Identification of Biotransformation Products of Veterinary Drugs Present in Piggery Wastewater during Treatment with Photobioreactors based on Microalgae-Bacteria and Purple Phototrophic Bacteria Consortia

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Abstract
This work identified transformation products (TPs) from 16 antibiotics, 1 anti-parasitic, 1 analgesic and 1 hormone, present in spiked real piggery wastewater (PWW) before and after two different treatments in two open photobioreactors operated continuously with a consortium of microalgae-bacteria and purple photosynthetic bacteria. For this purpose, suspect and non-target strategies based on liquid chromatography quadrupole-time-of-flight mass spectrometry (LC-QTOF-MS) were used. The application of quantitative structure-retention relationship (QSRR) prediction models, in addition to a comprehensive evaluation of the obtained MS/MS spectra, provided valuable information to support the identifications. The confirmation of the TPs was carried out with the corresponding reference standards, when these were commercially available. Alternatively, probable structures of the TPs based on diagnostic evidence were proposed. To the best of our knowledge, some of the identified TPs have never been reported before. A transformation pathway for their biotransformation has been proposed. The presence of the identified TPs was assessed in real PWW samples through retrospective analysis. Ultimately, the potential ecotoxicological risk posed by these nineteen veterinary drugs and their TPs was evaluated by means of risk quotients.

Keywords: Algal-bacterial processes · Contaminants of emerging concern · PPB · Suspect list · Swine manure · Transformation products

1. Introduction

as disease control is still widespread. Pharmaceutical loads in swine manure may vary between 0.01 and 100 mg Kg^{-1} (or mg L^{-1}). Assessment of the ecotoxicity of many metabolites of certain pharmaceuticals has provided

Pig farms have industrialized during the last decades and hundreds of pigs per unit are raised in stabled conditions. The resulting piggery wastewater (PWW), containing pig urine and feces, is made up to 98% of water and high concentrations of organic matter and nutrients (Makara and Kowalski, 2015).

The European Directive 91/676/EEC, concerning the protection of waters against pollution caused by nitrates from agricultural sources, has established a limit of discharge of 170 Kg ha^{-1} y^{-1} for total nitrogen. Therefore, management strategies are urgently needed. One of the most popular swine manure treatments has been anaerobic digestion due to its energy recovery potential (Almeida Streitwieser, 2017). However, PWW contains a low carbon to nitrogen (C:N) ratio that limits nutrients recovery (Liu et al., 2017). Thus, new approaches based on phototrophic microorganisms have recently emerged (García et al., 2019). Algal-bacterial consortia (AB) have shown efficient removal of organic matter and nutrients, as a result of their dual autotrophic and heterotrophic metabolism (Rittmann and McCarty, 2012). Additionally, this symbiosis entails a low energy consumption and carbon footprint since the generated carbon dioxide (CO_2) is photosynthetically fixed (Cheah et al., 2016). On the other hand, purple phototrophic bacteria (PPB), which use the infra-red spectrum of solar radiation, can also support high rates of organic matter and nutrients assimilation (Hülsen et al., 2016).

Nutrients are not the only environmental concern that pig manure entails. Despite the fact that sub-therapeutic use of antimicrobial growth promoters is prohibited in the European Union, their use

evidence that acute and chronic toxicity can be greater than for the parent compounds. Furthermore, the connection between antibiotic residues and antibiotic resistance in pathogenic bacteria has been consistently reported in recent years, especially with respect to antibiotic use in animal production.

In the present work, a suspect list containing 19 veterinary drugs and 88 tentative transformation products (TPs) have been searched before and after two different treatments in two open photobioreactors operated continuously with a consortium of microalgae-bacteria and purple photosynthetic bacteria.

Table 1. Suspect list

	Veterinary drugs	Therapeutic class	# Associated TPs
1	**Amoxicillin**	**Antibiotics**	6
2	**Penicillin**		5
3	**Oxytetracycline**		11
4	**Doxycycline**		4
5	**Marbofloxacin**		5
6	**Ciprofloxacin**		16
7	**Enrofloxacin**		2
8	**Danofloxacin**		2
9	**Sulfadiazine**		2
10	**Sulfathiazole**		4
11	**Sulfamethizole**		1
12	**Sulfadimidine**		3
13	**Sulfamethoxazol**		8
14	**Tiamulin**		4
15	**Trimethoprim**		5
16	**Apramycin**		1
17	**Fenbendazole**	**Antiparasitics**	4
18	**Dexamethasone**	**Analgesics**	4
19	**Progesterone**	**Hormones**	20
Total			**107**

2. Material and Methods

One hundred millilters of 0.45-μm-filtered samples (n=4) were spiked at 0.1% before solid phase extraction (SPE) using Oasis® HLB cartridges (60 mg, 3 cc; Waters Chromatography). Then, cartridges were eluted with 6 mL of ACN, and the resulting organic solutions were subsequently evaporated and reconstituted in 1 mL of 0.01% FA in a mixture H_2O/MeOH (95:5). Finally, the extracts were analyzed by ultra-high performance liquid chromatography (UHPLC) – Q-ToF in MS^{All} mode. More specifically, chromatographic separation was carried out by a Waters Aquity UHPLC and a Waters Chromatography reversed-phase column HSS T3 (100 mm × 2.1 i.d., 1.8 μm particle size), making use of H2O- and MeOH-based mobile phases containing 0.01% FA as modifier. Mass detection was performed by a Xevo G2-S QToF from Waters.

3. Results and Discussion

Candidates to the suspect list were compiled by using two different in silico prediction tools: (1) the Eawag-Biocatalysis/Biodegradation Database Pathway Prediction System (Eawag-BBD/PPS) (http://eawag-bbd.ethz.ch/predict/), an artificial intelligence system, which predicts microbial metabolic reactions based on biotransformation rules set in the Eawag-BBD and scientific literature. Eawag PPS was used with the "relative reasoning mode" switched off, and (2) the MetabolitePredict software (Metabolite Tools 2.0, Bruker Daltonics, Bremen, Germany), a rule based expert system, which predicts metabolites from Phase I, II and Cytochrome P450 reactions. The prediction results from both programs included the molecular formula as well as the structures of the generated TPs from two subsequent reactions in the metabolic pathway. Already known and reported metabolites from the literature were also added to the suspect database.

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