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Removal of pharmaceuticals from human urine during storage, aerobic biological treatment, and activated carbon adsorption to produce a safe fertilizer

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ABSTRACT

Urine has great potential to be an effective fertilizer due to its high nutrient content, however, it can contain potentially worrying pharmaceuticals. Our objective was to study whether urine storage and aerobic biological treatment, i.e. nitrification, was sufficient to remove pharmaceuticals or an additional treatment with activated carbon was necessary to produce a fertilizer from urine. We investigated the abatement of twelve pharmaceuticals, including antibiotics and antivirals, in laboratory experiments representing the treatment steps of anaerobic storage of source-separated human urine, stabilization using partial and full nitrification under acclimatized and non-acclimatized conditions, and treatment of nitrified urine using powdered activated carbon (PAC). Two-month-long-term storage of urine was insufficient to substantially degrade the pharmaceuticals, except for hydrochlorothiazide (>90%). In the partial and full nitrification fed-batch reactors, atazanavir, ritonavir, and clarithromycin were rapidly removed, with biotransformation rate constants greater than 10 L $g_{SS}^{-1} d^{-1}$. Darunavir, emtricitabine, trimethoprim, N4-acetylsulfamethoxazole, sulfamethoxazole, atenolol, diclofenac, and hydrochlorothiazide were degraded slowly, with biotransformation rate constants of < 1 L $g_{SS}^{-1} d^{-1}$. With 200 mg PAC L^{-1}, at least 90% of each investigated pharmaceutical was removed. Yeast estrogen screen tests and bioluminescence inhibition tests revealed efficient removal of estrogenicity (99%) and toxicity (56%) using nitrification, and a reduction of 89% and 64%, respectively, using 200 mg PAC L^{-1}. With our study, we provide biotransformation rate constants of compounds never previously investigated. We also show that a combination of nitrification and PAC adsorption enables the production of a safe fertilizer with sufficiently low pharmaceutical concentrations and no removal of beneficial nutrients.

1. Introduction

Urine can be an effective fertilizer because it is rich in nutrients. It accounts for up to 88% of total nitrogen, 67% of total phosphorus, and 73% of total potassium found in wastewater (Friedler et al., 2013; Karak and Bhattacharyya, 2011; Larsen and Gujer, 1996). However, the majority of the pharmaceuticals consumed by humans which go unassimilated are also excreted via urine (Lienert et al., 2007). The presence of pharmaceuticals raises concerns for the application of urine as a fertilizer because they may enter the food chain and pose risk for the environment and public health. Also, the first commercial urine fertilizer in Switzerland, Aurin, was licensed by FOAG (Vuna GmbH 2020) with the obligation of complete removal of pharmaceuticals. Treatment processes to remove pharmaceuticals from source-separated human urine therefore need to be assessed.

Various urine treatment options have been proposed and tested (Landry and Boyer, 2019; Maurer et al., 2006; Udert et al., 2015). The World Health Organization proposes a urine storage time of at least six months to achieve pathogen inactivation (WHO, 2006). Vinneråas et al. (2008) showed that two-month urine storage at temperatures above 20°C was sufficient to inactivate *Cryptosporidium* protozoa, gram-positive bacteria, and viruses. The process of combining biological

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nitrification in a moving bed biofilm reactor (MBBR) with consecutive distillation was developed to recover all the nutrients from urine in a single, highly concentrated solution. This concept was proven at a laboratory scale (Udert and Wächter, 2012) and pilot reactors were operated successfully for several years (Fumasoli et al., 2016). However, further investigations are required to reveal the removal of pharmaceuticals in these urine treatment processes. MBBR processes—which use plastic biofilm carriers suspended in the reactor—have been found to be more effective at micropollutant removal from biological municipal wastewater treatment than activated sludge, e.g. to remove diclofenac and trimethoprim (Escolà Casas et al., 2015b; Falås et al., 2012, 2013). Falås et al., 2013. Zupanc et al. (2013) also found strikingly higher removal rates for diclofenac (74–85%) in MBBR than in activated sludge (36%). More than 50% removal of diclofenac and atenolol from activated sludge effluent as well as higher removal rate constants for a wide range of micropollutants were found in MBBRs intermittently fed with primary wastewater (Tang et al., 2017, Tang et al., 2021). The MBBR systems were also found to be suitable for removing pharmaceuticals (21 of 26 compounds had a > 20% removal rate in batch experiments) from hospital wastewater (Escolà Casas et al., 2015b). Therefore, it can be expected that some pharmaceuticals will be degraded during nitrification based on an MBBR.

Nevertheless, an additional post-treatment is likely to be necessary for the maximum removal of pharmaceuticals. Zhang et al. (2015) investigated the degradation of pharmaceuticals in human urine samples under ultraviolet (UV) light alone and UV combined with advanced oxidation processes. UV radiation alone was found to be insufficient for degrading pharmaceuticals, while UV/peroxide (H_2O_2) or UV/peroxydisulfate (PDS) processes were effective in degrading aromatic pharmaceuticals such as sulfamethoxazole and trimethoprim but were insufficient for removing N4-acetylsulfamethoxazole (Zhang et al., 2015).

Ozonation and activated carbon adsorption are also considered feasible and efficient post-treatment options for reducing micropollutant levels in municipal wastewater (Eggen et al., 2014; Joss et al., 2008; Schindler Wildhaber, 2015). The ozone or carbon required is dependent on the concentration of bulk organic compounds. Efficient abatement of micropollutants in wastewater requires a dose of 0.4–0.7 g O_3 g DOC^{-1} (Bourgin et al., 2018; Dodd et al., 2008; Margot et al., 2013). The critical aspects related to ozonation are by-product formation, such as nitrosamines (Marti et al., 2015), and the possible toxicity of ozonation reaction products (Dodd et al., 2010; Stalter et al., 2010; Zimmermann et al., 2011). Sorption to granular or powdered activated carbon (PAC) is a suitable wastewater treatment option because activated carbon can adsorb a wide range of pharmaceuticals without producing transformation products (Boehler et al., 2012; Bonvin et al., 2016; Kårelid et al., 2017; Margot et al., 2013; Nowotny et al., 2007). Adsorption of eleven pharmaceuticals onto granular activated carbon was recently tested in nitrified source-separated human urine by Köpping et al. (2020). However, to the best of our knowledge, no previous study has applied a PAC treatment to source-separated urine.

