



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; Hansen, 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., Kragelund, C., Andersen, H. R., & 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.
<https://doi.org/10.1016/j.scitotenv.2015.05.099>

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

1
2
3
4
5
6
7
8
9
10 **Biodegradation of pharmaceuticals in hospital**
11
12
13 **wastewater by a hybrid biofilm and activated sludge**
14
15
16
17 **system (Hybas)**
18
19
20
21
22
23
24

25 **Mònica Escolà Casas¹, Ravi Kumar Chhetri², Gordon Ooi¹, Kamilla M. S. Hansen², Klaus**
26 **Litty³, Magnus Christensson⁴, Caroline Kragelund³, Henrik R. Andersen² and Kai Bester^{1*}**
27
28
29
30
31

32 1) Environmental Science, Aarhus University, Frederiksborgsvej 399, 4000 Roskilde, Denmark
33

34 2) Department of Environmental Engineering, Technical University of Denmark, Miljøvej 113,
35
36 2800 Kgs. Lyngby, Denmark
37

38
39 3) Department of Chemistry and Biotechnology, Danish Technological Institute, Kongsvang Allé
40
41 29, 8000 Aarhus C, Denmark
42

43
44 4) AnoxKaldnes, Klosterängsvägen 11A, 226 47 Lund, Sweden
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

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 (HybasTM)) 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 HybasTM 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, β -blockers, analgesics and antibiotics) were fitted to a single first-order kinetics degradation function, giving degradation rate constants from 0 to 1.49 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 HybasTM 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

1
2
3
4
5 al., 2013; Kovalova et al., 2012). Fungal fluidized-bed bioreactors were also tested for
6
7 pharmaceuticals removal in hospital wastewater (Cruz-Morató et al., 2014), and presented better
8
9 efficacy than the activated sludge systems. Finally, suspended biofilm systems such as MBBRs
10
11 (Moving Bed Biofilm Reactors) have appeared to directly remove pharmaceuticals from hospital
12
13 wastewater (Escolà Casas et al., 2015).
14
15

16
17
18 Different biofilm technologies have recently appeared as an effective tool to remove micro-
19
20 pollutants with the main focus being on porous media biofilm processes i.e., slow sand filter e.g.
21
22 (Bester and Schafer, 2009; Escolà Casas and Bester, 2015; Heberer et al., 2004; Hijosa-Valsero et
23
24 al., 2011; Janzen et al., 2009; Li et al., 2014; Reungoat et al., 2011; Zearley and Summers, 2012).
25
26 Along with such biofilm technologies, MBBR also seemed to be a promising solution to remove
27
28 micro-pollutants, which cannot be removed with activated sludge treatment (Hapeshi et al. 2013;
29
30 Zupanc et al. 2013; Falås et al. 2012; Falås et al. 2013; Escolà Casas and Bester 2015).
31
32
33

34
35
36 HybasTM is a hybrid process, based on the integrated fixed-film activated sludge technology, where
37
38 polyethylene carriers for biofilm growth are suspended within activated sludge in one reactor
39
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, HybasTM contains two separate biomasses:
43
44 one with low sludge age (activated sludge flocks), and one with high-sludge age (attached biofilm
45
46 on MBBR-carriers). This fact allows fast growing biomass to be in the form of activated-sludge
47
48 flocks, while the slow-growing biomass develops on the MBBR carriers. The presence of attached
49
50 biofilm in wastewater treatment systems have shown to improve the activated sludge performance
51
52 for nutrient removal (Debabrata, 2010, Randall and Sen, 1996). Falås et al., 2013 demonstrated that
53
54
55
56
57
58
59
60
61
62
63
64
65

1
2
3
4
5 attached biofilm contributes significantly to the overall removal of micro-pollutants in a Hybas™
6
7 system.
8

9
10 According to the literature, Hybas™ treatment seems to be a promising option to treat hospital
11
12 wastewater. Therefore, this study tested and evaluated for the first time the performance of a pilot
13
14 Hybas™ plant for the removal of pharmaceuticals from mechanically pre-treated hospital
15
16 wastewater.
17

20 21 **2. Materials and Methods**

22 23 24 **2.1 Treatment system**

25
26
27
28 A lab-scale treatment line was established to treat a portion of the wastewater produced at the
29
30 oncology section of Aarhus University Hospital. The line consisted of four three liter reactors in
31
32 series (H1, H2, H3 and P). H1 contained only activated sludge, H2 and H3 contained activated
33
34 sludge and biofilm carriers and P was a polishing step containing only biofilm carriers. Each reactor
35
36 (H2, H3 and H4) contained 500 AnoxKaldnes™ K5 carriers (AnoxKaldnes, Lund, Sweden) and
37
38 resulted in a filling ratio of 50%. The set-up is drawn in Figure 1. The mixing was performed using
39
40 aeration and the wastewater was pumped at 0.95 L h⁻¹ flow into the treatment line. Sludge recycling
41
42 rate from the settler to H1 was 100%.
43
44

45
46
47 The raw hospital wastewater was passed through an 80 µm filter from Amiat Water Systems
48
49 (Bochum, Germany) using a monopump (Pump 1) from Seepex (Hillerød, Denmark) into the
50
51 mixing tank (100 L). This equalization tank maintained a temperature between 15°C and 18°C and
52
53 its function was to level out the flow and dynamics in the loading over the day as there was
54
55 regularly low flow during nights in the sewage pipe. Using a peristaltic pump (Pump 2) from
56
57 Watson-Marlow (Ringsted, Denmark) the water was pumped from H0 to H1. The main parameters
58
59
60
61
62

1
2
3
4
5 of the wastewater (pH, oxygen, temperature, COD, DOC, NH₄-N, NO₂-N and NO₃-N) were
6
7 measured regularly.
8

9
10 It was intended to perform BOD removal and nitrification in H1 and have biofilms operating on low
11
12 BOD loading in H2, H3 and P being more adapted to degrade complex and difficult to degrade
13
14 organic matter that the sludge cannot degrade. It was hypothesised that this might induce an ability
15
16 to degrade pharmaceuticals that are recalcitrant to sludge treatment.
17
18

19 20 21 **2.2 Experimental design** 22

23
24 The study used two different methodologies to experimentally investigate the system's capacity to
25
26 degrade pharmaceuticals from hospital wastewater. First, a batch experiment was conducted to test
27
28 which of the spiked compounds could be potentially degraded in the treatment train and to study
29
30 their degradation kinetics. Then, a continuous flow experiment was performed to mimic the real
31
32 performance of the system
33
34

35 36 37 38 **2.2.1 Batch experiment** 39

40
41 To work with each reactor of the system as a unit, the flow was stopped by turning off the two
42
43 pumps (See figure 1). The connecting tubes were also blocked with clamps. As not all compounds
44
45 are used in a hospital at all times of the each reactor was spiked with a stock solution containing all
46
47 the target compounds, which achieved nominal concentrations of 14 µg L⁻¹ for the pharmaceuticals
48
49 and 200 µg L⁻¹ for the X-ray contrast media to make sure in all reactors was enough for kinetic
50
51 assessment. After the spiking, from 1 minute to 24 h, 10 mL samples were taken with a glass pipette
52
53
54 from each reactor. The concentrations of pharmaceuticals were analysed by HPLC-MS and the
55
56
57
58
59
60
61
62
63
64
65

1
2
3
4
5 determined concentrations (resulting from the original plus spiked) were used for further
6
7 assessment).

