Enantioselective degradation of amphetamine-like environmental micropollutants (amphetamine, methamphetamine, MDMA and MDA) in urban water

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ABSTRACT

This paper aims to understand enantioselective transformation of amphetamine, methamphetamine, MDMA (3,4-methylenedioxy-methamphetamine) and MDA (3,4-methylenedioxyamphetamine) during wastewater treatment and in receiving waters. In order to undertake a comprehensive evaluation of the processes occurring, stereoselective transformation of amphetamine-like compounds was studied, for the first time, in controlled laboratory experiments: receiving water and activated sludge simulating microcosm systems. The results demonstrated that stereoselective degradation, via microbial metabolic processes favouring S-(-)-enantiomer, occurred in all studied amphetamine-based compounds in activated sludge simulating microcosms. R- (+)-enantiomers were not degraded (or their degradation was limited) which proves their more recalcitrant nature. Out of all four amphetamine-like compounds studied, amphetamine was the most susceptible to biodegradation. It was followed by MDMA and methamphetamine. Photochemical processes facilitated degradation of MDMA and methamphetamine but they were not, as expected, stereoselective. Preferential biodegradation of S-(-)-methamphetamine led to the formation of S-(-)-amphetamine. Racemic MDMA was stereoselectively biodegraded by activated sludge which led to its enrichment with R-(-)-enantiomer and formation of S-(-)-MDA. Interestingly, there was only mild stereoselectivity observed during MDMA degradation in rivers. This might be due to different microbial communities utilised during activated sludge treatment and those present in the environment. Kinetic studies confirmed the recalcitrant nature of MDMA.

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1. Introduction

Amphetamine based compounds: amphetamine, methamphetamine, MDMA and MDA (Table S1) are characterised as "psychoactive substances with stimulant, euphoric, anorectic, and, in some cases, empathogenic, entactogenic, and hallucinogenic properties" (Carvalho et al., 2012). The α-methyl group prevents the oxidation of the amine group by monoamine oxidases increasing the ability of the molecule to cross membranes, including the blood-brain barrier resulting in its pharmacokinetics (Young and Glennon, 1986). Amphetamine based compounds have been used for several millennia, through the consumption of Catha edulis (Khat) and various plants in the genus Ephedra (family Ephedraceae) (Carvalho et al., 2012). Only S-(-)-amphetamine is prescribed by the UK National Health Service (NHS) (NHS, 2013). Un-prescribed use and possession of amphetamine also is illegal, as is possession or use of all other amphetamine based compounds included within this article. The quantity of amphetamines (not including MDMA) seized by UK police and border force agencies increased by 30% to 1.4 tonnes in 2012/13 compared to the previous 12 months (Coleman, 2013).

Amphetamines have high oral bioavailability, high volume of distribution, and low plasma protein binding (usually less than 20%) with renal and/or hepatic excretion occurring between 6 and 12 h. Excretion may occur post-hepatic metabolism. However for several amphetamine based compounds a significant proportion is not biotransformed (Kraemer and Maurer, 2002), meaning the detection of parent drugs within wastewater is feasible. The metabolic pathway of amphetamine (Fig. S1) produces a conjugated hippuric acid or a conjugated hydroxyamphetamine as the major metabolites, although hydroxynorephedrine is also a minor
metabolite (Kraemer and Maurer, 2002). The half-life of S-(+)-amphetamine, due to its faster metabolism, is shorter than that of R-(−)-enantiomer (Cody et al., 2004). Consequently excreted amphetamine is enriched with R-(−)-amphetamine. The metabolism of methamphetamine (Fig. S1) into either 4-hydroxymethamphetamine or into amphetamine is catalysed by CYP2D6 leading to inter-individual variability (Lin et al., 1997). Methamphetamine is also poorly metabolised with up to 55% excreted as the parent drug (de la Torre et al., 2004), with S-(+)-methamphetamine having a shorter half-life than that of the R-(−)-enantiomer. MDMA (Fig. S2) is extensively metabolised in humans resulting in only around 20% excreted unchanged (de la Torre et al., 2004). The two major human metabolic pathways are through the opening of the methylenedioxy ring followed by methylation and then phase II conjugation, or the N-dealkylation into MDA followed by deamination and oxidation (Lim and Foltz, 1989).