The present study's goal was to study the processes necessary to produce a safe fertilizer by investigating the abatement of a selection of pharmaceuticals in the process units along a treatment chain, consisting of urine storage and biological urine treatment using nitrification. Furthermore, we investigated, whether PAC adsorption as an additional treatment step could ensure pharmaceutical removal. All experiments were conducted on laboratory scale. Several of the biological treatment conditions chosen had not been comparatively investigated before, including full and partial nitrification with and without the acclimatization of biota by pharmaceuticals. The use of urine-based fertilizers can bring significant economic and environmental benefits to low- and middle-income countries. We therefore chose compounds based on the priority substances found at a study site in Durban (South Africa), where the presence of antiretroviral (ARV) drugs and antibiotics in the water is of particular concern, because a growing part of the population takes these as treatment for HIV (Bischel et al., 2015). In addition to being prescribed for the treatment of bacterial infections, antibiotics are frequently used as prophylaxis to prevent infection in patients who have suppressed immune systems (e.g., HIV+). ARV drugs and antibiotics in source-separated urine were also investigated elsewhere (Jaatinen et al., 2016; Pynnonen and Tuhkanen, 2014). Beside four ARV drugs (atazanavir, darunavir, emtricitabine, and ritonavir), three antibiotics and one corresponding human metabolite (clarithromycin, trimethoprim, sulfamethoxazole, and its human metabolite N4-acetylsulfamethoxazole), other pharmaceuticals commonly used in industrial countries were selected (diclofenac, hydrochlorothiazide, atenolol, and its biological biotransformation product atenolol acid) (Bourgin et al., 2018). Furthermore, we used bioassays as reported in literature for urine testing to evaluate estrogenicity and toxicity of urine during storage and biological treatment.

2. Materials and methods

2.1. Target pharmaceuticals and analytical methods

Compounds were chosen based on their relevance in low- and middle-income (Bischel et al., 2015) and industrialized countries (See introduction). Individual spike concentrations of the 12 target pharmaceuticals in urine were chosen with concentrations (100–800 μg L^{-1}) in the range of predicted average concentrations based on consumption in Switzerland (Table 1), to work in realistic and comparable concentration ranges. Measured concentrations can vary strongly depending on the medicine that individuals took at the moment of sampling. The source-separated human urine used in this study contained only small amounts of atenolol acid (4–14 μg L^{-1}), diclofenac (11–14 μg L^{-1}), and hydrochlorothiazide (66–110 μg L^{-1}). The physicochemical properties of the study's 12 selected pharmaceuticals, their isotope-labeled internal standards, and their suppliers are provided in Supplementary Information (SI) Table S1. The preparation of the pharmaceutical mixtures (Text S2) and their analysis (Text S3) are explained in detail in the SI. The methods for determining further chemical and physical parameters, e.g. COD or pH, are given in the SI (Text S4) as well as the method for biomass quantification in the fed-batch MBBRs (Text S6).

2.2. Urine solution

The source-separated human urine for this study was collected from the NoMix system urine storage tanks in Eawag's Forum Chriesbach building. The chemical oxygen demand ($COD_{dissolved}$), dissolved organic carbon (DOC), total ammonia, and phosphate levels were around 3300 mg L^{-1}, 1500 mg L^{-1}, 2500 mg L^{-1}, and 200 mg L^{-1}, respectively. The type of urine used for the experiments (e.g., stored urine or nitrified urine collected from men's or women's collection tanks; Table S5) and their composition in comparison to domestic or hospital wastewater (Table S6) are given in SI Text S5. Nitrification experiments were carried out with urine collected from either men's storage tank (partial nitrification) or women's storage tank (full nitrification and PAC experiments) as shown in Table S5. The main difference between men's and women's urine storage tanks was dilution as toilets for disabled for both sexes are connected to women's urine storage tank. The analytical methods used for measuring general parameters and inorganic compounds are described in SI Text S4.

2.3. Urine storage experiments

The urine samples (from the storage tank's top and bottom layers) were split into 250 mL aliquots in amber-colored glass bottles (screw cap Schott bottles, Silicone Cream/PTFE). The batch reactors were prepared in duplicate and spiked with the pharmaceutical mixture. For the abiotic control, a batch reactor was prepared by filtering the urine aliquot (top layer of the tank) through a 0.45 μm pore-size filter (Macherey-Nagel,

Table 1
Alphabetical list of the target compounds with their usage, measured concentrations in source-separated urine samples from a study in eThekwini, South Africa, (Bischel et al., 2015), average predicted concentrations in urine in Switzerland from annual consumption and excretion ratio, target spike concentrations in this study, and measured concentrations in the spiked human urine samples.

Compound name	Usage	Measured conc. in eThekwini (μg L^{-1}) (Avg ± 95% CI; n = 20) (Bischel et al., 2015)	Predicted average conc. in Switzerland (μg L^{-1}) (Bischel et al., 2015)	Target spike conc. in this study (μg L^{-1})	Range of measured conc. in the spiked human urine samples (μg L^{-1})
Atazanavir (ATA)	Antiviral	< 2.5	No Data	100	79–101
Atenolol (ATE)	Beta-blocking agent	31 ± 33	233	200	157–200
Atenolol acid (AA)	Human metabolite and transformation product of atenolol or metoprolol	98 ± 110	No Data	0	0–14
Clarithromycin (CLR)	Macrolide antibacterial	17 ± 29	112	200	157–200
Darunavir (DAR)	Antiviral	< 1	No Data	100	79–100
Diclofenac (DIC)	Analgesic: anti-inflammatory or antirheumatic	30 ± 10	15	200	157–200
Emtricitabine (EMT)	Antiviral	101 ± 97	No Data	100	79–100
Hydrochlorothiazide (HCT)	Diuretic	42 ± 18	574	200	157–197
N4-Acetylsulfamethoxazole (NSMX)	Human metabolite of sulfamethoxazole	360 ± 340	342	800[a]/ 400[b]	622–792[a]/ 311–396[b]
Ritonavir (RIT)	Antiviral	1.6 ± 0.5	2	100	79–101
Sulfamethoxazole (SMX)	Sulfonamide antibacterial	2300 ± 1000	137	200[a]/ 400[b]	156–198[a]/ 311–396[b]
Trimethoprim (TRI)	Antibacterial	190 ± 140	82	200	158–201

[a] spike concentration in partial nitrification experiments. [b] spike concentration in full nitrification experiments.

GF-S) and then spiking the pharmaceutical mixture and 1 mL sodium azide (NaN_3) (3 g L^{-1} stock solution, 12 mg L^{-1} final concentration in the batch reactors) to prevent biological activity. The batch reactors were covered with aluminum foil, the caps were screwed on tightly, and they were not opened during sampling. They were magnetically stirred at lab temperature (25 °C) for 77 days and sampled at 7-day intervals. To maintain anaerobic conditions in the reactors, samples were collected using a syringe and the air in the headspace was replaced with nitrogen gas (Alphagas, 99.99% purity) through inlet and outlet needles during sampling. COD, DOC, total inorganic carbon, sulfate, and sulfite concentrations in the anaerobic batch reactors are shown in SI Figure S1.

