

Technical University of Denmark



## Abiotic and biofilm-mediated transformation of heroin biomarkers in wastewater under aerobic and anaerobic conditions

Ramin, Pedram; Causanilles, A.; Polese, Fabio; Emke, E.; de Voogt, P.; Plósz, Benedek G.

*Publication date:*  
2015

*Document Version*  
Peer reviewed version

[Link back to DTU Orbit](#)

*Citation (APA):*

Ramin, P., Causanilles, A., Polese, F., Emke, E., de Voogt, P., & Plósz, B. G. (2015). Abiotic and biofilm-mediated transformation of heroin biomarkers in wastewater under aerobic and anaerobic conditions. Abstract from 2nd International Conference on "Wastewater-based drug epidemiology", Ascona, Switzerland.

**DTU Library**  
Technical Information Center of Denmark

---

**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.

# Abiotic and biofilm-mediated transformation of heroin biomarkers in wastewater under aerobic and anaerobic conditions

Pedram Ramin<sup>a</sup>, A. Causanilles<sup>b</sup>, F. Polese<sup>a</sup>, E. Emke<sup>b</sup>, P. de Voogt<sup>b</sup> and B.G. Plósz<sup>a</sup>

<sup>a</sup> Technical University of Denmark, Department of Environmental Engineering, Kgs. Lyngby, Denmark

<sup>b</sup> KWR Watercycle Research Institute, Nieuwegein, The Netherlands

pear@env.dtu.dk

Wastewater-based epidemiology is a new paradigm to monitor trends of community-wide illicit drug use based on analysis of urinary drug biomarkers in raw sewage. For reliable estimations of drug use based on observations in wastewater treatment plant (WWTP) influents, it is necessary to consider in-sewer processes, mainly biological transformation and sorption (Plósz et al., 2013). Several studies investigated the transformation of drugs by testing drug stability in raw wastewater influent. However, only few experimental studies assessed fate in full-scale sewer pipelines (Jelic et al. 2015) and the role of biofilm attached on sewer pipe walls in transformation processes (Thai et al. 2014). Sewer systems (rising pressurized mains and gravity sewers) sustain microbial growth under aerobic and/or anaerobic conditions. Biological activity substantially differs under different redox conditions (Hvitved-Jacobsen et al., 2013). It is unknown how these conditions can affect the biotransformation kinetics of drug biomarker. In this study, we assessed the transformation of selected illicit drugs (Heroin—HER) and its human metabolites (6-monoacetylmorphine—sixMAM, morphine—MOR, Morphine-3-glucuronide—MORG, Codeine—COD and Nor Codeine—NCOD) in annular rotating biofilm reactors (ARBRs), reproducing sewers under aerobic and anaerobic conditions. ARBRs consist of an inner rotating drum and outer stationary cylinder, supporting the growth of attached biomass and simulating in-sewer conditions by controlling e.g., shear stress on biofilm and substrate diffusion. Biofilm were formed in two ARBRs (one aerobic and one anaerobic) by continuously feeding the reactors with pre-clarified wastewater from external cooled ( $T \leq 4^\circ\text{C}$ ) containers sparged with air or nitrogen over one year prior to experiments. As a result, 0.75 and 1 mm biofilm were enhanced in aerobic and anaerobic reactors respectively. For biotransformation experiments, 5-L wastewater inoculum were prepared in external feeding containers (vacuum filtered,  $0.6 \mu\text{m}$ ), and continuous recirculation ( $4 \text{ Lh}^{-1}$ ) was established with ARBRs and external containers. During all operation time, rotation speeds of reactors were kept at 20 rpm. Experiments were started by spiking standards to the feed reactor (final concentration of  $10 \mu\text{gL}^{-1}$ ). Nine samples (240 ml) were taken from ARBR outlet over 48 hrs. Immediately after sampling, samples were spiked with deuterated standards (final concentration  $360 \text{ ngL}^{-1}$ ) and transported to a freezer at  $-20^\circ\text{C}$ . Control experiments were also performed using mineral water under the same experimental conditions. During the experiments, temperature ( $T_{ave} = 17.5^\circ\text{C}$ ) and pH ( $pH_{ave} = 8.9$ ) were continuously measured. Sample preparation and analysis consisted of solid phase extraction (SPE) with Oasis HLB cartridges followed by liquid chromatography coupled to high resolution mass spectrometry.  $20 \mu\text{L}$  of the sample extract were injected into the HPLC-LTQ Orbitrap, and after chromatographic separation of targeted drugs on a C18 column, full scan accurate mass data was acquired under positive electrospray ionization mode. The Activated Sludge Model for Xenobiotics (ASM-X) (Plósz et al., 2013) was used to simulate biotransformation of spiked chemicals, considering variable volume in external container and assuming no mass transfer limitation in the intact biofilm. Monte Carlo method employing Latin

Hypercube Sampling—LHS of parameter space, was used to estimate first-order abiotic ( $k_{\text{abiotic}}$ ,  $\text{d}^{-1}$ ) and biotransformation rates ( $k_{\text{bio}}$ ,  $\text{Lg}^{-1}\text{d}^{-1}$ ), with root mean square normalized error as objective function.