The ecotoxicological effects of amphetamines are unknown. Human toxicological pathways include oxidative and/or nitrodative stress and neurotoxicity. It is suggested that these may be potential modes of action in other species. It is also known, through laboratory animal studies, that hepatotoxicity occurs across organisms (Carvalho et al., 2012).

All amphetamines have been identified in wastewater, although to date there are no known regulations addressing the concentrations of illicit drugs in wastewater or receiving environments. Amphetamine and methamphetamine appear to be readily degraded (usually >75% removal) during conventional activated sludge treatment wastewater treatment (Bijlsma et al., 2014; Baker and Kasprzyk-Hordern, 2013; Huerta-Fontela et al., 2008). This is reflected in very low concentrations found in effluent and receiving waters and usually not exceeding 20 ng L\(^{-1}\) of amphetamine and 100 ng L\(^{-1}\) of methamphetamine. However during periods of higher drug usage (e.g. during a music festival) removal rates dropped to <15% (Bijlsma et al., 2014) resulting in discharge of amphetamine into the receiving environment. Compared to other amphetamine based compounds MDMA has generally poor and varying removal rates from 12% to 99% (Bijlsma et al., 2012, 2014; Baker and Kasprzyk-Hordern, 2013; Huerta-Fontela et al., 2008). This poor and wide removal rate is further exacerbated during high drug loading in the influent, e.g. due to a music festival, with rates dropping to <20% (Bijlsma et al., 2014). Due to poor removal rates during wastewater treatment, event related high usage of MDMA and (un)intentional release of unused MDMA (e.g. during police raid at illegal production facility) into the sewage system might result in widespread occurrence of MDMA in the receiving environment.

Degradation of amphetamines is thought to be highly stereoselective in wastewater. The limited work undertaken has revealed that, similarly to human metabolism, microbial metabolic processes during wastewater treatment favour S-(−)-amphetamine (Kasprzyk-Hordern and Baker, 2012a). Degradation of amphetamine and methamphetamine has also been studied in river simulating microcosm experiments (Bagnall et al., 2013). The experiments demonstrated that under biotic conditions stereoselective microbial degradation, which favours S-(−)-enantiomers, takes place. The above results could have significant ecotoxicological consequences as amphetamines are known to have enantiomers showing different potency and, potentially, markedly different ecotoxicity. Research in this area aiming at a full understanding of the stereoselective transformation of amphetamines in the environment is therefore of key importance. This paper aims to verify enantioselective transformation of amphetamine, methamphetamine, MDMA and MDA during wastewater treatment and in receiving waters. In order to undertake a comprehensive evaluation of processes occurring, stereoselective transformation of amphetamines was studied, for the first time, in controlled laboratory experiments - receiving water and activated sludge simulating microcosm systems.

2. Experimental

2.1. Chemicals and materials

The reference standards: R/S-(±)-amphetamine, R/S-(±)-methamphetamine, R/S-(±)-MDMA (3,4-methylenedioxyamphetamine) and R/S-(±)-MDMA (3,4-methylenedioxy-methamphetamine), were purchased from LGC Standards (Teddington, UK). All solvents were of HPLC grade and were purchased from Sigma-Aldrich. All glassware was silanised with dimethyldichlorosilane (5% DDMCS in toluene, Sigma-Aldrich) to minimise sample loss through adsorption of basic analytes onto –OH sites present on glass surface. The surrogate/internal standards (IS): R/S-(±)-amphetamine-d11, R/S-(±)-methamphetamine-d14, R/S-(±)-MDMA-d5, R/S-(±)-MDA-d5 were purchased from LGC standards (Middlesex, UK). Stock solutions of each compound (1 mg mL\(^{-1}\)) were prepared in methanol and stored in the dark at –16 °C. Working solutions were prepared by diluting stock solution in mobile phase and stored at –16 °C.

2.2. Sample collection and storage

Time proportional 24 h composite samples of wastewater were collected (10 mL every 15 min) using a 3700 portable sampler (ISCO, Lincoln USA). Recent results have indicated that acidification to pH 4 with hydrochloric acid preserves compounds, with no significant EF change and no mobilisation of the compounds from solid particles (unpublished results). Therefore pH of each sample was maintained at 4.0 during sampling, transportation and storage, and then returned to pH 7 prior to SPE. Grab samples were collected from the river. All samples were transported back to the laboratory in cool boxes packed with ice blocks and frozen immediately upon arrival. Due to equipment failure samples were collected continuously from Thursday 10th July to Sunday 13th July, then restarted from Monday 21st July to Thursday 24th July.