2.4. Aerobic biological urine treatment in nitrification reactors

The biological treatment of source-separated human urine was conducted in two bench-top MBBRs operated in continuous flow-through mode with partial or full nitrification. In this study, *partial nitrification* stands for the oxidation of 50% of the ammonia to nitrate, whereas *full nitrification* stands for the oxidation of nearly 100% of the ammonia to nitrate. The MBBRs were prepared using nitrified urine and Kaldnes™ biofilm carriers (made of polyethylene with a slightly lower density than water, small cylinders with 10 mm in diameter, and 8 mm in length) taken directly from an in-house, pilot-scale nitrification reactor already carrying nitrifying organisms (Fumasoli et al., 2016). Air diffusers were placed at the bottom of the MBBRs to maintain good mixing of the biofilm carriers and to avoid denitrification. The tops of the MBBRs were covered to reduce evaporation. The MBBRs' operational parameters are summarized in Table 2. Stored urine was transferred from the collection tanks to the lab in a 10-liter glass container and used as the sole influent for the MBBR in the partial nitrification stage. Inflow was controlled via a peristaltic pump (Watson-Marlow sciQ400, Falmouth, USA), which was automatically switched on and off at the target pH values of 6.0 and 6.1, respectively. For the full nitrification stage MBBR, 2 M potassium bicarbonate ($KHCO_3$) (Merck KGaA, Darmstadt, Germany) was mixed with the stored urine at a molar $KHCO_3$:NH_4^+— ratio of 1:1 and the mixture was dosed by an automated peristaltic pump (Ismatec Reglo Analog, IDEX Health & Science GmbH, Wertheim, Germany) to ensure complete nitrification. In this case, pH was kept between 6.9 and 7.0. The MBBRs' performances were monitored by measuring COD, ammonia, nitrate, and nitrite levels. The flow-through MBBRs were run for 16 days before continuous spiking with the pharmaceutical mixture to start the acclimatization phase (see Figure S2 for MBBRs' performances). In the partial nitrification stage, the pharmaceutical mixture was directly spiked into the MBBR with a high precision pump (Gynkotek, Model 480). In the full nitrification stage, the pharmaceutical mixture was first mixed with the inflow in a U-shaped glass vessel (Gerber Instruments, K. Schneider&Co.AG, Effretikon, Switzerland) and then spiked into the MBBR. The phases before and after continuous spiking of the MBBRs with the pharmaceutical mixture are called the *non-acclimatized phase* and *acclimatized phase*, respectively (Fig. S2).

Table 2
Operational parameters of the flow-through MBBRs. Biomass (suspended solids, SS) in the MBBRs was in the range of 0.9–2.62 mg_{SS} L^{-1}.

Parameters	Unit	Partial nitrification MBBR	Full nitrification MBBR
Urine volume	L	2.7	7.0
Kaldnes™ filling ratio	%	40	50
Kaldnes™ volumetric surface area	m^2 m^{-3}	460	460
Total surface area	m^2	0.50	1.66
Airflow	L h^{-1}	250	250
Dissolved oxygen concentration	mg L^{-1}	6–8	6–8
T emperature	°C	21.3 ± 1	20.4 ± 0.8
Target pH range	pH	6.0–6.1	6.9–7.0
Average hydraulic retention time (HRT)	Days	9[a] and 20[b]	9[a] and 7[b]
Experiment duration	Days	218 (106[a] and 112[b])	140 (58 [a] and 82[b])
Time points of operation of batch experiments	Days after start of experimental phase	(49, 55, 60)[a] and (26, 47, 91)[b]	(22, 27, 34)[a] and (16, 28, 42)[b]
	Number of reactor volumes replaced	(7, 8, 9)[a] and (2, 3, 5)[b]	(1, 2, 3)[a] and (2, 3, 5)[b]

[a] Phase before continuous spiking of the pharmaceutical mixture (non-acclimatized phase). [b] Phase with continuous spiking of the pharmaceutical mixture (acclimatized phase).

The fed-batch MBBRs (500 mL volume) used 250 mL of nitrified urine and Kaldnes™ from the flow-through MBBRs. The Kaldnes™ filling ratio was the same as in the flow-through MBBRs. The decrease in pH caused by ammonia nitrification was balanced by a continuous inflow of stored urine to the partial nitrification stage fed-batch MBBR via a peristaltic pump (Watson-Marlow sciQ400, Falmouth, UK). With a flow rate of 0.2 mL h^{-1}, the result was a pH of 6.3 ± 0.2. In the full

nitrification stage, the fed-batch MBBR stored-urine inflow was spiked with 2M $KHCO_3$ solution with a molar $KHCO_3$:NH_4^+ ratio of 1:1, and the flow rate ranged between 0.64 and 1.21 mL h^{-1} to maintain a pH between 6.9 and 7.0. Since the fed-batch MBBRs did not have an outflow, a maximal dilution of 7% was expected in the pharmaceutical concentration after 24 h. After spiking the pharmaceutical mixture, sampling was performed at 0.5, 2, 4, 6, 8, 10, and 24 h in the partial nitrification stage. In the full nitrification stage, the pharmaceutical mixture's solvent was evaporated by a factor of 10 to minimize the increase in DOC (see SI Text S2). The reactor was sampled at 0.3, 1, 3, 6, 12, and 24 h.

An abiotic control batch reactor (brown Schott bottle) was operated in parallel, without Kaldnes™. It was prepared at the same time with 250 mL of nitrified urine from the flow-through MBBR, which was filtered through 0.45 μm GF/F glass microfibre filters (Whatman, Dassel, Germany) and spiked with NaN_3 to prevent biological activity. The pharmaceutical mixture was added to the reactor at the beginning of the experiments. For aeration, the control reactor was magnetically stirred at 300 rpm throughout the experiment.

2.5. Determination of kinetic parameters

A biotransformation rate constant k_{bio} for each pharmaceutical in the fed-batch MBBRs was calculated assuming a pseudo-first-order reaction (Joss et al., 2006a) with respect to the pharmaceutical concentration. We assumed that change in the biotransformation due to microbial growth was negligible, and the microorganisms were not limited by substrates, nutrients, or electron acceptors. The estimation of the k_{bio} was performed using a nonlinear regression analysis in the nlmrt package of R software version 3.3.0. More details are given in Text S7.

2.6. Powdered activated carbon (PAC) experiments

The sorption experiments used Norit® SAE Super brand PAC (pH at point of zero charge (pH_{PZC}) of 9.8, 94009–7, Cabot Norit Americas Inc., Marshall, TX, USA). Nitrified urine from the biological nitrification reactor operating in partial nitrification mode in Eawag's Forum Chriesbach building (Fumasoli et al., 2016) was filtered through a 0.45 μm pore size GF/F filter (Whatman, Dassel, Germany) and split into aliquots in 20 Erlenmeyer flasks (including ten control samples spiked with NaN_3). Two sets of experiments were run: in the first set, the background DOC concentration in the flasks rose from 100 mg L^{-1} to around 1400 mg L^{-1} after spiking the pharmaceutical mixture due to its methanol/ethanol content. Since high background DOC concentrations might influence the sorption of pharmaceuticals to the PAC (Delgado et al., 2012), the pharmaceutical mixture solvent was evaporated down by a factor of 10 for the second set of experiments. Here, the DOC concentration in the batches rose from 62 mg L^{-1} to 158 ± 33 mg L^{-1}. PAC was added in duplicates to achieve concentrations of 50, 100, 200, and 400 mg PAC L^{-1} (250 mL aliquots) and 25, 50, 100, and 150 mg PAC L^{-1} (40 mL aliquots) in the first and second set of experiments, respectively. The flasks were shaken at 150 rpm at 25 °C for 24 h. For the analysis of the pharmaceuticals, the samples were filtered through 0.45 μm GF/F filters and the filtrate was stored at −20 °C until measurement. Details on the calculated Freundlich adsorption isotherm constants (K_F) are shown in SI Text S8, Table S11, and Figure S7.