11 12 **2.2.2 Continuous flow experiment**

13
14
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.
23
24
25
26
27
28
29
30
31
32
33

34 35 **2.3 Chemicals**

36
37
38
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.
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1
2
3
4
5 The following compounds were analysed:
6

7 Antibiotics: Azithromycin, Ciprofloxacin, Clarithromycin, Clindamycin, Erythromycin,
8
9 Sulfadiazine, Sulfamethizole, Sulfamethoxazole, Trimethoprim
10

11 Blood pressure regulators: Atenolol, Metoprolol, Propranolol, Sotalol
12

13 Diverse: Carbamazepine, Diclofenac, Ibuprofen, Phenazone, Tramadol, Citalopram, Venlafaxine
14

15 X-ray contrast media: Diatrizoic acid, Iohexol, Iopromide, Iomeprol, Iopamidol
16
17

18 And the metabolite Acetyl-Sulfadiazine
19
20
21
22

23 Formic acid and gradient grade methanol were from Merck (Darmstadt, Germany); water was
24
25 obtained from an in-house Millipore apparatus.
26
27
28
29

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 (NO_2^-N) and nitrate nitrogen (NO_3^-N) were collected and analysed for all reactors.
36
37 Subsamples of approximately 50mL were filtered with a 0.45 μm filter. Samples for DOC were
38 preserved in the freezer until analysis, while samples for COD, $\text{NH}_4^+\text{-N}$ and NO_2^-N and NO_3^-N
39
40 were analysed the same day.
41
42
43
44
45
46

47 COD, $\text{NH}_4^+\text{-N}$, NO_2^-N and NO_3^-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),
50
51 $\text{NH}_4^+\text{-N}$ (LCK 303/304), NO_2^-N (LCK 341) and NO_3^-N (LCK 339) following the manufacturer's
52
53 instructions (HACH LANGE GMBH, Düsseldorf, Germany).
54
55
56
57
58
59
60
61
62

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 m².

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

1
2
3
4
5 analysing, the samples were left to reach room temperature and homogenized. 1.5 mL subsamples
6
7 of each sample were transferred to HPLC vials and centrifuged for 10 minutes at 6000 rpm. 900 μ L
8
9 of the aqueous phase were taken with a syringe and transferred to a new HPLC vial. 100 μ L of
10
11 internal standard were added to each sample with a glass syringe. Finally, the samples were
12
13 analysed by means of HPLC-MS/MS. Injections of 10 and 50 μ L were used for the batch and
14
15 concentration profile experiments correspondingly.
16
17

21 22 **2.6.2 HPLC-MS/MS**

23
24
25 The concentrations of the pharmaceuticals in the hospital wastewater and the treatment reactors
26
27 were quantified by means of HPLC-MS/MS. The HPLC had a dual low-pressure mixing ternary-
28
29 gradient system Ultimate 3000 from Dionex. The system operated with a pump of the 3000 series
30
31 (DGP-3600 M), a 3000 TSL autosampler (WPS 3000 TSL) and a column oven and degasser also
32
33 from Dionex 3000. The mass spectrometer model was an API 4000 (ABSciex, Framingham, MA,
34
35 USA). HPLC and MS/MS conditions are stated in the supplementary information (S2).
36
37
38
39

40 41 **2.6.3 Data treatment**

42
43
44 The concentrations of all the compounds over time in the wastewater were plotted. For each
45
46 experiment, the data was treated in a different way as described in the following lines.
47
48
49

50 51 **2.6.3.1 Batch experiment**

52
53
54 Concentrations were plotted for each reactor and compound. Figure 2 shows nine illustrative
55
56 compounds while the remaining compounds are shown in the supplementary material (Figure S1).
57
58 First-order degradation equation (Equation 1) was fitted using GraphPad Prism, with no weighting:
59
60
61
62

1
2
3
4
5
6
7 Equation 1: $C = (C_0) \cdot e^{-kt}$
8
9

10
11
12 C expresses initial and concentration, k is the removal rate constant, t stands for time.

13
14 The k values of each reactor were used to calculate a removal (%) of each of the compounds within
15
16 the treatment train by using Equation 2:
17

18 Equation 2:
19

20
21
22
$$\frac{C_P}{C_{H0}} = \frac{v_i}{(1 + k_P \cdot \tau_P) \cdot ((1 + k_{H1} \cdot \tau_{H1}) \cdot (1 + k_{H2} \cdot \tau_{H2}) \cdot (1 + k_{H3} \cdot \tau_{H3}) \cdot (v_i + v_r) - v_r)}$$

23
24
25
26

27 C_P and C_{H0} express the concentrations in the reactors H0 and P [$\mu\text{g/L}$] correspondingly, k_i express
28
29 the removal rate constants [h^{-1}] in the H1, H2, H3 and P reactors. τ_i refers to the hydraulic residence
30
31 times [h] of H1, H2, H3 and P reactors while v_i and v_r describe the inflow and recirculation flows
32
33 [L/h] respectively.
34
35
36
37

38
39 As expressed on Equation 3, the hydraulic residence time for H1, H2 and H3 was the same and can
40
41 be found as the tank volume divided with the sum of inflow and recirculation flows. For the last
42
43 reactor (P), the hydraulic residence time was different than H1-H3 and can be calculated as tank
44
45 volume divided with inflow.
46
47
48
49

50 Equation 3:
51

52
$$\tau_{H1} = \frac{\text{Tank volume}}{v_i + v_r} = \tau_{H2} = \tau_{H3} \neq \tau_P; \tau_P = \frac{\text{Tank volume}}{v_i}$$

53
54
55
56
57
58
59
60
61
62

1
2
3
4
5 Compounds giving estimated removals over 20% during the treatment system were considered to be
6
7 degradable.
8
9

10 **2.6.3.2 Continuous flow experiment**

11
12
13
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
18
19
20

21 Equation 4:

$$22 \text{Removal (\%)} = \left(1 - \frac{\text{Concentration last tank (P)}}{\text{Concentration initial tank (H0)}} \right) \times 100$$

23
24
25
26
27
28
29
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 $\pm 10\%$.
33
34
35
36
37
38
39
40

41 **3. Results and discussion**

42 **3.1 General Parameters for the treatment system**

43
44
45
46
47
48 The system had operated continuously for 10 months before the experiments were performed. All
49 flows and set points of the system were kept constant for the last two months after which the
50 experiments were performed. The parameters that characterise the general water treatment
51 performance in this period are summed up in Table 1.
52
53
54
55
56
57
58
59
60
61
62

1
2
3
4
5 Oxygen was never below 2.5 mg/L in H1 (sludge only system) and 4.5 mg/L in H2-H4 (biofilm and
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

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. HybasTM 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

1
2
3
4
5 a parent compound could happen via transformation of metabolites from other parent compounds
6
7 (Kovalova et al. 2012).
8

9
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.
19
20
21
22
23
24
25
26
27
28

29
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.
37
38
39
40
41
42
43
44
45

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
52
53
54
55
56
57
58
59
60
61
62

1
2
3
4
5 was the first reactor in the treatment train, most of the total removal was occurring there (Figure 4).
6
7 When correcting the k_i values with the amount of biomass per reactor volume (k_{bio}) the most
8
9 efficient reactor appeared to be the polishing MBBR (P). This is an indication that P was actually
10
11 highly adapted to remove micro-pollutants.
12
13
14
15