2.3. Sample preparation

The method has been described in detail by Evans et al. (Evans et al., 2015). Briefly, liquid samples were filtered and 50 mL samples were collected and passed through Oasis HLB cartridges (Waters, UK) at a rate of 6 mL min\(^{-1}\) and eluted in 4 mL methanol under gravity prior to evaporation to dryness. This process was carried out in duplicate; each duplicate was then reconstituted in one of the two mobile phases detailed below.

2.4. Sample analysis

All samples were analysed, as described by Evans et al. (Evans et al., 2015) using HPLC performed on Waters ACQUITY UPLC™ system (Waters, Manchester, UK). The chiral separation was carried out with the use of two columns. The CBH, an enzyme based column packed with Cellobiohydrolase (100 × 2 mm, 5 μm, Sigma Aldrich, UK) was run isocratically with 90:10 water:isopropanol, 1 mM ammonium acetate at a rate of 0.075 mL min\(^{-1}\). Each injection was 20 μL.

All analytes were identified and quantified using a Xevo TQD Triple Quadrupole Mass Spectrometer (Waters, UK), equipped with an electrospray ionisation source in positive ion mode. Nitrogen was used as the nebulising and desolvation gas, supplied by a high purity nitrogen generator (Peak Scientific, UK). Argon (99.998%)
was the collision gas supplied by a BOC cylinder (BOC, UK). MassLynx 4.1 (Waters, UK) was used to control the Waters ACQUITY system and the Xevo TQD. Optimised MS parameters were as follows: the capillary voltage set at 3.49 kV, source temperature at 150 °C, desolvation gas flow at 300 L h⁻¹. Nitrogen was used as nebulising and desolvation gas, while argon was used as a collision gas. MassLynx 4.1 (Waters, UK) was used to control the Waters ACQUITY system and the Xevo TQD. Data processing was carried out on TargetLynx software (Waters, Manchester, UK) (Evans et al., 2015).

2.5. Quantification and confirmation

Each compound was quantified by multiple reaction monitoring (MRM), using the protonated molecular ion as the precursor ion. The most abundant transition product ion was used to quantify, whilst confirmation was carried out using the lesser abundant product ion (Table S2). In addition the ratio of quantifier to confirmatory ion was used according to limits set by EC guidelines (2002). Deuterated surrogate/internal standards were used to compensate for ion suppression/enhancement, loss during sample preparation and/or stereoselective mechanisms during sample preparation (Evans et al., 2015). Enantiomeric fractions were calculated using Equation (1) (Evans et al., 2015).

\[
EF = \frac{(+) - (-)}{(+) + (-)}
\]

Where: EF denotes enantiomeric fraction, (+) denotes concentration of S-(+)-enantiomer and (-) indicates concentration of R-(−)-enantiomer, in relation to the compounds targeted within this paper. An EF > 0.5 therefore indicates an enrichment of the S-(+)-enantiomer and EF < 0.5 indicates an excess of R-(−)-enantiomer.

Full validation data are presented in Tables S3–4 and are described in detail elsewhere (Evans et al., 2015).

2.6. Full scale study

In order to verify stereoselective transformation of chiral drugs during wastewater treatment and in receiving waters, a large WWTP3 serving 910 thousand people and a 33 km long stretch of a river were monitored. The river was sampled in July 2011; four sampling points were chosen: location R1 and R2 (upstream and downstream from two discharges from small WWTPs 1&2 respectively), location R3 and location R4 (Fig. 1). Grab samples were collected in the morning and afternoon for 1 week from four river sites whose locations in relation to the WWTPs can be seen in Fig. 1. A monitoring campaign of WWTP3 was undertaken in July 2014, with time proportional samples collected from three locations: after screens (location W1), after settling tanks (location W2) and after activated sludge treatment (location W4). Grab samples of the activated sludge reactors were collected at location W3 at the WWTP (Fig. 1). Main characteristics of the WWTP are provided in Table S5.