2.7. Bioassays

Urine samples were enriched by performing solid-phase extraction (SPE), as described by Escher et al. (2005b) for yeast estrogen screen (YES) and bioluminescence inhibition tests. The recombinant YES was performed based on the description by Routledge and Sumpter (1996). In the bacterial bioluminescence inhibition test, *Vibrio fischeri* bacteria were exposed to the extracted samples in 96-well plates. Detailed information is given in SI Text S9, Figure S8, and Table S12.

3. Results and discussions

3.1. Degradation of pharmaceuticals during urine storage

Significant target pharmaceutical degradation during urine storage (anaerobic conditions and pH 9, concentrations see Table S5 in the Supplementary Information) was only observed for hydrochlorothiazide (Fig. 1). In 50 days, its concentration decreased by 90% in both the biotic and abiotic batch reactors, indicating that chemical hydrolysis was the major removal pathway. The first-order transformation rate constant for hydrochlorothiazide (k_{hydro}) under both biotic and abiotic conditions was 0.04 day^{-1}.

Other compounds showed slightly decreasing patterns during storage. Since degradation under biotic and abiotic conditions did not differ significantly, the degradation was assumed to be abiotic. Concentrations of atazanavir, atenolol, clarithromycin, and darunavir decreased by 6%, 20%, 27%, and 35%, respectively, over 77 days (Figure S3; see Table 3 for rate constants). The concentration of the human metabolite N4-acetylsulfamethoxazole decreased by 30–50% in 77 days. However, the concentration of its parent compound, sulfamethoxazole, simultaneously increased, suggesting a transformation of N4-acetylsulfamethoxazole into sulfamethoxazole, as found elsewhere (Göbel et al., 2007). The sum of sulfamethoxazole and N4-acetylsulfamethoxazole together decreased slightly, by 20%, up to day 77. The trimethoprim concentration had not diminished in either the biotic or abiotic batch reactor by day 42. At day 49, however, an unexplainable decrease of approximately 40% was observed in the biotic batch reactor, followed by no further decrease by day 77. The concentration of emtricitabine and diclofenac did not significantly change in 77 days. Few literature references are available on the degradation of pharmaceuticals during urine storage. Gajurel et al. (2007) found that diclofenac was not removed in a year at pH levels of 4, 7, and 10, whereas sulfamethoxazole decreased by 30% in a year (Butzen et al., 2005) or by 24% during 6 months of urine storage (Jaatinen et al., 2016) at pH 9. Winker et al. (2009) reported that after 1.5 years of storage, several beta-blockers and antibiotics were still present in urine. To conclude, our results confirm literature data that two-month storage is insufficient to substantially degrade pharmaceuticals.

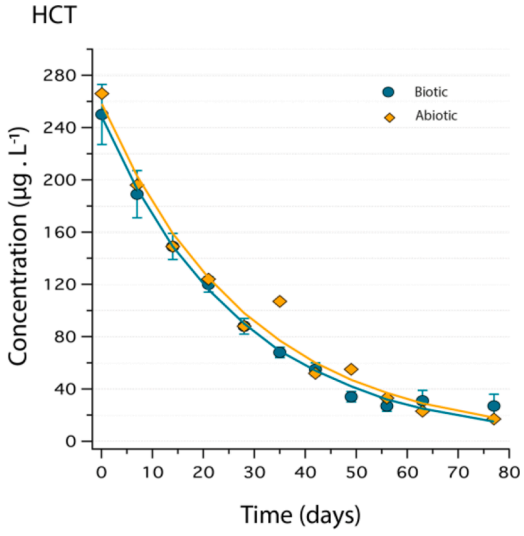

Fig. 1. Concentrations of hydrochlorothiazide (HCT) during storage of source-separated human urine in anaerobic batch reactors (pH 9). Error bars correspond to deviations from the average values of duplicate batches operated using urine from the top and bottom of the storage tank (n_{batch} = 4). An abiotic control experiment was carried out using urine from the top of the storage tank in a single batch reactor.

Table 3
Summary of the kinetic rate constants in anaerobic urine storage batch experiments (k_{hydro} in d^{-1}), in urine nitrification experiments (k_{bio} in L g_{SS}^{-1} d^{-1} and half-lives $t_{1/2}$ in hours; ranges for non-acclimatized and acclimatized triplicate sets; details on each set are given in Tables S7 and S8), and in the nitrification of wastewater (conventional activated sludge; CAS, membrane bioreactor; MBR, and MBBR) from literature. *The minus sign in front of k_{bio} indicates the formation of sulfamethoxazole in the experiments. $^{+}$sign indicates that the removal during nitrification may be due to both biotransformation and adsorption. NA: not analyzed.

Experiment:	Urine storage anaerobic (pH 9)	Partial nitrification (pH 6)		Full nitrification (pH 7)		Rate constants in CAS or MBR	Rate constants in MBBR
Compounds	k_{hydro} (d^{-1})	k_{bio} (L g_{SS}^{-1} d^{-1})	$t_{1/2}$ (h)	k_{bio} (L g_{SS}^{-1} d^{-1})	$t_{1/2}$ (h)	k_{bio} (L g_{SS}^{-1} d^{-1})	k_{bio} (L g_{SS}^{-1} d^{-1})
Atazanavir^{+}	0.002	3.5–93	0.18–4.8	50–180	0.01–0.33	NA	NA
Darunavir	0.01	0.26–1.7	7.3–> 24	0.36–2.0	0.6–> 24	NA	NA
Emtricitabine	0	0.00–0.06	> 24	0.05–0.37	> 24	NA	NA
Ritonavir^{+}	NA	4.7–69	0.24–3.52	24–150	0.005–0.71	NA	NA
Clarithromycin	0.005	1.9–37	0.45–8.6	1.9–71	0.24–8.4	(< 0.5)[1], (0.034–0.2)[2]	(0.63–2.05)[12]
Sulfamethoxazole	−0.01*	−0.01*–0.29	> 24	0.01–0.20	> 24	(0.3)[3], (0.083)[4]	(0.19–0.43)[12], (0.24–0.38)[13], (0.10)[14]
N4-acetylsulfamethoxazole	0.01	0.02–0.44	> 24	0.16–0.28	> 24	(5.9–7.6)[1], (3.2–5)[1], (>2.9)[2]	NA
Sulfamethoxazole+N4-acetylsulfamethoxazole	0.003	0.01–0.04	> 24	0.03–0.23	> 24	NA	NA
Trimethoprim	0	0.02–0.05	> 24	0.09–0.61	> 24	(0.15)[3], (0.22)[2], (0.05–0.9)[5], (pH6;9.2, pH7;5.7, pH8;1.6)[6], (<0.3)[7]	(1–3.3)[7], (0.67–1.51)[12], (0.26–0.41)[13], (0.26)[14]
Diclofenac	0	0.03–0.11	> 24	0.02–0.13	> 24	(1.2)[3], (0.04)[1], (< 0.02)[2], (<0.1)[7], (0.01)[8]	(1.3–1.7)[7], (0.26–0.38)[8], (0.36–1.37)[12], (5.28–5.52)[13], (0.07)[14]
Hydrochlorothiazide	0.04	0.00–0.07	> 24	0.02–0.17	> 24	(<0.2)[7]	(<0.1)[7]
Atenolol	0.003	0.08–0.50	> 24	0.36–1.66	10–> 24	(0.69)[9], (1.1–1.9)[10], (1.5)[11], (pH6;0.33, pH7;0.99, pH8; 2.7)[6]	(1.97–4.32)[12], (0.31–0.7)[13], (1.20)[14]

[1] (Joss et al., 2006b).
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[13] (Tang et al., 2017).
[14] (Escolà Casas et al., 2015a).