16 **3.3 Pharmaceuticals Removal: Trends**

17
18
19 Different trends were observed through the different compounds in terms of removal and
20
21 degradation kinetics.
22
23
24
25

26 **3.3.1 Beta-blockers**

27
28
29 Beta-blockers (atenolol, metoprolol, propranolol and sotalol) could be degraded in the batch
30
31 experiment. For atenolol (Supplementary information S2 and S 3, numerical data in Table 2) a
32
33 removal close to 100% was determined while it was a bit over 20% for the other beta-blockers.
34
35 During the continuous flow experiment atenolol, metoprolol and propranolol were detected in H0
36
37 and degraded later during the treatment train (Metoprolol and Propranolol in Figure 3). The
38
39 degradation rates were fairly matching the estimated ones from the batch experiment.
40
41
42
43
44
45

46
47 Propranolol concentrations were higher in H1 in comparison to H0, such formation of propranolol
48
49 was compensated later by the following reactors removal. This gave an apparent no-removal of
50
51 propranolol when comparing inlet (H0) and outlet (P) concentrations during the continuous flow
52
53 experiment (Figure 3). Propranolol is known to be the only beta-blocker forming conjugates
54
55 (Documed 2014) and, furthermore it was degraded in the batch experiment. Therefore, this apparent
56
57 no-removal was most likely the result of propranolol being formed by de-conjugation at a similar
58
59
60
61
62
63
64
65

1
2
3
4
5 rate than it was being degraded. This finding would also be in agreement with a previous MBR
6
7 study (Kovalova et al., 2012) that also observed negative removals of propranolol in hospital
8
9 wastewater treatments operating at similar hydraulic retention times.
10

11
12
13
14 Remarkably, atenolol was removed better than the other three beta-blockers. Such high removals
15
16 might be explained by co-metabolism. Unlike metoprolol and sotalol, the biodegradation of atenolol
17
18 has been previously observed and linked to the activity of ammonia-oxidizing bacteria and
19
20 heterotrophs (Sathyamoorthy et al., 2013). This hypothesis of co-metabolism fits the present study
21
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
25
26 than the removal for other beta-blockers in previous studies on hospital wastewater treatment
27
28 (Nielsen et al., 2013).
29
30
31
32
33

34
35 The degradation of propranolol, metoprolol and sotalol occurred in different tanks than the
36
37 nitrification. This points out that nitrification and degradation of beta-blockers (except perhaps
38
39 atenolol) are independent processes. On the other side, the the removal of atenolol, coinciding with
40
41 nitrification.
42
43
44
45

46 **3.3.2 Iodinated X-ray Contrast Media (ICM)**

47
48

49
50 Iopamidol, iohexol and iomeprol could be degraded following first-order kinetics (Figure 2,
51
52 supplementary material, S2 and S3, numerical data in Table 2), with both, estimated and real
53
54 removal between 60 and 80% (Figure 4). Iopromid and diatrizoic acid were not removed. ICM are
55
56 not metabolized or conjugated in humans (Bourin et al., 1997). Therefore the fact that the data for
57
58
59
60
61
62

1
2
3
4
5 ICMs from the batch experiment matches the continuous flow results better than most other groups
6
7 of pharmaceuticals investigated might be due to the lack of de-conjugation processes.
8

9
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
19
20 system that are the common factor (Escolà Casas and Bester 2015; Hapeshi et al., 2013; this study).
21
22 In this study biodegradation was detected in the sludge-only reactor (H1). This result is supporting
23
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
29
30 in BOD gives a low sludge production and thus the possibility for slow growing microorganisms to
31
32 survive in the sludge without being washed out.
33
34
35

36
37 Iohexol, iomeprol and iopamidol presented the highest k_{bio} in H1 (k_{H1bio} in table 2), which contained
38
39 only sludge, and the lowest k_{bio} in P, which contained only MBBR. The k_{bio} values in H2 and
40
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
44
45 such values do not differ so much from each other (Tables 1 and 2). According to the k_{bio} values, it
46
47 seems that in this set-up, the sludge biomass community was more efficient in removing ICM than
48
49 the MBBR community.
50
51

52
53 ICM are usually persistent to conventional WWTP treatment as well as realistic ozone treatment
54
55 (Kovalova et al., 2012, Hapeshi et al., 2013). MBR treatment showed negligible removals of ICM
56
57 from hospital wastewater, except for iopromide which was removed up to 31% (Kovalova et al.,
58
59
60
61
62
63
64
65

1
2
3
4
5 2012). Also other hospital wastewater-treatment studies employing MBR (Nielsen et al., 2013) or
6
7 fungal fluidized-bed bioreactor in non-sterile conditions (Cruz-Morató et al., 2014) found low
8
9 removal (ranging from 0 and 44%) for ICM. The direct degradation of the three X-ray contrast
10
11 media by the present treatment train can position Hybas™ technology as a solution to remove this
12
13 kind of compounds.
14
15

16 17 18 **3.3.3 Sulfonamides** 19

20
21
22 Sulfadiazine, sulfamethoxazole and sulfamethizole as well as the sulfadiazine metabolite (acetyl-
23
24 sulfadiazine) are a group related structures (supplementary information, S1) that behaved differently
25
26 during the wastewater treatment.
27

28
29 During the batch experiment, the concentration of sulfadiazine decreased in H1, H2 and H3
30
31 reactors, while it remained stable in reactor P. Acetyl-sulfadiazine, its main metabolite, seemed to
32
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
40
41 that both compounds can be degraded in the Hybas™ set-up.
42
43
44

45
46
47 In the continuous flow experiment sulfadiazine concentrations remained stable, while the
48
49 concentration of acetyl-sulfadiazine increased by $0.6\mu\text{g L}^{-1}$ (Figure 3). Thus, the expectations that
50
51 acetyl-sulfadiazine would de-conjugate to form sulfadiazine were not fulfilled. This suggests that
52
53 other interactions might occur between sulfadiazine and sulfadiazine metabolites.
54
55
56
57
58
59
60
61
62

1
2
3
4
5 The batch experiment showed that both, sulfamethoxazole and sulfamethizole could be degraded by
6
7 the treatment system (Figure 2 and supplementary information S3). In humans, sulfamethoxazole is
8
9 usually also excreted in acetylated form while sulfamethizole is only acetylated in small amounts
10
11 (3%) (Hekster and Vree 1982). When operating the system at continuous flow, 25% of
12
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
18
19 MBR treatment.
20
21
22
23
24

25 Therefore an explanation for stable concentrations of sulfamethoxazole during the continuous flow
26
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).
34
35
36
37
38

39 Finally, sulfamethizole concentrations diminished by circa $0.7\mu\text{g L}^{-1}$. The calculated removal of
40
41 sulfamethizole matched well the experimental removal during the continuous flow experiment. This
42
43 was expected as this compound is not excreted as an acetylated conjugate.
44
45
46
47

48 **3.3.4 Analgesics**

49
50

51 During the batch experiment, phenazone concentrations decreased following first order kinetics but
52
53 the overall removal was under 20%.. In the present study the pure MBBR reactor (P) showed to be
54
55 the one with the lowest removal. This finding might indicate that the degradation of phenazone is a
56
57 co-metabolic process. Phenazone was not detected during the continuous flow experiment.
58
59
60
61
62

1
2
3
4
5
6
7 In the batch experiments the concentrations of tramadol were decreasing but the removal calculated
8
9
10 with the resulting reaction rate constants was below 20% (not significant). The removal determined
11
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
17
18 study, on pure MBBR operations, tramadol could not be removed. Also Kovalova et al., (2012)
19
20 found that tramadol was not degraded in an MBR.
21
22

