An interdisciplinary approach to reduce the antibiotic load in waterbodies (PharmCycle)

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Abstract

The key objective of the research project „PharmCycle“ is to reduce prospective impacts of pharmaceuticals to the aquatic environment and drinking water supply. PharmCycle addresses therefore the life cycle of pharmaceuticals, from the sustainable design and production, enhanced ecotoxicological risk assessment to the improved wastewater treatment.

The first cycle runs with antibiotics, which are selected from different prioritization lists due to their environmental concern. For these antibiotics an sustainable ecotoxicological risk assessment is performed, integrating different chronic bacteria tests. A set of wastewater treatment pilot studies aims to reduce the concentration of the selected antibiotics in effluents.

The second cycle runs with sustainable antibiotics designed with physical-chemical and in silico methods.

The third cycle is executed with sustainable antibiotics, including newly designed antibiotic peptides, produced within PharmCycle using molecular and biotechnological methods.

Every cycle includes the upgraded ecotoxicologial risk assessment and investigations on wastewater treatment procedures. The approach of the project and first results are shown.

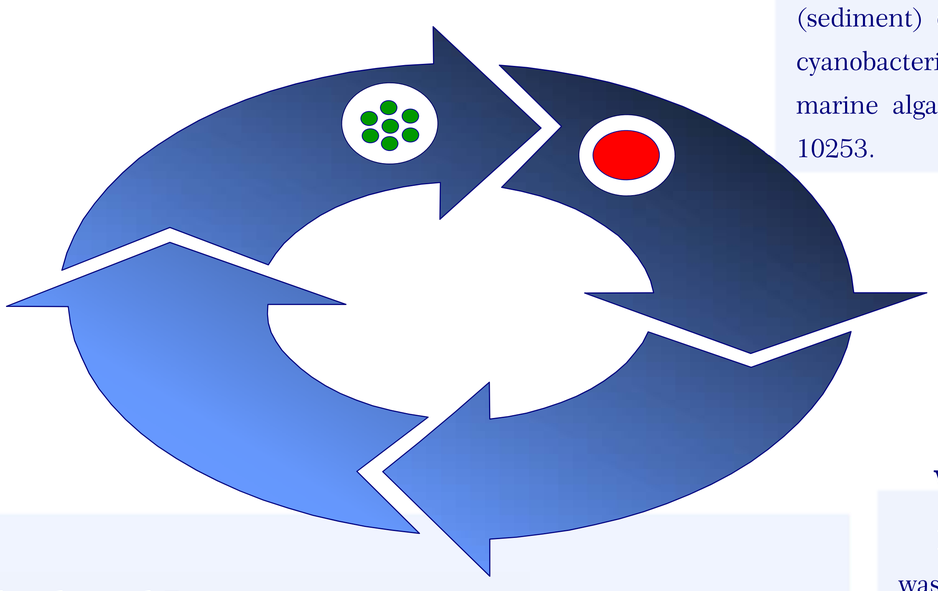
The interdisciplinary approach of PharmCycle

Sustainable Pharmacy

Sustainable pharmacy considers the complete life cycle of pharmaceuticals – from the design, processing, use, disposal to the occurrence in the environment. It is of high importance to develop active substances, which adequately fulfil their purpose and after its use will be fast and completely degraded in the environment. This approach focuses on the primary emission source and considers the complete life cycle of pharmaceuticals – Benign by Design. For the chemical design an intensive research on the transformation products with experimental *and in-silico* methods is undertaken.

Environmental law & management

§ Recommendations for the implementation of sustainable pharmacy in environmental law and environmental management will be proposed.



Sustainable ecotoxicological risk assessment

The aim is to develop a more sustainable environmental risk assessment of pharmaceuticals, especially of antibiotics. Therefore further biological test methods will be developed beside the bioassays required from the EU (EMA) to identify chronic ecotoxicity, behavioural effects, effects on symbiosis and on marine (sediment) organisms will be developed. Within PharmCycle a chronic marine cyanobacteria test was developed, which was significantly more sensitive than the marine algae test with *Phaeodactylum tricornutum* according to DIN EN ISO 10253.