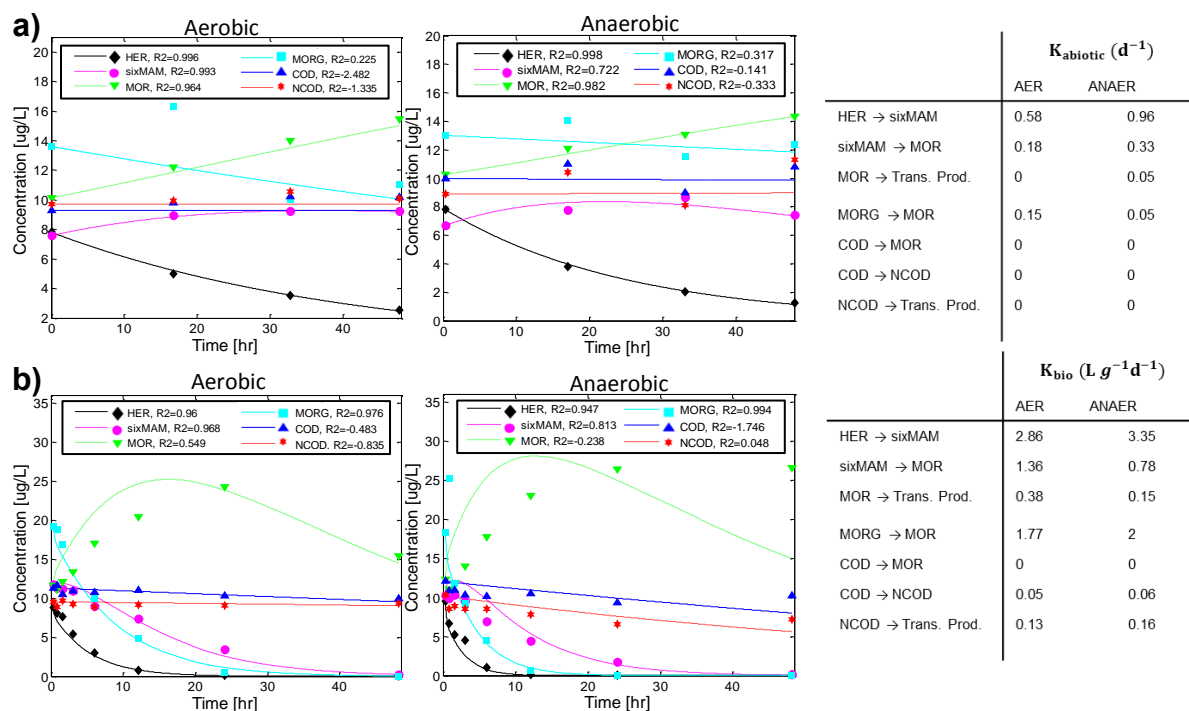


Fig. 1. Concentrations of illicit drugs in ARBR batch experiments over the course of experiments: (a) abiotic control; (b) and using pre-clarified wastewater. Values of the abiotic and biotic transformation rate parameters estimated are shown in tables to the right

As for sorption onto biofilm or suspended solids, amongst all the heroin biomarkers, only NCOD was found to sorbe onto biofilm  $k_D = 0.14 \text{ Lg}^{-1}$ . Our results suggest that beside biofilm mediated biotransformation, abiotic transformation rate for HER, sixMAM, MOR and MORG are significant. The biotransformation rate for COD and NCOD are obtained, at least, a magnitude lower than the other metabolites. Biotransformation rate for HER, sixMAM and MORG are found to be high under both aerobic and anaerobic conditions. Taken together, to back-calculate heroin abuse rate separately from MOR, 6-MAM seems to be an ideal candidate. Additionally, for assessing MOR abuse rate, the amount of transformation product derived from COD seems to be negligible, compared to MORG and 6-MAM, thereby allowing for simplifying the biokinetic process model identified to back-calculate HER abuse rate. The experimental methodology tested in this study allowed to assess biotransformation kinetics of illicit drugs under conditions typically occurring in sewer systems. Estimated kinetic parameters offer the possibility of (i) predicting the fate of illicit drugs in full-scale sewer systems and (ii) eventually refining estimations of heroin abuse in urban areas.

## References

- Hvitved-Jacobsen, T., Vollertsen, J., Haaning Nielsen, A (2013) Sewer Processes - Microbial and Chemical Process Engineering of Sewer Networks, Second Edition. CRC Press.
- Jelic, A., Rodriguez-Mozaz, S., Barceló, D., Gutierrez, O. (2015). Impact of in-sewer transformation on 43 pharmaceuticals in a pressurized sewer under anaerobic conditions. *Water Res.* 68, 98–108.
- Plósz, B.G., Reid, M.J, Borup, M., Langford, K.H., Thomas, K.V. (2013). Biotransformation kinetics and sorption of cocaine and its metabolites and the factors influencing their estimation in wastewater. *Water Res.*, 47, 2129–40.
- Thai, P. K., Jiang, G., Gernjak, W., Yuan, Z., Lai, F. Y., Mueller, J. F. (2014). Effects of sewer conditions on the degradation of selected illicit drug residues in wastewater. *Water Res.* 48, 538–547.