2.7. Microcosms

River microcosms were carried out with water collected from location R1 shown in Fig. 1. Receiving water microcosms were 90:10 river water (also collected from location R1); WWTP effluent. Activated sludge microcosms were set up with activated sludge taken during the mixing phases of the batch reactors (location W3). River and receiving water microcosms were carried out in 2 L cylindrical flasks under continuous mixing with magnetic stirrers. Each flask was kept under specific conditions of either dark (wrapped in foil), light (exposed to an Osram 400 W HQI BT daylight lamp for 12 h per day, through borosilicate 3.3 glass with no visible light absorption and UV light cut-off at <275 nm, intensity was measured as 320 μmol/S/m²) and biotic or abiotic (sterilised with 1 g L⁻¹ sodium azide). All reactors had cotton wool stoppers and were agitated by magnetic stirrers to maintain high O₂ levels. Samples were collected every day for 2 weeks. Dissolved oxygen, pH and temperature were measured daily. Dissolved oxygen was in the range of 7–8 mg L⁻¹. The pH ranged between 8 and 9. Temperature of microcosms was within the range: 21–29 °C.

Activated sludge microcosms were carried out in 2 L cylindrical flasks with air pumped to below the surface of the liquid. All reactors were kept in the dark with rubber bungs to allow controlled provision of air and exhausts to be filtered. Magnetic stirrers were employed to prevent stratification. Eleven samples were collected over 24 h. Physicochemical parameters of wastewater such as ammoniacal nitrogen, nitrate, nitrite and chemical oxygen demand are provided in Table S5.

Microcosms were carried out in duplicate and were either spiked with a single compound or with a mixture of compounds.

2.8. Kinetics – activated sludge simulating microcosms

The compounds studied are characterised as having low volatility and therefore volatilisation was not considered as a potential removal pathway in studied microcosms. Photodegradation was also not considered (not relevant) under tested activated sludge conditions. Therefore the two important degradation mechanisms to consider were biodegradation and sorption to sludge. Sorption of studied compounds was previously reported as <10% (Baker and Kasprzyk-Hordern, 2011) and therefore could be considered negligible. Several reports utilised pseudo-first-order kinetics for degradation of micropollutants in activated sludge reactors (Joss et al., 2006; Suarez et al., 2010; Collado et al., 2012). Indeed when applying pseudo-first order kinetics (OECD 303) in this work, ln(Ce/Ci) plotted as a function of time yielded a straight line (R² > 0.9). Pseudo-first order biodegradation rate k₁ [L g⁻¹ h⁻¹] (normalised for concentration of suspended solids) was therefore calculated using the following formula (Equation (2)).

\[
\ln \frac{C_e}{C_i} = -k_1*t*SS
\]

where: t = aeration time (24 h), C_e = concentration at time point t (μg L⁻¹), C_i = initial concentration (μg L⁻¹), SS = concentration of activated sludge solids (g L⁻¹).

3. Results and discussion

3.1. Occurrence of chiral amphetamines in receiving waters and during wastewater treatment

3.1.1. Occurrence of chiral amphetamine-like drugs in rivers

The environmental monitoring programme carried out in a large river in the UK showed no quantifiable results for amphetamine or methamphetamine. On the other hand, MDMA was found to be ubiquitous in the receiving waters (Fig. 2). Concentrations did generally rise in the river downstream from WWTP1 and 2 and after activated sludge treatment (location W4). The enantiomeric fraction of MDMA was consistently below 0.3, showing a persistence of the R-(−)-enantiomer in the aqueous environment. This is in agreement with excretion patterns by humans and with the values reported by
Regarding MDA, only S-\((+)-MDA\) was identified in analysed samples. This is most probably due to metabolism of MDMA, which favours degradation of S-\((+)-MDMA\) leading to the formation of S-\((+)-MDA\). This indicates that MDA present in analysed environmental samples is due to the metabolism of MDMA and not as a result of illegal use of MDA. In the case of illegal use, MDA in the environmental matrix would be either enriched with R-\((-)-enantiomer\) (due to stereoselective metabolism of MDA favouring S-\((+)-enantiomer\) or it would remain racemic (because MDA is distributed as racemate) (Kasprzyk-Hordern and Baker, 2012a, 2012b). As enantiomers of MDA and MDMA are known to have different pharmacological actions (e.g. according to Marquardt et al., 1978) S-\((+)-MDA\) behaves more like the stimulant amphetamine and R-\((-)-MDA\) behaves more like the hallucinogen LSD) there is high probability that two enantiomers of MDA or MDMA will trigger different (potentially toxic) biological responses in the environment. Detailed ecotoxicological studies are therefore required to verify the significance of stereoselective degradation of these drugs.