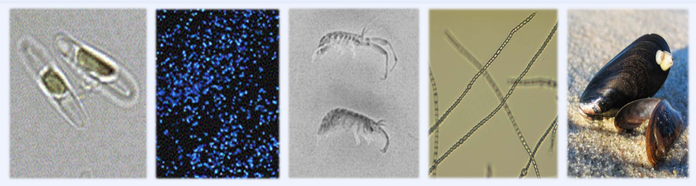


Fig. 1: Testorganism

Biotechnological Processing

An antibiotic peptide (Fig. 5) was chosen for biotechnological production, for which a fast degradation in the environment is assumed and which already proved antibacterial activity against e.g. *Staphylococcus aureus* by maintaining rather low hemolysis. Corresponding DNA was cloned and expression as a single peptide or as a peptide-onconase fusion product was achieved in *Pichia pastoris* and in *E. coli*, respectively. Cultivation and peptide production were performed in a highly instrumented 5 L bioreactor (Fig. 4). Peptide was purified from inclusion bodies (*E. coli*) or from the cultivation supernatant (yeast) by immobilized metal ion affinity chromatography and/or RP-HPLC (Fig. 6). Peptide identity was confirmed by mass spectrometry.

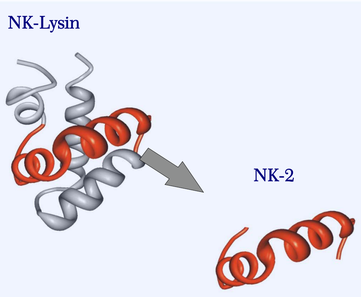


Fig. 5: Antibiotic Peptide
(Andrä & Leippe et al. 1999)

Fig. 4: 5 L Bioreactor

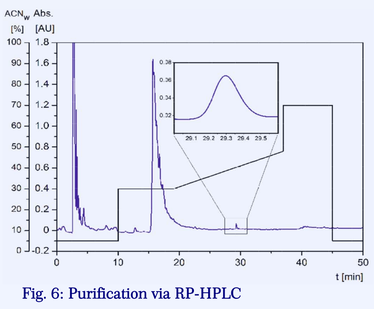


Fig. 6: Purification via RP-HPLC

Wastewater Treatment

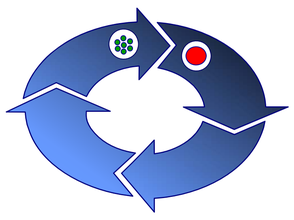
Process steps like biological wastewater treatment, various adsorption technologies and membrane filtration are suitable individually and in combination to remove pharmaceuticals from wastewater.

Among the objectives of PharmCycle the boundaries of the removability of pharmaceuticals from wastewater and the comparison to the behavior of sustainable developed pharmaceuticals in such treatment steps will be determined.

Fig. 2: Biological Treatment

Fig. 3: Membrane Filtration

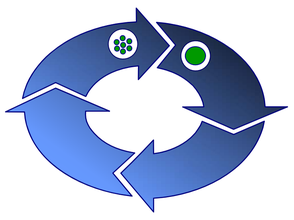
PharmCycles



- Ciprofloxacin
- Clarithromycin
- Doxycyclin
- Erythromycin
- Sulfamethoxazol
- Trimethoprim
- …

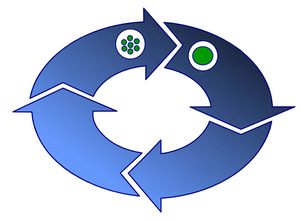
1. PharmCycle with priority antibiotics

Existing antibiotics were selected as priority antibiotics according to different criteria, e.g. occurrence in the environment, market volume, mode of action, degradation and toxicity as priority antibiotics. The priority antibiotics were subject of an enhanced ecotoxicological risk assessment and wastewater treatment.



2. PharmCycle with sustainable, chemically processed antibiotics

In the second PharmCycle sustainable, chemical processed antibiotics will be studied with enhanced ecotoxicological risk assessment and wastewater treatment methods.



3. PharmCycle with sustainable, biotechnologically processed antibiotics

In the third PharmCycle sustainable, biotechnological processed peptide antibiotics will be investigated with enhanced ecotoxicological risk assessment and waterwater treatment methods.

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