23
24
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.,
28
29 2009). Further studies regarding the degradation conditions for diclofenac should be conducted in
30
31 the future.
32
33

3.3.5 Antidepressants/Antiepileptic

34
35
36
37
38
39
40 For batch and continuous flow experiments, venlafaxine removal was under 20%. Previously
41
42 described treatment systems (Nielsen et al., 2013, Kovalova et al., 2012) and data from municipal
43
44 WWTPs (Metcalf et al., 2010) also described poor removal for this compound.
45
46

47
48 The removal of carbamazepine was calculated to be close to 20% during the batch. This
49
50 concentration decrease only occurred in the P reactor during the continuous flow experiment. A
51
52 previous project operating MBBR reactors in series showed similar results for carbamazepine. The
53
54 fact that carbamazepine could be degraded by this MBBR is a small success as carbamazepine is
55
56 usually a very recalcitrant compound in activated sludge WWTP and it has not been degraded in
57
58
59
60
61
62

1
2
3
4
5 other hospital wastewater-treatment projects (Nielsen et al., 2013, Kovalova et al., 2012, Falås et
6 al., 2013, Onesios et al., 2009).
7
8
9

10
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.
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36

37 **3.3.6 Macrolides and other antibiotics**

38
39

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
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1
2
3
4
5 previous literature might rely on the sludge/biofilm characteristics of the system that, somehow,
6 developed efficient clindamycin degraders.
7
8
9

10
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).
15
16 Trimethoprim was produced in H1 but degraded during the following steps of the treatment train.
17
18
19
20
21
22

23 24 25 **5. Conclusion** 26

27
28 A train of four reactors combining sludge, HybasTM and a polishing MBBR reached similar or better
29 removal efficiencies of pharmaceuticals compared to other technologies (i.e activated sludge or
30 MBR), particularly when considering specific compounds (like X-ray contrast media).
31
32

33
34 The degradation of organic matter and nitrification mainly occurred in the first treatment reactor
35 indicating that the system was a low loaded one compared to the systems for treating general
36 wastewater. In addition, HybasTM also reached low organic matter values in the outlet through a
37 consistent (but small) stepwise removal in the following treatment steps which maintains a low
38 loaded biofilm adapted for growing on hardly degradable organic matter. This process results in an
39 effluent which is low in DOC and thus more suitable for a cost effective polishing by ozonation
40 (Antoniou, 2013).
41
42
43
44
45
46
47
48
49
50

51
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
56
57
58
59
60
61
62

1
2
3
4
5 metabolites are facts that have to be taken in account in such wastewater systems. – It is, however
6
7 expected that the reaction rate constants in full scale will not 100% match those determined in the
8
9 small pilot. For these reasons currently a large pilot with 1m³ tanks is under construction that will
10
11 be able to treat wastewater for a whole hospital department before applying these principles to
12
13 fullscale plants.
14

15
16 This study also evaluated the effectiveness and role of each reactor. The first three reactors were
17
18 well designed for COD, nitrogen removal and for compounds that might degrade preferably through
19
20 sludge biomass (e.g. X-ray contrast media). On the other hand, the specific activity (k_{bio}) of the
21
22 biomass for micro-pollutants removal was the highest in the last reactor in most cases. Therefore,
23
24 further designs such as up-scaling or addition of ozonation steps should envision a larger polishing
25
26 reactor in order to maximize the removal of recalcitrant pharmaceuticals. Another design option
27
28 could be to operate a high-load HybasTM for BOD removal and a secondary HybasTM for removal of
29
30 organic micro-pollutants.
31
32

33
34 The tested combination of sludge, HybasTM and MBBR resulted to be a compact, robust and easy-
35
36 to-operate technology that showed to remove not only COD and nitrogen but also some recalcitrant
37
38 micro-pollutants. Therefore, this type of wastewater treatment can be seen as a clever solution for
39
40 hospital wastewater treatment.
41
42

43 44 45 46 **Acknowledgments**

47 The authors want to acknowledge the AUFF grant “Advanced water purification using bio-
48
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.
58
59
60
61
62

References

- Bester, K. and Schafer, D. (2009) Activated soil filters (bio filters) for the elimination of xenobiotics (micro-pollutants) from storm- and waste waters. *Water Research* 43, 2639-2646.
- Christensson M. and Welander T. (2004). Treatment of municipal wastewater in a hybrid process using a new suspended carrier with large surface area. *Water Science and Technol.*, 49, 207–214.
- Cruz-Morató, C., Lucas, D., Llorca, M., Rodriguez-Mozaz, S., Gorga, M., Petrovic, M., Barceló, D., Vicent, T., Sarrà, M. and Marco-Urrea, E. (2014) Hospital wastewater treatment by fungal bioreactor: Removal efficiency for pharmaceuticals and endocrine disruptor compounds. *Science of The Total Environment* 493, 365-376.
- Debabrata M. (2010) Removal of high organics containing wastewater in a shaft-type activated sludge hybrid reactor. *International Journal of Environmental Sciences*, 1, 368-382.
- Escolà Casas, M. and Bester, K. (2015) Can those organic micro-pollutants that are recalcitrant in activated sludge treatment be removed from wastewater by biofilm reactors (slow sand filters)? *Science of The Total Environment* 506–507, 315-322.
- Escolà Casas, M., Ravi Kumar Chhetri, Gordon Ooi, Kamilla M. S. Hansen, Klaus Litty, Magnus Christensson, Caroline Kragelund, Henrik R. Andersen and Kai Bester: Biodegradation of pharmaceuticals in hospital wastewater by staged Moving Bed Biofilm Reactors (MBBR), *Water Research*, submitted, 2015
- Falås, P., Baillon-Dhumez, A., Andersen, H.R., Ledin, A. and la Cour Jansen, J. (2012) Suspended biofilm carrier and activated sludge removal of acidic pharmaceuticals. *Water Research* 46, 1167-1175.
- Falås, P., Longrée, P., la Cour Jansen, J., Siegrist, H., Hollender, J. and Joss, A. (2013) Micropollutant removal by attached and suspended growth in a hybrid biofilm-activated sludge process. *Water Research* 47, 4498-4506.
- Hapeshi, E., Lambrianides, A., Koutsoftas, P., Kastanos, E., Michael, C. and Fatta-Kassinos, D. (2013) Investigating the fate of iodinated X-ray contrast media iohexol and diatrizoate during microbial degradation in an MBBR system treating urban wastewater. *Environ Sci Pollut Res Int.* 20,3592–3606.
- Heberer, T., Mechlinski, A., Fanck, B., Knappe, A., Massmann, G., Pekdeger, A. and Fritz, B. (2004) Field studies on the fate and transport of pharmaceutical residues in bank filtration. *Ground Water Monitoring and Remediation* 24, 70-77.