### 3.1.2. Occurrence and fate of chiral amphetamine-like drugs during wastewater treatment

The 24-composite wastewater influent samples from WWTP3 showed consistently quantifiable concentrations of amphetamine with an average EF = 0.6 (Fig. 3) indicating its enrichment with S-\((+)-enantiomer\). The drug persisted through the settling process; however the concentration drastically dropped during activated sludge treatment. The EF was found to decrease to racemic. The EF of amphetamine continued to decrease slightly as the activated sludge process continued and the wastewater was transferred for discharge containing amphetamine with EF of 0.4 indicating enrichment of amphetamine with R-\((-)-enantiomer\). This is an outcome of significant importance showing that enantiomeric composition of amphetamine could be reversed during activated
sludge treatment. This could potentially be of significant ecotoxicological importance as an assumption is usually made during environmental risk assessment that drugs remain in the environment in the same enantiomeric form as marketed. The WWTP process was found to be effective with the removal of 93 ± 2% of amphetamine (accounting for 95 ± 1% and 89 ± 4% removal of S-(-) and R-(-)-amphetamine respectively) from wastewater.

Methamphetamine was detected at relatively low concentrations (<50 ng L⁻¹) in wastewater (Fig. 3). It was mostly enriched with potent S-(-)-enantiomer in raw wastewater. The range of removal rates for methamphetamine was found to be relatively large: 23% ± 88%. Stereoselectivity in the removal was observed and led, similarly to amphetamine, to an enrichment of methamphetamine with R-(-)-enantiomer.

MDMA was the most prevalent of all the illicit drugs targeted in wastewater influent with concentrations exceeding 1 μg L⁻¹ (Fig. 3). This supports the literature data suggesting that this is a relatively popular drug within the UK. Its concentration was higher in the weekend samples, which is to be expected from a recreational drug. The concentration of MDMA was also higher in the settled wastewater than in the influent, which was unexpected and cannot be explained at this stage. MDMA was also detected in the effluent. The settling process was found not to change the EF of MDMA. However, the activated sludge process showed high stereoselectivity and resulted in an enrichment of MDMA with R-(-)-enantiomer which is in agreement with the EF recorded in receiving waters. The removal rate of MDMA was on average 40% ± 24% and is mainly attributed to the removal of S-(-)-MDMA (mean removal for S-(-)- and R-(-)-MDMA was 53% ± 29% and 1% ± 20% respectively). This poor removal concurs with the literature values which have been reported previously (Bijlsma et al., 2012, 2014; Baker and Kasprzyk-Hordern, 2013; Huerta-Fontela et al., 2008). The above indicates that removal of MDMA is attributed to S-(-)-MDMA. R-(-)-MDMA is not degraded under the studied conditions and therefore it should be considered as a pollutant of recalcitrant nature.

MDMA was also detected in wastewater (Fig. 3). The concentration profile appears to mirror MDMA concentrations with peaked concentrations falling towards the end of the weekend and just after. The concentration of MDA increased sharply between the influent and the activated sludge stage, indicating that MDA is produced by the breakdown of MDMA considering the complementary reduction in concentration of this compound during the same time frame. The EF of MDA in influent and settled wastewater tends to be in excess of 0.5. This is to be expected considering the anticipated human metabolic pathway of MDMA, favouring S-(-)-enantiomer and leading to the formation of S-(-)-MDA. However, activated sludge treatment brings this enantiomeric fraction, due to microbial activity, to below 0.5, most likely through the preferential degradation of S-(-)-MDA. This is contradictory to the data gathered from the July river water samples which only detected S-(-)-MDA. The overall increase of S-(-)- and R-(-)-MDA during activated sludge treatment accounted for 89 ± 35% and 254 ± 69% respectively, which confirms the recalcitrant nature of R-(-)-MDA.

3.2. River (and receiving waters) and activated sludge simulating microcosms

In order to study the mechanisms of transformation and to understand which processes are responsible for stereoselective degradation of amphetamines, several river, receiving waters and activated sludge simulating microcosms were undertaken under controlled laboratory conditions. The following phenomena were studied: microbial metabolic transformation (in dark and light biotic reactors – DBRs and LBRs), photolysis (in light biotic and light
abiotic reactors — LBRs and LARs) and other physicochemical processes, e.g. hydrolysis, sorption (in dark and light abiotic reactors — DARs and LARs).