3.2. Removal of pharmaceuticals during aerobic biological urine treatment

The concentrations of a few pharmaceuticals decreased substantially during aerobic biological treatment. These decreases can be attributed to biotransformation or adsorption on biofilm because no elimination was observed in the abiotic control batch reactors (without carriers). However, the shape of the removal curves fit to biotransformation assuming a pseudo-first-order reaction, so biotransformation rate constants were calculated. Compounds for which adsorption cannot be excluded will be discussed below. Among the ARV drugs, atazanavir and ritonavir were removed by approximately 90% within 2 h (Fig. 2). In some experiments, the removal of these compounds was so rapid that the initial experimental concentrations (< 10 min) could not be determined. For these two compounds, partial sorption cannot be excluded, since log $Dow_{pH=6/7}$ is quite high (4.54 for atazanavir and 5.22 for ritonavir). Biotransformation rate constants were > 3.5 L g_{SS}^{-1} d^{-1} and > 4.7 L g_{SS}^{-1} d^{-1} for atazanavir and ritonavir, respectively, with no significant differences between the partial and full nitrification experiments (Table 3). However, biotransformation seems to be slightly faster with acclimatized biomass. Darunavir was degraded by 30–90% within 12 h across the different experiments, showing some variation between the replicates. Graphs of all the slowly degraded pharmaceuticals, along with their best fitting curves during each fed-batch experiment, are provided in SI Figures S4–S6. The biotransformation rate constants ranged from 0.26 to 2.0 L g_{SS}^{-1} d^{-1}, but without any specific trends observable among the different conditions. Emtricitabine was mostly stable over 24 h under all conditions. To the best of our knowledge, there is no literature available on the elimination efficiencies of the investigated ARV drugs in urine or wastewater treatment, except for ritonavir, which was 78% removed by the biological treatment of hospital wastewater in a membrane bioreactor (solid retention time of 30–50 days) (Kovalova et al., 2012) and 11–21% removed in the conventional activated-sludge treatment of municipal wastewater (Bourgin et al., 2018). Clarithromycin was degraded relatively quickly, with rate constants > 2 L g_{SS}^{-1} d^{-1}, but it showed significant variation across the triplicates in the fed-batch experiments. Slightly faster transformation was observed with acclimatized biomass, with a transformation of > 76% within 12 h (Fig. 2). In wastewater treatment, no sorption to sludge was found (Göbel et al., 2007). The concentration did not drop below detection limits but instead remained constant on a low plateau after the first decrease. This trend cannot be explained by first-order biotransformation kinetics (which were still used to calculate a rate constant by omitting these points). Gulde et al. (2018) saw the same phenomena for amines and explained it as ion trapping in protozoa: neutral compounds diffuse through their cell membrane and become trapped in acidic vesicles because diffusion of the newly formed, positively charged species is strongly hindered. This phenomenon may also hold for atazanavir

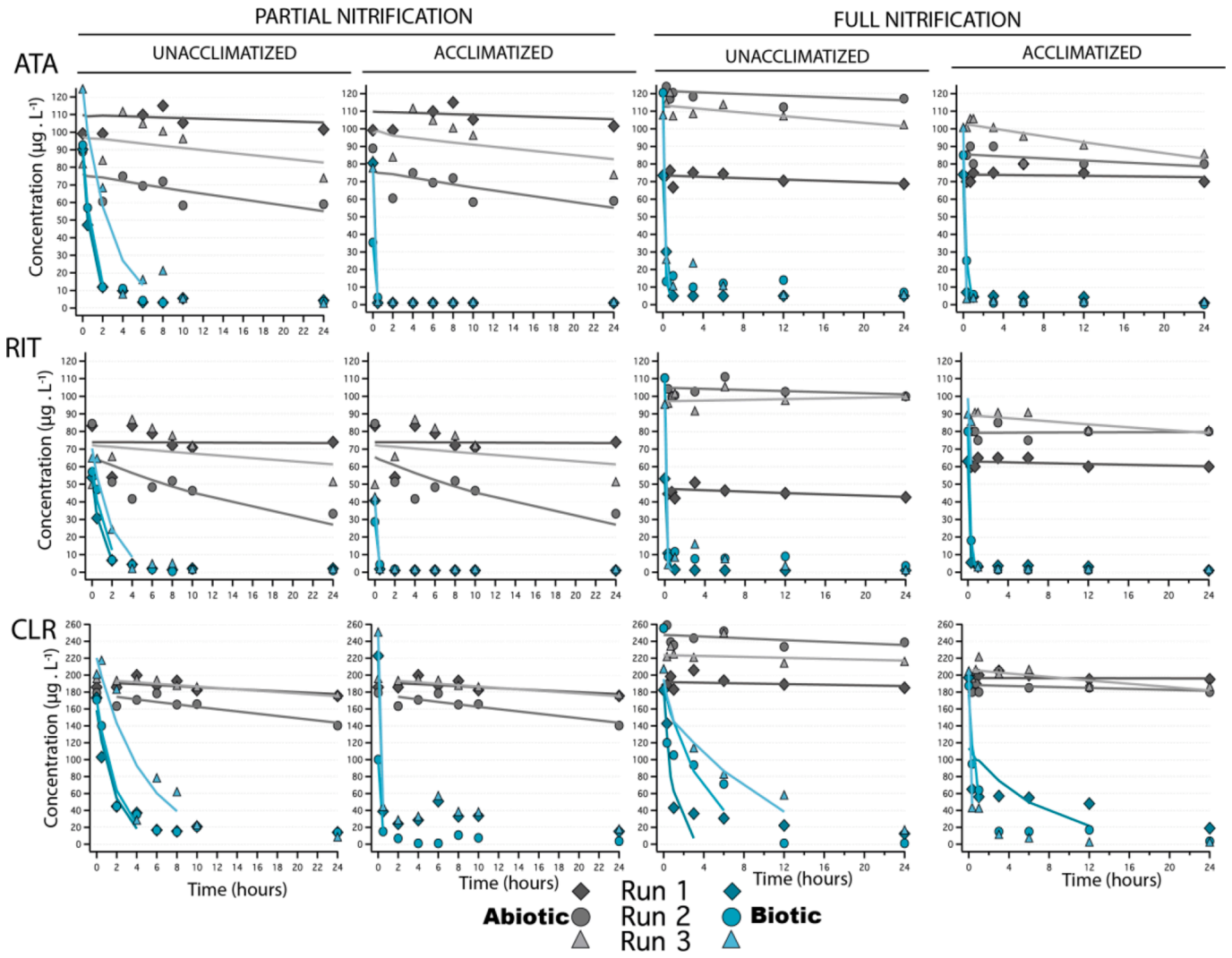

Fig. 2. Concentrations of the fast-degrading pharmaceuticals atazanavir (ATA), ritonavir (RIT), and clarithromycin (CLR) in fed-batch MBBRs operated in the partial or full nitrification stages, with or without acclimatized microbiota.