1
2
3
4
5 Hekster, C.A. and Vree, T.B. (1982) Clinical pharmacokinetics of sulphonamides and their N4-
6 acetyl derivatives. *Antibiot Chemother* (1971) 31, 22-118.
7
8 Hijosa-Valseiro, M., Matamoros, V., Sidrach-Cardona, R., Pedescoll, A., Martin-Villacorta, J.,
9 Garcia, J., Bayona, J.M. and Becares, E. (2011) Influence of design, physico-chemical and
10 environmental parameters on pharmaceuticals and fragrances removal by constructed wetlands.
11 *Water Science and Technology* 63, 2527-2534.
12
13 Janzen, N., Banzhaf, S., Scheytt, T. and Bester, K. (2009) Vertical flow soil filter for the
14 elimination of micro pollutants from storm and waste water. *Chemosphere* 77, 1358-1365.
15
16 Joss, A., Siegrist, H. and Ternes, T.A. (2008) Are we about to upgrade wastewater treatment for
17 removing organic micropollutants? *Water Science and Technology* 57, 251-255.
18
19 Joss, A., Zabczynski, S., Gobel, A., Hoffmann, B., Löffler, D., McArdell, C.S., Ternes, T.A.,
20 Thomsen, A. and Siegrist, H. (2006) Biological degradation of pharmaceuticals in municipal
21 wastewater treatment: Proposing a classification scheme. *Water Research* 40, 1686-1696.
22
23 Ødegaard, H., Christensson, M. & Sørensen, K. 2014 Hybrid systems. In: *Activated Sludge- 100*
24 *Years and Counting*. (Jenkins, D. & Wanner, J., eds). IWA Publishing.
25
26 Köhler, C., Venditti, S., Igos, E., Klepizewski, K., Benetto, E. and Cornelissen, A. (2012)
27 Elimination of pharmaceutical residues in biologically pre-treated hospital wastewater using
28 advanced UV irradiation technology: A comparative assessment. *Journal of Hazardous Materials*
29 239–240, 70-77.
30
31 Kosma, C.I., Lambropoulou, D.A. and Albanis, T.A. (2010) Occurrence and removal of PPCPs in
32 municipal and hospital wastewaters in Greece. *Journal of Hazardous Materials* 179, 804-817.
33
34 Kovalova, L., Siegrist, H., von Gunten, U., Eugster, J., Hagenbuch, M., Wittmer, A., Moser, R. and
35 McArdell, C.S. (2013) Elimination of Micropollutants during Post-Treatment of Hospital
36 Wastewater with Powdered Activated Carbon, Ozone, and UV. *Environmental Science &*
37 *Technology* 47, 7899-7908.
38
39 Kovalova, L., Siegrist, H., Singer, H., Wittmer, A. and McArdell, C.S. (2012) Hospital wastewater
40 treatment by membrane bioreactor: performance and efficiency for organic micropollutant
41 elimination. *Environmental Science & Technology* 46, 1536-1545.
42
43 Li, D., Alidina, M. and Drewes, J.E. (2014) Role of primary substrate composition on microbial
44 community structure and function and trace organic chemical attenuation in managed aquifer
45 recharge systems. *Applied Microbiology and Biotechnology*, 98, 5747-5756.
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1
2
3
4
5 Liu, Q., Zhou, Y., Chen, L. and Zheng, X. (2010) Application of MBR for hospital wastewater
6 treatment in China. *Desalination* 250, 605-608.
7
8 Mazumder, D. (2010) Removal of high organics containing wastewater in a shafttype activated
9 sludge hybrid reactor. *International Journal of Environmental Sciences* 1, 368-382.
10
11 Metcalfe, C.D., Chu, S., Judt, C., Li, H., Oakes, K.D., Servos, M.R. and Andrews, D.M. (2010)
12 Antidepressants and their metabolites in municipal wastewater, and downstream exposure in an
13 urban watershed. *Environmental Toxicology and Chemistry*, 29, 79-89.
14
15 Nielsen, U., Hastrup, C., Klausen, M.M., Pedersen, B.M., Kristensen, G.H., Jansen, J.L., Bak, S.N.
16 and Tuerk, J. (2013) Removal of APIs and bacteria from hospital wastewater by MBR plus O(3),
17 O(3) + H(2)O(2), PAC or ClO(2). *Water Science and Technology*, 67, 854-862.
18
19 Onesios, K., Yu, J. and Bouwer, E. (2009) Biodegradation and removal of pharmaceuticals and
20 personal care products in treatment systems: a review. *Biodegradation*, 20, 441-466.
21
22 Pauwels, B. and Verstraete, W. (2006) The treatment of hospital wastewater: an appraisal. *Journal*
23 *of Water Health* 4, 405-416.
24
25 Pills, P. (2012) Pills Project: Pharmaceutical residues in the aquatic system– a challenge for the
26 future. A European partnership project of: Emschergenossenschaft (DE), Waterschap Groot Salland
27 (NL), Centre de Recherche Public Henri Tudor (LU), Eawag (CH), Glasgow Caledonian University
28 (UK) and Université de Limoges (FR). [http://www.pills-](http://www.pills-project.eu/content/136/documents/PillsBrochure-en.pdf)
29 [project.eu/content/136/documents/PillsBrochure-en.pdf](http://www.pills-project.eu/content/136/documents/PillsBrochure-en.pdf)
30
31 Randall, C.W. and Sen, D. (1996) Full-scale evaluation of an integrated fixed-film activated sludge
32 (IFAS) process for enhanced nitrogen removal. *Water Science and Technology* 33, 155-162.
33
34 Reungoat, J., Escher, B.I., Macova, M. and Keller, J. (2011) Biofiltration of wastewater treatment
35 plant effluent: Effective removal of pharmaceuticals and personal care products and reduction of
36 toxicity. *Water Research* 45, 2751-2762.
37
38 Santos, L.H.M.L.M., Gros, M., Rodriguez-Mozaz, S., Delerue-Matos, C., Pena, A., Barceló, D. and
39 Montenegro, M.C.B.S.M. (2013) Contribution of hospital effluents to the load of pharmaceuticals
40 in urban wastewaters: Identification of ecologically relevant pharmaceuticals. *Science of The Total*
41 *Environment* 461–462, 302-316.
42
43 Sathyamoorthy, S., Chandran, K. and Ramsburg, C.A. (2013) Biodegradation and Cometabolic
44 Modeling of Selected Beta Blockers during Ammonia Oxidation. *Environmental Science &*
45 *Technology* 47, 12835-12843.
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

- 1
2
3
4
5 Thomas, K.V., Dye, C., Schlabach, M. and Langford, K.H. (2007) Source to sink tracking of
6 selected human pharmaceuticals from two Oslo city hospitals and a wastewater treatment works.
7 Journal of Environmental Monitoring, 9, 1410-1418.
8
9
10 Ternes, T.A., Joss, A. and Siegrist, H. (2004) Scrutinizing pharmaceuticals and personal care
11 products in wastewater treatment. Environmental Science & Technology 38, 392A-399A.
12
13 Verlicchi, P., Galletti, A., Petrovic, M. and Barceló, D. (2010) Hospital effluents as a source of
14 emerging pollutants: An overview of micropollutants and sustainable treatment options. Journal of
15 Hydrology 389, 416-428.
16
17 Verlicchi, P., AlAukidy, M., Zambello E. (2015) What have we learned from worldwide experiences
18 on the management and treatment of hospital effluent? — An overview and a discussion on
19 perspectives, Science of the Total Environment 514, 467–491.
20
21 Zearley, T.L. and Summers, R.S. (2012) Removal of Trace Organic Micropollutants by Drinking
22 Water Biological Filters. Environmental Science & Technology 46, 9412-9419.
23
24 Zupanc, M., Kosjek, T., Petkovšek, M., Dular, M., Kompare, B., Širok, B., Blažeka, Ž. and Heath,
25 E. (2013) Removal of pharmaceuticals from wastewater by biological processes, hydrodynamic
26 cavitation and UV treatment. Ultrasonics Sonochemistry 20, 1104-1112.
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