3.2.1. MDMA

3.2.1.1. River and receiving water microcosms. The results of the mixed-compound river water microcosms (Fig. S3) indicated that MDMA was only slightly degraded (<50%) over the course of two weeks in abiotic conditions both in the presence and absence of light (see DAR and LAR reactors in Fig. S3). On the other hand, microbial degradation in the presence of light led to the complete removal of MDMA within the two weeks study period (see LBR reactor in Fig. S3). Only slight stereoselectivity was observed in biotic conditions in the second week of the experiment and led to the enrichment of MDMA with R-(-)-enantiomer.

Microbial metabolic degradation in the presence of light was also the most successful in removing MDMA in the mixed-compound receiving water microcosms (see DBR and LBR reactors in Fig. 4), although photolysis was also found to reduce the concentration of MDMA by approximately 80% over the two weeks. The microorganisms did not degrade MDMA under anoxic conditions (see DABR reactor in Fig. S4). The biological degradation resulted in only slight enrichment of MDMA with R-(-)-enantiomer under dark biotic conditions in the second week of the experiment.

3.2.1.2. Activated sludge microcosms. In contrast to the river-water microcosms, the mixed-compound activated sludge microcosms resulted in high stereoselectivity of microbial metabolic degradation (Fig. 5). Due to faster degradation of S-(+)-MDMA, which was almost completely removed in 9 h, a significant dominance of R-(-)-MDMA would be expected to be present in effluent discharges considering the 16 h activated sludge retention time (as observed at the sampled WWTP).

The single-compound activated sludge microcosm confirmed that stereoselective microbial metabolism favours degradation of S-(+)-MDMA with the formation of S-(+)-MDA (Fig. 6a). No degradation of R-(-)-MDMA was observed during the 24 h duration of

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**Fig. 4.** Mixed-compound receiving waters microcosms — MDMA, MDA, amphetamine and methamphetamine degradation under dark biotic (DBR) and light biotic (LBR), conditions (concentrations are represented by bars, enantiomeric fractions are represented by symbols). Note: See Figs. S4 – S7 for results from all studied microcosm conditions: abiotic (DAR), dark biotic (DBR), light biotic (LBR), light anoxic (LAR), dark anoxic biotic (DABR) and dark anoxic biotic (DAAR) conditions.
the microcosm experiment. An increase of MDA concentration aligned with a decrease of MDMA concentration further supports our hypothesis. Interestingly, MDA’s enantiomeric fraction was found to decrease in time from 0.92 to 0.62, which might suggest that S-(+)-MDA, formed as a by-product of MDMA metabolism, is subsequently preferentially metabolised, which then leads to the enrichment of MDA with R-(−)-enantiomer.

The above results are of significance regarding the fate and possible effects of MDMA in the aqueous environment. Based on our results, there is only mild stereoselectivity observed during MDMA degradation in rivers. However, a pronounced stereoselective biodegradation favouring degradation of S-(+)-MDMA and formation of S-(+)-MDA is observed in activated sludge simulating microcosms. This might be due to the different microbial communities utilised during activated sludge treatment and those present in the environment. MDMA is produced as a racemate but due to human metabolism and then microbial metabolic processes during wastewater treatment it becomes enriched with R-(−)-enantiomer. Kinetic studies (Table 1) confirmed the recalcitrant nature of MDMA. $K_{bio}$ and $t_{1/2, bio}$ of S-(+)-MDMA degradation were 1.36 $\text{L g}^{-1} \text{h}^{-1}$ and 0.51 h respectively. Due to the lack of degradation of R-(−)-MDMA no kinetic studies could be undertaken.

3.2.2. MDA

3.2.2.1. River and receiving waters microcosms. MDA was not degraded under abiotic and/or anoxic conditions in the mixed-compound receiving water microcosm, as shown in Fig. S5. Aerobic biological degradation did significantly degrade MDA and resulted in an excess of S-(+)-MDA in dark conditions, although not significantly in light conditions. Photochemical processes were not found to degrade either enantiomer, so this difference in enantiomeric fractions between biotic light and biotic dark is thought to be due to the development of different microorganism populations and/or different metabolic pathways utilised in the differing conditions. Increased formation of S-(+)-MDA in dark biotic conditions within the second week is likely to be associated with metabolism of MDMA showing slight preference towards S-(+)-enantiomer under the same conditions (see Fig. 4 and Fig. S4).