and ritonavir, where such a plateau was also observed. Previous reports of clarithromycin transformation varied from 0 to 80% in municipal or hospital wastewaters treated using conventional activated sludge or membrane bioreactors (Bourgin et al., 2018; Michael et al., 2013). The k_{bio} was generally reported as lower in municipal wastewater (< 2 L g_{SS}^{-1} d^{-1}) than in the present study's urine treatment (2–71 L g_{SS}^{-1} d^{-1}, Table 3). Higher clarithromycin removal rates were related to longer sludge retention times (Göbel et al., 2007), which might have allowed specialized bacteria to grow. For all the other compounds, where available, the k_{bio} determined in urine were in a similar range as in municipal or hospital wastewater treatment (Table 3). Sulfamethoxazole concentrations increased in partial nitrification batch bioreactors, whereas its human metabolite, N4-acetylsulfamethoxazole, decreased stoichiometrically, with transformation rate constants from 0.02 to 0.44 L g_{SS}^{-1} d^{-1}. This indicates a conversion of one to the other which had been reported previously in municipal wastewater treatment (Göbel et al., 2005). Under full nitrification conditions, sulfamethoxazole and N4-acetylsulfamethoxazole were both slightly degraded. It should be noted that the inflow concentration ratios of sulfamethoxazole to N4-acetylsulfamethoxazole were 1:4 and 1:1 in the partial and full nitrification modes, respectively, which may have influenced their transformation. Moreover, sulfamethoxazole and N4-acetylsulfamethoxazole speciation (pK_a around 6) are different under partial (pH 6) and full nitrification (pH 7). Gulde et al. (2014) observed a pH dependency for the biotransformation of such polar, ionizable micropollutants (Table 3). Varying transformation efficiencies for sulfamethoxazole have also been reported in the literature on wastewater, such as from −280% (i.e., the formation of sulfamethoxazole) to 98% (Kern et al., 2010) (Table 3). Data for N4-acetylsulfamethoxazole were not always given in the literature, therefore, it is hard to judge the deconjugation of this compound. The concentration of trimethoprim decreased very slowly, reaching a maximum 44% removal within 12 h under full nitrification. Biotransformation rate constants for trimethoprim (pK_a around 7) ranged from 0.01 to 0.05 L g_{SS}^{-1} d^{-1} in partial-nitrification MBBRs and, slightly higher, from 0.1 to 0.6 L g_{SS}^{-1} d^{-1} under full-nitrification conditions. Previous studies reported similar removal rates in wastewater (0.05–0.9 L g_{SS}^{-1} d^{-1}, Table 3) and the removal of less than 30% was found in wastewater treatment with full nitrification (Göbel et al., 2007; Perez et al., 2005). However, higher elimination, of up to 90%, was obtained with longer solid retention times, such as 60–80 days (Göbel et al., 2007), or above 40% in MBBR fed with wastewater (Escolà Casas et al., 2015b) (k_{bio} up to 1.51 L g_{SS}^{-1} d^{-1}, Table 3). Atenolol was degraded by 15–70% under full nitrification (k_{bio} 0.4–1.7 L g_{SS}^{-1} d^{-1}) and between 8–60% under partial nitrification (0.08–0.5 L g_{SS}^{-1} d^{-1}) within 12 h. Under partial nitrification conditions, atenolol acid was produced as a transformation product at a 1:1 ratio, whereas under full nitrification, atenolol acid was found in lower amounts, indicating other transformation pathways and products

that had not been analyzed. In the literature, similar k_{bio} were reported for atenolol in wastewater (0.69 to 1.9 L g_{SS}^{-1} d^{-1}, Table 3), but higher removal rates were reported in staged MBBR (up to 4.32 L g_{SS}^{-1} d^{-1}, Table 3). Atenolol transformation in membrane bioreactors and conventional activated sludge (Kovalova et al., 2012) was found to vary between 0–99%. Hydrochlorothiazide and diclofenac were degraded less than 20% within 12 h, and k_{bio} were below 0.2 L g_{SS}^{-1} d^{-1} in every MBBR batch. Diclofenac was also found to be slowly degraded in wastewater, with rate constants in a similar or slightly higher range (< 0.02–1.2 L g_{SS}^{-1} d^{-1}, Table 3). We did not find such high rates as found by Tang et al. (2017) in an MBBR intermittently fed with raw wastewater (up to 5.52 L g_{SS}^{-1} d^{-1}, Table 3). Diclofenac removal has been reported from 0 to 85% (Joss et al., 2006a; Radjenovic et al., 2007; Ternes, 1998; Zupanc et al., 2013); hydrochlorothiazide removal has been found from 0 to 66% in membrane bioreactors (Kovalova et al., 2012; Radjenovic et al., 2007) and 0–77% in conventional activated sludge (Castiglioni et al., 2006; Radjenovic et al., 2007)—there is substantial variation depending on the treatment conditions.

Overall, biological urine treatment by nitrification proved to be more effective than urine storage at eliminating pharmaceuticals. Similar removal rates were found in batch experiments conducted with non-acclimatized and acclimatized biota, except for the three compounds with the fastest transformation (atazanavir, ritonavir, and clarithromycin), where acclimatization slightly increased the rate constants, especially in the experiments involving partial nitrification. Since the reactor volume flowing through our MBBR, after starting acclimatization, was only exchanged 2 to 5 times before the fed-batch experiments with acclimatized biota were started, a longer lag time for acclimatization may have further increased the transformation of pharmaceuticals in the acclimated biota. (Wang and Wang, (2018)) observed that biotransformation of, e.g., sulfamethoxazole, trimethoprim, and diclofenac, was significantly enhanced in acclimated activated sludge. To the best of our knowledge, the literature contains no further studies investigating the biotransformation of pharmaceuticals using acclimatized microbiota in a human urine matrix.

The removal of pharmaceuticals from source-separated human urine using nitrification can be considered as a beneficial side effect of that process because its main purpose is the stabilization of nitrogen in urine for use as fertilizer and the removal of easily degradable organic substances. Full nitrification of source-separated human urine has the advantage of higher process stability because nitrite accumulation is less critical, as shown by (Jiang et al., (2011)), than in partial nitrification, as investigated by (Udert and Wächter, (2012)). Furthermore, the distillation product is more thermally stable (Udert et al., 2015). Pharmaceutical removal was very similar under full and partial nitrification conditions, except for slightly higher trimethoprim and atenolol elimination under full nitrification. Six out of eleven pharmaceuticals were mostly stable under both the partial and full nitrification processes. A supplementary treatment, therefore, e.g., using PAC, is necessary when a pharmaceutical-free fertilizer is desired.