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 ₄ ⁺ -N	NO ₂ ⁻ -N	NO ₃ ⁻ -N
		Sludge [g/L]	Carriers [g/m ²]	[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]

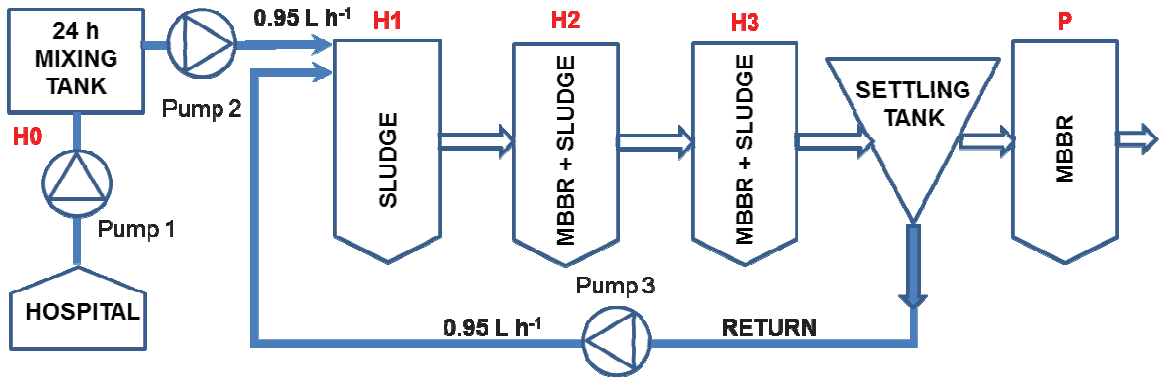
Table 2: Parameters from curve fitting (Eq. 1). R2 relates to the fit for determining the rate constant k , k_i : reactor removal rate constant, k_{bio} : removal rate constant per gram of biomass per liter (according to Table 1). Bold values: highest k_i or k_{bio} among the three reactors. na=mathematically 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	1.49×10⁰	4.77×10⁻¹	0.99	7.78×10 ⁻¹	1.41×10 ⁻¹	0.97	7.86×10 ⁻¹	2.01×10 ⁻¹	0.20	1.13×10 ⁻²	1.09×10 ⁻²
Atenolol	0.94	1.14×10⁰	3.66×10⁻¹	0.97	6.82×10 ⁻¹	1.23×10 ⁻¹	0.98	7.37×10 ⁻¹	1.88×10 ⁻¹	0.90	5.19×10 ⁻²	4.99×10 ⁻²
Azitromycin	na	na	na	na	na	na	na	na	na	na	na	na
Carbamazepine	0.44	1.57×10 ⁻²	5.03×10 ⁻³	0.57	1.81×10 ⁻²	3.28×10 ⁻³	0.61	1.88×10⁻²	4.81×10 ⁻³	0.54	1.68×10 ⁻²	1.62×10⁻²
Clarithromycin	0.86	9.10×10⁻²	2.92×10 ⁻²	0.90	7.62×10 ⁻²	1.38×10 ⁻²	0.93	8.23×10 ⁻²	2.10×10 ⁻²	0.75	3.59×10 ⁻²	3.45×10⁻²
Ciprofloxacin	na	na	na	na	na	na	na	na	na	na	n.a	na
Citalopram	0.57	1.66×10 ⁻²	5.32×10 ⁻³	0.71	1.50×10 ⁻²	2.72×10 ⁻³	0.59	1.52×10 ⁻²	3.87×10 ⁻³	0.85	2.14×10⁻²	2.06×10⁻²
Clindamycin	0.81	5.04×10⁻²	1.62×10 ⁻²	0.92	4.80×10 ⁻²	8.69×10 ⁻³	0.90	3.45×10 ⁻²	8.80×10 ⁻³	0.68	1.87×10 ⁻²	1.80×10⁻²
Diatrizoic acid	0.03	4.61×10 ⁻³	1.48×10 ⁻³	0.07	5.05×10 ⁻³	9.14×10 ⁻⁴	0.09	5.57×10⁻³	1.42×10 ⁻³	0.09	5.41×10 ⁻³	5.20×10⁻³
Diclofenac	0.23	6.67×10 ⁻³	2.14×10 ⁻³	0.76	1.09×10 ⁻²	1.97×10 ⁻³	0.69	1.33×10⁻²	3.39×10⁻³	0.13	3.48×10 ⁻³	3.34×10 ⁻³
Erythromycin	0.68	3.28×10 ⁻²	1.05×10 ⁻²	0.84	2.88×10 ⁻²	5.22×10 ⁻³	0.93	3.32×10⁻²	8.47×10 ⁻³	0.71	2.09×10 ⁻²	2.01×10⁻²
Iohexol	0.94	2.40×10⁻¹	7.68×10⁻²	0.99	1.96×10 ⁻¹	3.55×10 ⁻²	0.99	1.97×10 ⁻¹	5.03×10 ⁻²	0.39	1.29×10 ⁻²	1.24×10 ⁻²
lomeprol	0.91	1.48×10⁻¹	4.73×10⁻²	0.93	1.34×10 ⁻¹	2.42×10 ⁻²	0.95	1.31×10 ⁻¹	3.33×10 ⁻²	0.32	1.13×10 ⁻²	1.08×10 ⁻²
lopamidol	0.89	2.03×10 ⁻¹	6.51×10⁻²	0.96	1.79×10 ⁻¹	3.24×10 ⁻²	0.97	1.72×10 ⁻¹	4.39×10 ⁻²	0.58	2.22×10⁻²	2.14×10 ⁻²

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

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

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



1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

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).

