.25 \times 10^{-2}$	$4.08 \times 10^{-3}$	0.73	$2.22 \times 10^{-2}$	$5.66 \times 10^{-3}$	0.88	$2.15 \times 10^{-2}$	<b><math>2.07 \times 10^{-2}</math></b>
Sotalol	0.76	<b><math>2.29 \times 10^{-2}</math></b>	$7.34 \times 10^{-3}$	0.96	$2.20 \times 10^{-2}$	$3.99 \times 10^{-3}$	0.98	$2.19 \times 10^{-2}$	$5.59 \times 10^{-3}$	0.88	$1.01 \times 10^{-2}$	<b><math>9.72 \times 10^{-3}</math></b>
Sulfadiazine	0.71	<b><math>3.68 \times 10^{-2}</math></b>	<b><math>1.18 \times 10^{-2}</math></b>	0.76	$3.40 \times 10^{-2}$	$6.16 \times 10^{-3}$	0.64	$2.80 \times 10^{-2}$	$7.14 \times 10^{-3}$	0.00	$1.42 \times 10^{-3}$	$1.36 \times 10^{-3}$
Sulfamethizole	0.72	$3.05 \times 10^{-2}$	$9.78 \times 10^{-3}$	0.88	$3.39 \times 10^{-2}$	$6.14 \times 10^{-3}$	0.94	<b><math>3.93 \times 10^{-2}</math></b>	<b><math>1.00 \times 10^{-2}</math></b>	0.56	$8.95 \times 10^{-3}$	$8.61 \times 10^{-3}$
Sulfamethoxazole	0.81	$3.12 \times 10^{-2}$	<b><math>9.99 \times 10^{-3}</math></b>	0.93	$3.17 \times 10^{-2}$	$5.75 \times 10^{-3}$	0.95	<b><math>2.99 \times 10^{-2}</math></b>	$7.62 \times 10^{-3}$	0.48	$3.75 \times 10^{-3}$	$3.60 \times 10^{-3}$
Trimethoprim	0.74	$2.40 \times 10^{-2}$	$7.68 \times 10^{-3}$	0.91	$2.98 \times 10^{-2}$	$5.39 \times 10^{-3}$	0.88	<b><math>3.02 \times 10^{-2}</math></b>	$7.70 \times 10^{-3}$	0.57	$1.17 \times 10^{-2}$	<b><math>1.12 \times 10^{-2}</math></b>
Tramadol	0.67	$1.75 \times 10^{-2}$	$5.62 \times 10^{-3}$	0.93	$1.70 \times 10^{-2}$	$3.08 \times 10^{-3}$	0.96	<b><math>1.80 \times 10^{-2}</math></b>	$4.59 \times 10^{-3}$	0.73	$6.10 \times 10^{-3}$	<b><math>5.86 \times 10^{-3}</math></b>
Venlafaxine	0.34	<b><math>7.87 \times 10^{-3}</math></b>	$2.52 \times 10^{-3}$	0.93	$6.87 \times 10^{-3}$	$1.25 \times 10^{-3}$	0.86	$7.69 \times 10^{-3}$	$1.96 \times 10^{-3}$	0.82	$6.48 \times 10^{-3}$	<b><math>6.23 \times 10^{-3}</math></b>





Figure  
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