3.3. Post-treatment using PAC

PAC's removal efficiency for 11 pharmaceuticals (including one human metabolite) is presented in Fig. 3. Similar results were obtained in the first and second sets of experiments (despite the significant addition of solvent from the spiked pharmaceutical mixture in the first set), showing that the solvent did not influence sorption. At the lowest concentration of PAC applied, 25 mg L^{-1}, more than 70% of atazanavir, ritonavir, and trimethoprim were removed, whereas less than 20% of emtricitabine, N4-acetylsulfamethoxazole, or sulfamethoxazole were removed. Removal of the other five compounds ranged from 20 to 70%. Pharmaceutical removal efficiencies increased steadily with increasing concentrations of PAC (Fig. 3, Table S10). With 100 mg PAC L^{-1}, more

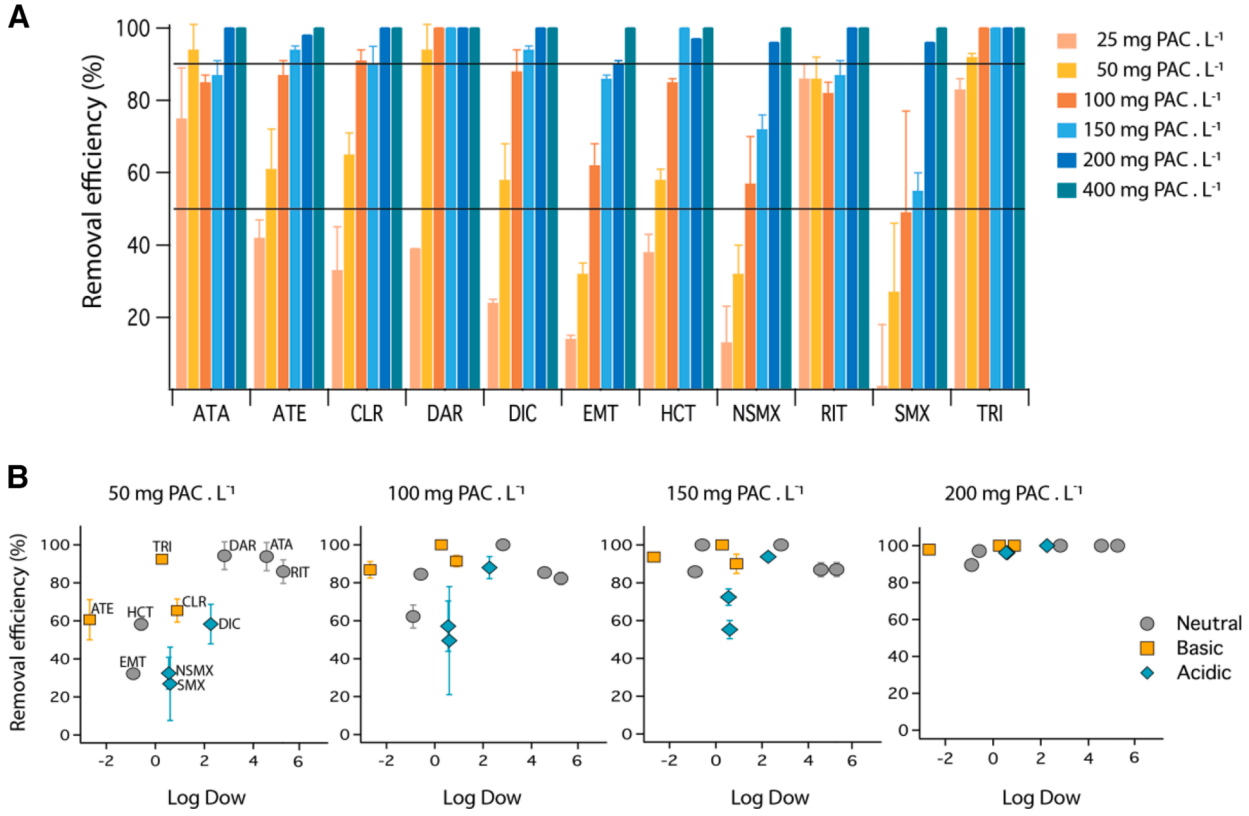

Fig. 3. Removal efficiencies (A) for each pharmaceutical and (B) depending on the pharmaceutical's log $Dow_{pH=6}$ at varying PAC dosages in partially nitrified urine (pH 6; 24 h contact time). Average values for both set of experiments with standard deviations are presented. Horizontal lines correspond to 50% and 90% removal efficiencies.

than 50% of each compound was removed, and 90% removal of each compound could be achieved with a dose of 200 mg PAC L^{-1}. The removal efficiency for sulfamethoxazole using 100 mg PAC L^{-1} showed the largest variation between the two sets of experiments but similar values in duplicates (72% and 76%, and 22% and 28%, in the first and second sets, respectively), which may have been influenced by the different initial spike concentrations in the two sets (Table 1). A comparison of hydrophobicity (log $Dow_{pH=6}$) and pharmaceutical removal efficiencies at varying concentrations of PAC revealed no clear correlations between the charged pharmaceuticals' physicochemical properties and their adsorption on PAC (Fig. 3), as also found by others (Kovalova et al., 2013). Other properties, e.g. molecular size, presence of specific functional groups, electrostatic interactions, may play a role, but such effects were not further investigated. Experimental adsorption data were fitted to the most commonly examined models—the linear, Langmuir, and Freundlich isotherms. Freundlich isotherms fitted best to the experimental data (Text S8, Figure S7) and the constants are given in the SI (Table S11).

The pharmaceutical removal efficiencies were compared to previously reported values in a wastewater matrix for the same PAC type (Table S10). Note that the comparison to the same PAC type is important, since sorption of pharmaceuticals to different PAC types can differ (Zietzschmann et al., 2014). A PAC dose of 10–20 mg L^{-1} was required to achieve an average 80% removal of micropollutants from wastewater with a typical DOC concentration of 5–10 mg L^{-1} (Boehler et al., 2012; Nowotny et al., 2007), so a PAC:DOC ratio of about 2:1 was needed. Since the DOC concentration of the present study's nitrified urine ranged from 100 to 200 mg L^{-1}, the concentration of PAC required for a similar removal would be expected to range from 200 to 400 mg PAC L^{-1}, which agrees well with our results. Considering that urine volumes range between 0.8 L and 2.0 L per person per day (WHO, 2006) and that per person wastewater volumes in Switzerland are typically 350 L per day, the volume of urine in domestic wastewater can be estimated at 0.2–0.6%. However, the amount of PAC required to treat a person-equivalent of urine is not 150–500 times higher, but only ten times higher in urine than in domestic wastewater. Hence, at least ten times less PAC is needed per person. As a result, eliminating pharmaceuticals at their source from biologically treated urine might be more economical than in wastewater treatment. Also compared to the costs of urine treatment, PAC dosage would be cheap: at typical costs of 1600 EUR t^{-1} PAC (DWA, (2019)) and 400 mg PAC L^{-1} urine, the costs would be 0.0007 EUR L^{-1} urine. This is substantially lower than the electricity costs for nitrification and distillation. Based on data given by (Fumasoli et al., (2016)) (electricity demand 71 Wh gN^{-1}, ammonium concentration in the influent 1.8 gN L^{-1}) and a typical electricity cost of 0.27 EUR kWh^{-1}, the electricity costs would be 0.03 EUR L^{-1} urine or about 50 times higher than the costs for PAC.