3.2.2.2. Activated sludge microcosms. Interestingly, in the mixed-compound activated sludge microcosms (Fig. 5) MDA did not appear to be degraded, although a significant enantiomeric fraction change was observed. This might be the result of two processes occurring: degradation of racemic MDA (spiked into the microcosm) and formation of S-(+)-MDA as a result of metabolism of S-(+)-MDMA.

3.2.3. Amphetamine

3.2.3.1. River water and receiving waters microcosms. The results of the mixed-compound receiving waters microcosm indicated that amphetamine is not degraded by abiotic processes (see DAR and LAR reactors in Fig. S6). However, aerobic biological degradation did not require light and resulted in almost complete degradation of amphetamine in 10 days (see DBR reactor in Fig. 4). Within 3 days, only R-(−)-amphetamine remained in the solution. Under light conditions amphetamine appears to have been produced in the second week of the experiment, possibly from the degradation of methamphetamine. Anoxic conditions were able to support microorganisms which did degrade amphetamine, although only by approximately 40%. A slight excess of R-(−)-amphetamine was also noted in one of the microcosms under dark anoxic biotic conditions.

Results of the single-compound receiving waters microcosm indicated complete degradation of amphetamine in under 3 days in light and under a week in the dark (Fig. 7a). In both cases the S-(+)-amphetamine was degraded first, resulting in an enantiomeric fraction of close to 0 prior to complete removal. These results are in agreement with previous results published by Bagnall et al. (2013).

3.2.2.2. Activated sludge microcosms. The mixed-compound activated sludge wastewater microcosms, as shown in Fig. 5, indicated...
that S(-)-amphetamine is preferentially degraded which led to the enrichment of amphetamine with R(-)-enantiomer. Formation of small quantities of S(-)-amphetamine at the later stage of the microcosm experiment might be because of preferential degradation of S(-)-methamphetamine.

In single-compound activated sludge microcosms, amphetamine spiked into sterile conditions did not significantly degrade amphetamine, as shown in Fig. 6b (see DAR reactor). However, biological processes were found to be responsible for rapid and stereoselective degradation resulting in only R(-)-amphetamine within 6 h. Kinetic studies (Table 1) confirmed, as in the case of MDMA and MDA, the more recalcitrant nature of R(-)-enantiomer. \( k_{\text{biol}} \) of R(-)-amphetamine degradation was 0.52 \( \text{L}_{\text{SS}} \text{g}^{-1} \text{h}^{-1} \), which is five times lower than \( k_{\text{biol}} \) of S(-)-amphetamine (2.76 \( \text{L}_{\text{SS}} \text{g}^{-1} \text{h}^{-1} \)).

3.2.4. Methamphetamine

3.2.4.1. River and receiving waters microcosms. In mixed-compound receiving water microcosms, methamphetamine degradation was limited and was only observed under biotic conditions (see DBR and LBR reactors in Fig. 4). Microbial metabolic processes did not show high stereoselectivity, although a slight excess of R(-)-methamphetamine was observed in the second week of the experiment.
n/a - not calculated due to no degradation of R-\((-\)) enantiomers.

<table>
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<tr>
<th>Chemical</th>
<th>(\text{Degradation rate constant} (k_1 \text{ and } k_{\text{biol}}))</th>
<th>E. coli</th>
<th>K. pneumonia</th>
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<tbody>
<tr>
<td>(\text{S-}(-)\text{-amphetamine})</td>
<td>(y = 0.198x + 0.198)</td>
<td>0.9688</td>
<td>0.25 0.20 3.49 0.81</td>
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<tr>
<td>(\text{R-}(-)\text{-amphetamine})</td>
<td>(y = 0.0759x + 0.1478)</td>
<td>0.9544</td>
<td>0.25 0.68 1.03 2.76</td>
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</table>

However, in the single-compound receiving water microcosms (Fig. 7b) a pronounced stereoselectivity was observed in dark biotic conditions. This is despite lower removal rate of methamphetamine in dark biotic conditions as opposed to light biotic conditions. Amphetamine was produced as a product of this degradation, however at relatively small amounts. Bagnall et al. (2013) also found that stereoselective degradation preferentially degraded S-\((-\))-methamphetamine, although this was noted in both light and dark reactors.