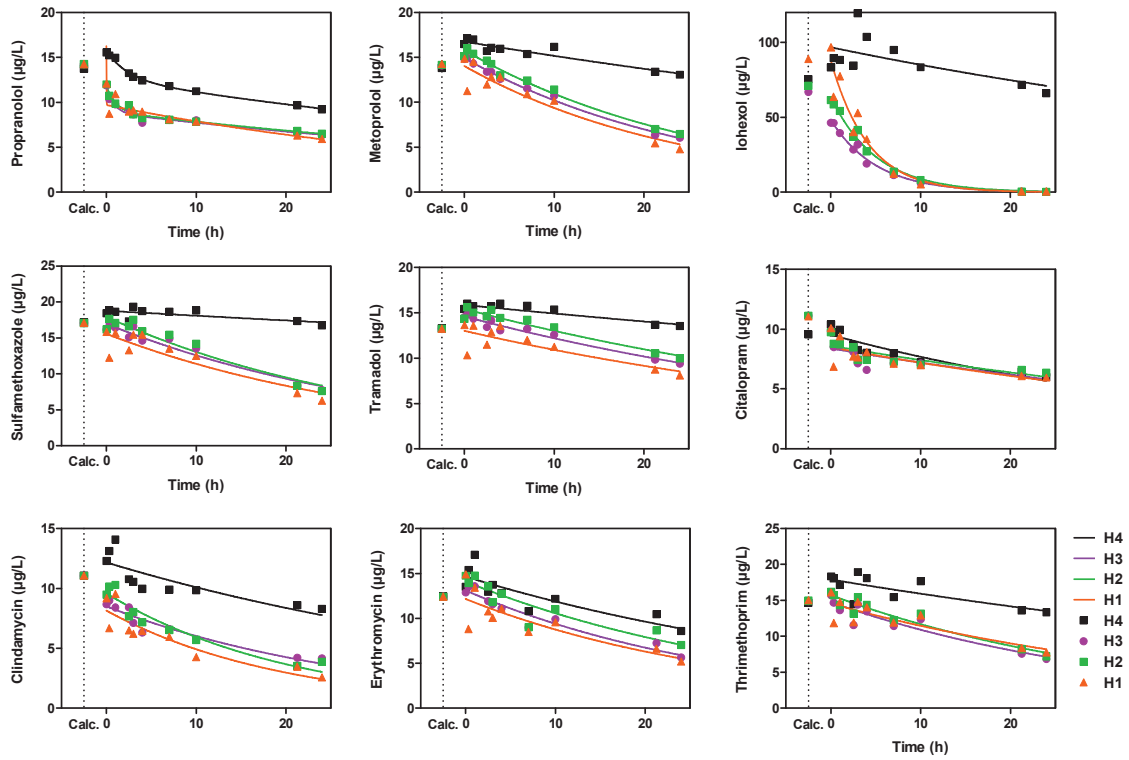
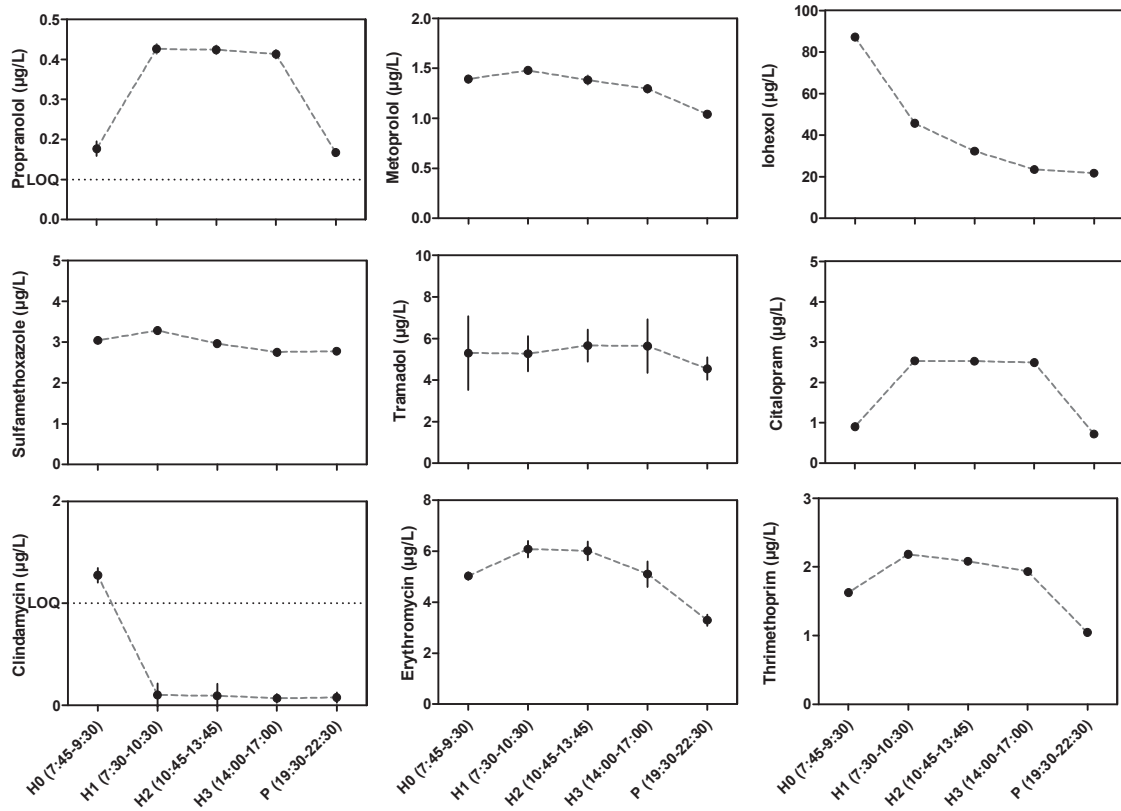


Figure 3: Average concentrations for selected compounds in the reactors during the continuous flow experiment. The error bars show the SD (n=2 samples for H0 and n=4 for the rest, each sample was analysed twice. SD was then derived from 4 measurements in H0 and 8 measurements in the rest of the tanks).



1
2
3
4
5 **Figure 4: Removal estimated from the batch experiment (Equation 2) compared to the measured**
6 **removal in the continuous flow experiment by applying Equation 4. *Known to be excreted as**
7 **conjugates, **known metabolites ***suggested to be formed from metabolites of other compounds**
8
9
10
11 **(Kovalova et al. 2012), ****suggested to be bound to faecal particles (Göbel et al. 2007).**
12

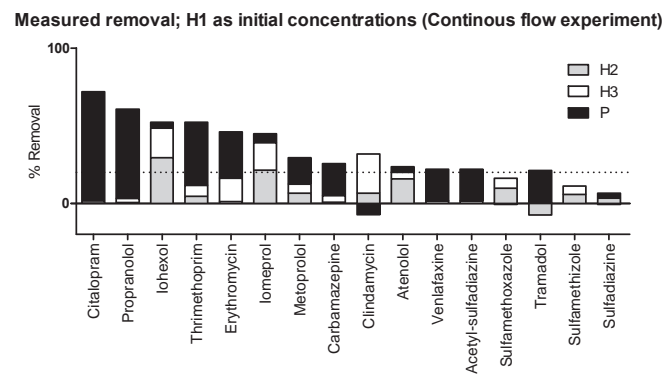
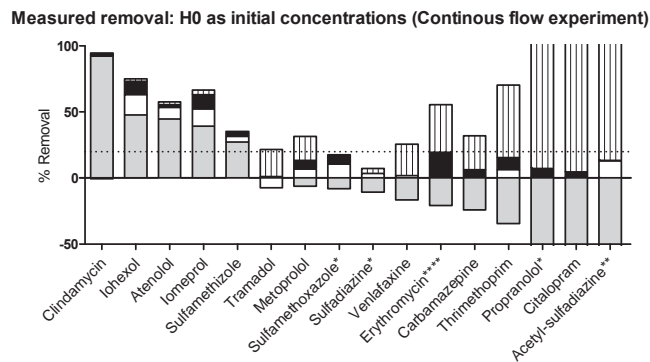
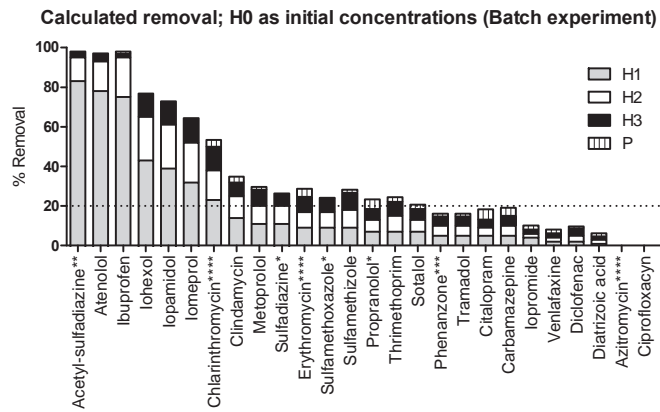


Table 1: General parameters \pm standard deviation for the HybasTM system during a two months period including the sampling for both experiments.