To ensure good quality fertilizer made from nitrified urine treated with PAC, nutrients should not be lost during the process. Our measurements showed that treating nitrified urine with 200 mg PAC L^{-1} did not remove significant amounts of ammonia, nitrate, and phosphate (Table S9).

3.4. Ecotoxicological evaluation of aerobic biological treatment

Estrogenic activity in non-spiked men's urine was measured at 923 ng EEQ L^{-1} using the YES test; partial nitrification removed 99% down to 11 ng EEQ L^{-1} (Figure S8 and Table S12). Estrogenic activity in stored men's urine taken from the waterless urinals and NoMix toilets at Eawag was previously reported as 84 ng EEQ L^{-1} (0.3 nM EEQ) (Escher et al., 2006), which is one order of magnitude lower than the level measured in this study. Different background estrogenic activity may not only arise from differences in pharmaceutical concentrations, but also from differences in the urine matrix such as salt and ammonia concentrations, but further investigations would be needed to clarify this assumption. Estrogenic activity in raw municipal wastewater was reported to range from 37 to 100 ng EEQ L^{-1} (Margot et al., 2013). The removal of estrogenicity from raw wastewater via biological treatment (activated sludge or MBBR) ranged from 75 to 99% (with a level of 0.7–8.3 ng EEQ L^{-1} in the effluent) depending on the extent of nitrification (Margot et al., 2013). In the present study, partially nitrified women's urine showed estrogenic activity of 33 and 53 ng EEQ L^{-1} before and after spiking the pharmaceutical mixture, respectively. After treatment with 100 and 200 mg PAC L^{-1}, sample estrogenicity had diminished to 5 and 6 ng EEQ L^{-1}, respectively, corresponding to a reduction of around 90%, whereas the blank value was 6 ng EEQ L^{-1}. For urine-based fertilizer production, the final urine is distilled, but for its later application to soils, (WHO, (2006)) recommends re-dilution by a factor of 100. Estrogenic activity was therefore also measured in the distilled urine product (enrichment by a factor of 100) after dilution with nanopure water (by a factor of 100), and it was found to be equivalent to the value before distillation (6 ng EEQ L^{-1}), which also corresponds to the blank value. The bacteria luminescence inhibition test found an EC_{50} value of 0.15 M in source-separated human urine from the men's storage tank (Figure S8). Source-separated human urine was previously reported to have an EC_{50} value of 0.31 M in a bioluminescence assay (Escher et al., 2005a). Effluent from the partial nitrification stage flow-through MBBR operated using stored men's urine clearly showed a lower toxicity (EC_{50} of 0.34 M) than the raw stored men's urine sample (EC_{50} of 0.15 M). The EC_{50} toxicity of nitrified-spiked women's urine was 13.9 M, and the value increased to 29 M and 39 M after treatment with 100 and 200 mg PAC L^{-1}, respectively. An EC_{50} value for the distilled urine product could not be calculated, indicating no toxicity. These results showed that nitrification resulted in a clear improvement in urine quality and that this could be increased even more by PAC sorption.

3.5. Consequences of urine treatment on fertilizer quality and environmental risk

According to literature, two-month urine storage can ensure the inactivation of *Cryptosporidium* protozoa, gram-positive bacteria, and various viruses (Bischel et al., 2015; Vinneras et al., 2008). However, our study shows that two-month urine storage is not sufficient to remove pharmaceuticals. To make a risk assessment of the urine to be used as fertilizer, we followed an EU technical guidance document (European Chemicals Bureau, 2003) to calculate the predicted environmental concentration (PEC_{soil}) of pharmaceuticals that would be found in soil after urine fertilizer application (typically 150 kg N $hectare^{-1}$ $year^{-1}$) (Jönsson et al., 2004). We used the average concentrations of the pharmaceuticals measured in the urine storage tanks in Durban and a post-treatment scenario involving a PAC concentration of 200 mg L^{-1} (Table S13), assuming no transformation in the biological treatment or in the soil. The resulting concentrations were compared to the predicted no-effect environmental concentrations in soil ($PNEC_{soil}$) as estimated by Martin et al., (2012) for trimethoprim (3102 μg kg^{-1}), diclofenac (1595 μg kg^{-1}), and sulfamethoxazole (1.19 μg kg^{-1}). For diclofenac and trimethoprim, the estimated PEC_{soil} after treatment with a PAC dose of 200 mg L^{-1} was well below the $PNEC_{soil}$, but higher by a factor of 1.5 for sulfamethoxazole. However, the PEC_{soil} was calculated for concentrations of sulfamethoxazole measured in Durban, which are very high (average 2300 μg L^{-1}) (Bischel et al., 2015). From the amounts of sulfamethoxazole consumed in Switzerland, Germany, France, and the USA, predicted average concentrations in urine would only range from 121 to 187 μg L^{-1} (Bischel et al., 2015). Starting from a concentration of 187 μg L^{-1} sulfamethoxazole and after treatment with 200 mg PAC L^{-1}, we calculated a PEC_{soil} of 0.15 μg kg^{-1}, resulting in a risk quotient below

1. These results are encouraging for urine's suitability as a fertilizer after enhanced treatment with activated carbon.

4. Conclusion

- e r In our study, we determined which steps in the treatment train are necessary to produce a safe fertilizer from urine.
- Long-term storage up to two months was not found to be suitable for pharmaceutical removal, as only one out of the 12 pharmaceuticals examined, hydrochlorothiazide, was degraded considerably.
- Nitrification, needed for nutrient stabilization and removal of bulk organics, generally resulted in biotransformation of pharmaceuticals at similar rates to those reported for wastewater treated with activated sludge. Nitrification was also an efficient way to decrease the estrogenicity and non-specific toxicity measured by bacteria bioluminescence inhibition of urine.
- To reliably remove pharmaceuticals from treated urine, a post-treatment using adsorption to PAC was necessary. Fortunately, this process showed no loss in nutrient content.
- A risk assessment of the treated urine used as fertilizer on soil resulted in a risk quotient below 1 for the concentrations of trimethoprim, diclofenac, and sulfamethoxazole predicted in European countries and the USA.
- These results, and results from studies with granular activated carbon (Köpping et al., 2020) have led to the production of a urine fertilizer (named *Aurin*), that is authorized for use on vegetables and flowers in Switzerland (Vuna GmbH 2020).

CRediT authorship contribution statement

Birge D. Özel Duygan: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Visualization. **Kai M. Udert:** Conceptualization, Methodology, Writing - review & editing, Supervision, Project administration, Formal analysis. **Annette Remmele:** Formal analysis, Investigation, Writing - review & editing. **Christa S. McArdell:** Conceptualization, Methodology, Visualization, Resources, Writing - review & editing, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Kai M. Udert is co-owner of the Eawag spin-off Vuna Ltd. Vuna Ltd. uses granular activated carbon for pharmaceutical removal. The study was not influenced by the relationship of Kai M. Udert with Vuna Ltd.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.resconrec.2020.105341.

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