**Table 1**

Degradation pseudo-first order constants (\(k_1\) and \(k_{\text{biol}}\)) in single-compound activated sludge simulating microcosms.

\[
R^2 \quad SS [\text{g L}^{-1}] \quad k_1 [\text{h}^{-1}] \quad t_{\frac{1}{2}} [\text{h}] \quad k_{\text{biol}} [\text{L gSS}^{-1} \text{h}^{-1}] \quad t_{\frac{1}{2}\text{biol}} [\text{h}]
\]

<table>
<thead>
<tr>
<th>Chemical</th>
<th>(k_1)</th>
<th>(t_{\frac{1}{2}})</th>
<th>(k_{\text{biol}})</th>
<th>(t_{\frac{1}{2}\text{biol}})</th>
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<tbody>
<tr>
<td>(\text{S-}(-)\text{-amphetamine})</td>
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<td>3.49 0.81</td>
<td>0.025 1.34</td>
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<tr>
<td>(\text{R-}(-)\text{-amphetamine})</td>
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<td>0.025 1.34</td>
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<td>3.50</td>
<td></td>
</tr>
<tr>
<td>(\text{R-}(-)\text{-methamphetamine})</td>
<td>0.9522 0.10</td>
<td>5.26 1.36</td>
<td>0.51</td>
<td></td>
</tr>
</tbody>
</table>

3.2.4.2. Activated sludge microcosms. The mixed-compound activated sludge wastewater microcosms (Fig. 5) led to almost complete removal of S-\((+)\)-methamphetamine in 9 h, suggesting a significant dominance of R-\((-)\)-enantiomers would be present in effluent discharges considering the 16 h activated sludge retention time exhibited at the sampled WWTP.

Similar results were observed in the case of single-component activated sludge microcosms (Fig. 6c). Significant S-\((+)\)-amphetamine production occurred under biotic conditions. This is to be expected due to the preferential microbial metabolic degradation of S-\((+)\)-methamphetamine. It should be noted that amphetamine formation was also observed in the abiotic reactor, which cannot be explained at this stage. Kinetic studies (Table 1) confirmed the more recalcitrant nature of R-\((-)\)-methamphetamine. \(k_{\text{biol}}\) and \(t_{\frac{1}{2}\text{biol}}\) of S-\((+)\)-methamphetamine degradation were 0.20 LgSS\(^{-1}\) h\(^{-1}\) and 3.60 h respectively. Due to the lack of degradation of R-\((-)\)-methamphetamine no kinetic studies could be undertaken.

4. Conclusions

The aim of this paper was to verify enantioselective transformation of amphetamine, methamphetamine, MDMA and MDA during wastewater treatment and in receiving waters in controlled laboratory experiments - receiving water and activated sludge simulating microcosm systems. The results demonstrated that stereoselective degradation, via microbial metabolic processes favouring S-\((-)\)-enantiomer, occurred in all studied amphetamine-based compounds in activated sludge simulating microcosms. R-\((-)\)-enantiomers were not degraded (or their degradation was limited) which proves their more recalcitrant nature. Out of all four
amphetamine-like compounds studied, amphetamine was the most susceptible to biodegradation. It was followed by MMDA and methamphetamine. Photochemical processes facilitated degradation of MDMA and methamphetamine but they were not, as expected, stereoselective.

Strong enantioselective degradation of amphetamine in favour of S-(+)-amphetamine was demonstrated in all environments. Preferential biodegradation of S-(+)-methamphetamine led to the formation of S-(+)-amphetamine. Racemic MDMA was stereoselectively biodegraded in activated sludge, which led to its enrichment with R-(−)-MDMA and formation of S-(+)-MDA. Interestingly, there was only mild stereoselectivity observed during MDMA degradation in rivers. This might be due to different microbial communities utilised during activated sludge treatment and those present in the environment. Kinetic studies confirmed the recalcitrant nature of MDMA.

To summarise, all the amphetamine based compounds studied here are stereoselectively metabolised by humans to produce an excreted product enriched with the R-(−)-enantiomers. Any stereoselective degradation carried out by organisms either in the treatment process or in the receiving environments has also been preferentially degrading the S-(+)-enantiomer in all the compounds studied here. Unfortunately, their enantiomer-specific toxicological pathways has never been documented. However, considering the toxicological pathways which they exhibit within humans it would be thought that a wide variety of organisms may be vulnerable to their toxic effects at relatively low concentrations. Toxicity tests, particularly those which identify enantiomer-specific and/or synergistic pathways, need to be carried out to assess the risk to receiving environments. This is of particular importance in the case of MDMA which is readily quantified in rivers around the world due to its poor removal rates and high usage.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2016.04.103.

References