Reactor	HRT	Biomass			pH	DOC	COD	NH ₄ ⁺ -N	NO ₂ ⁻ -N	NO ₃ ⁻ -N
		Sludge	Carriers			[mgC/L]	[mgO/L]	[mgN/L]	[mgN/L]	[mgN/L]
		[g/L]	[g/m ²]	[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

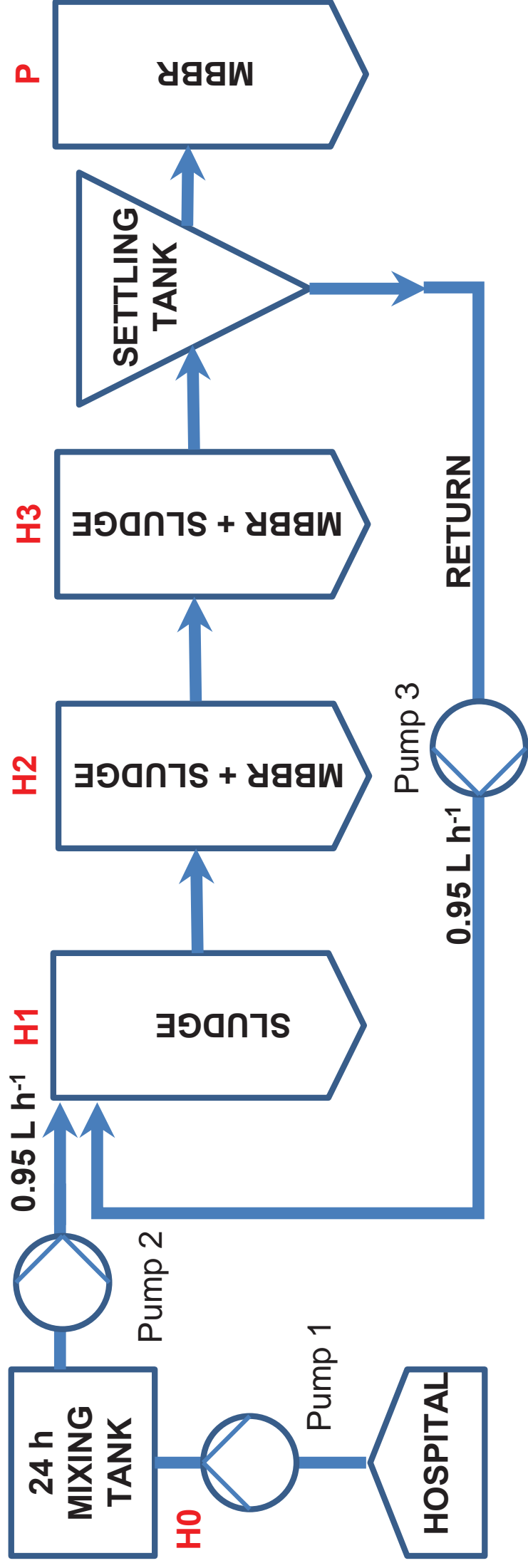
HRT= Hydraulic retention time [h]

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	1.49×10⁰	4.77×10⁻¹	0.99	7.78×10 ⁻¹	1.41×10 ⁻¹	0.97	7.86×10 ⁻¹	2.01×10 ⁻¹	0.20	1.13×10 ⁻²	1.09×10 ⁻²
Atenolol	0.94	1.14×10⁰	3.66×10⁻¹	0.97	6.82×10 ⁻¹	1.23×10 ⁻¹	0.98	7.37×10 ⁻¹	1.88×10 ⁻¹	0.90	5.19×10 ⁻²	4.99×10 ⁻²
Azithromycin	na	na	na	na	na	na	na	na	na	na	na	na
Carbamazepine	0.44	1.57×10 ⁻²	5.03×10 ⁻³	0.57	1.81×10 ⁻²	3.28×10 ⁻³	0.61	1.88×10⁻²	4.81×10 ⁻³	0.54	1.68×10 ⁻²	1.62×10⁻²
Clarithromycin	0.86	9.10×10⁻²	2.92×10 ⁻²	0.90	7.62×10 ⁻²	1.38×10 ⁻²	0.93	8.23×10 ⁻²	2.10×10 ⁻²	0.75	3.59×10 ⁻²	3.45×10⁻²
Ciprofloxacin	na	na	na	na	na	na	na	na	na	na	n.a	na
Citalopram	0.57	1.66×10 ⁻²	5.32×10 ⁻³	0.71	1.50×10 ⁻²	2.72×10 ⁻³	0.59	1.52×10 ⁻²	3.87×10 ⁻³	0.85	2.14×10⁻²	2.06×10⁻²
Clindamycin	0.81	5.04×10⁻²	1.62×10 ⁻²	0.92	4.80×10 ⁻²	8.69×10 ⁻³	0.90	3.45×10 ⁻²	8.80×10 ⁻³	0.68	1.87×10 ⁻²	1.80×10⁻²
Diatrizoic acid	0.03	4.61×10 ⁻³	1.48×10 ⁻³	0.07	5.05×10 ⁻³	9.14×10 ⁻⁴	0.09	5.57×10⁻³	1.42×10 ⁻³	0.09	5.41×10 ⁻³	5.20×10⁻³
Diclofenac	0.23	6.67×10 ⁻³	2.14×10 ⁻³	0.76	1.09×10 ⁻²	1.97×10 ⁻³	0.69	1.33×10⁻²	3.39×10⁻³	0.13	3.48×10 ⁻³	3.34×10 ⁻³
Erythromycin	0.68	3.28×10 ⁻²	1.05×10 ⁻²	0.84	2.88×10 ⁻²	5.22×10 ⁻³	0.93	3.32×10⁻²	8.47×10 ⁻³	0.71	2.09×10 ⁻²	2.01×10⁻²
Iohexol	0.94	2.40×10⁻¹	7.68×10⁻²	0.99	1.96×10 ⁻¹	3.55×10 ⁻²	0.99	1.97×10 ⁻¹	5.03×10 ⁻²	0.39	1.29×10 ⁻²	1.24×10 ⁻²
lomeprol	0.91	1.48×10⁻¹	4.73×10⁻²	0.93	1.34×10 ⁻¹	2.42×10 ⁻²	0.95	1.31×10 ⁻¹	3.33×10 ⁻²	0.32	1.13×10 ⁻²	1.08×10 ⁻²
lopamidol	0.89	2.03×10 ⁻¹	6.51×10⁻²	0.96	1.79×10 ⁻¹	3.24×10 ⁻²	0.97	1.72×10 ⁻¹	4.39×10 ⁻²	0.58	2.22×10⁻²	2.14×10 ⁻²
Iopromide	0.20	1.16×10⁻²	3.73×10 ⁻³	0.10	7.87×10 ⁻³	1.43×10 ⁻³	0.15	7.52×10 ⁻³	1.92×10 ⁻³	0.15	6.94×10 ⁻³	6.68×10⁻³
Metoprolol	0.89	4.04×10⁻²	1.29×10⁻²	0.98	3.66×10 ⁻²	6.64×10 ⁻³	0.99	3.79×10 ⁻²	9.67×10 ⁻³	0.88	9.95×10 ⁻³	9.57×10 ⁻³

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

Figure
Click here to download Figure: Figure1_Submit_Diagram tanksHYBAS.pdf



Supplementary material for on-line publication only

[Click here to download Supplementary material for on-line publication only: HYBAS_MS for submission AllSupplementaries.doc](#)