4th INTERNATIONAL CONFERENCE ON RISK ASSESSMENT OF PHARMACEUTICALS IN THE ENVIRONMENT

Barcelona, 9 - 10 October 2023

Biotransformation products of pharmaceuticals compounds in a two-phase (acidogenic-methanogenic) anaerobic bioreactor

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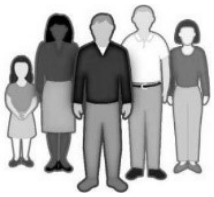
Sources of contamination of pharmaceutical compounds (PhCs)

Entrance of PhCs into the environment

PhC



Toxicity and adverse effects on human health?

BTP PhC

WWTPs

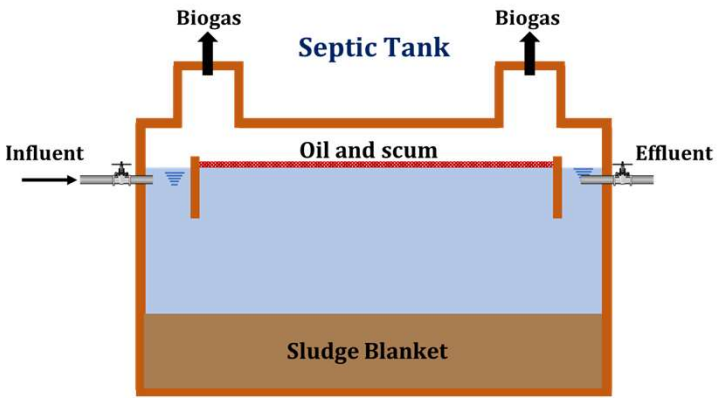
WWTPs ➔ unsatisfactory PhC removal
Biological metabolic processes ➔ biotransformation products (BTPs)

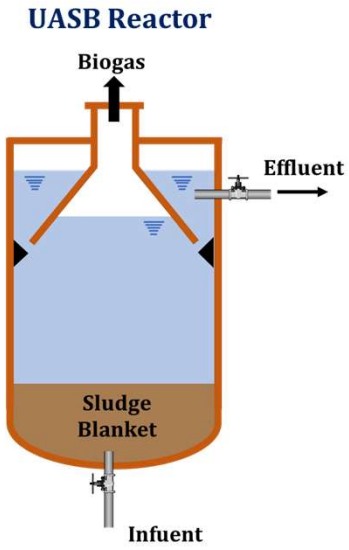
PhC BTP

Anaerobic digestion – conventional *vs* innovative bioreactors

- **Anaerobic digestion (AD)** ➔ widely applied around the world as unit of secondary biological treatment or to the sludge digestion;
- **Anaerobic fixed-film bioreactors (AFBR)** ➔ Longer SRT and biofilm formation can enhance the biodegradation of xenobiotics, such as PhCs

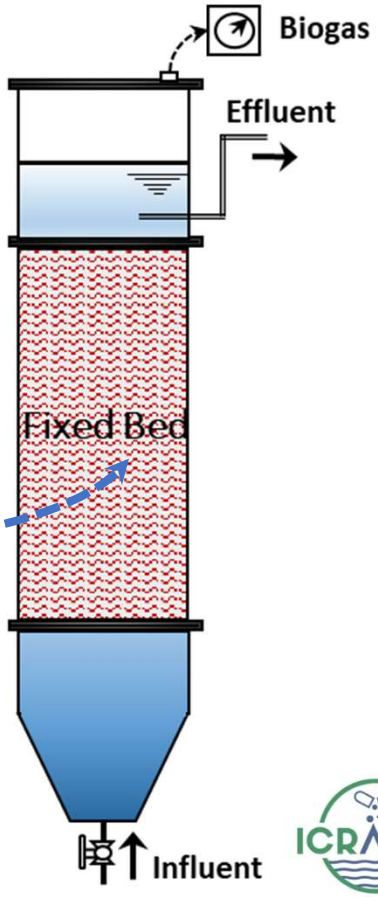
Conventional anaerobic systems





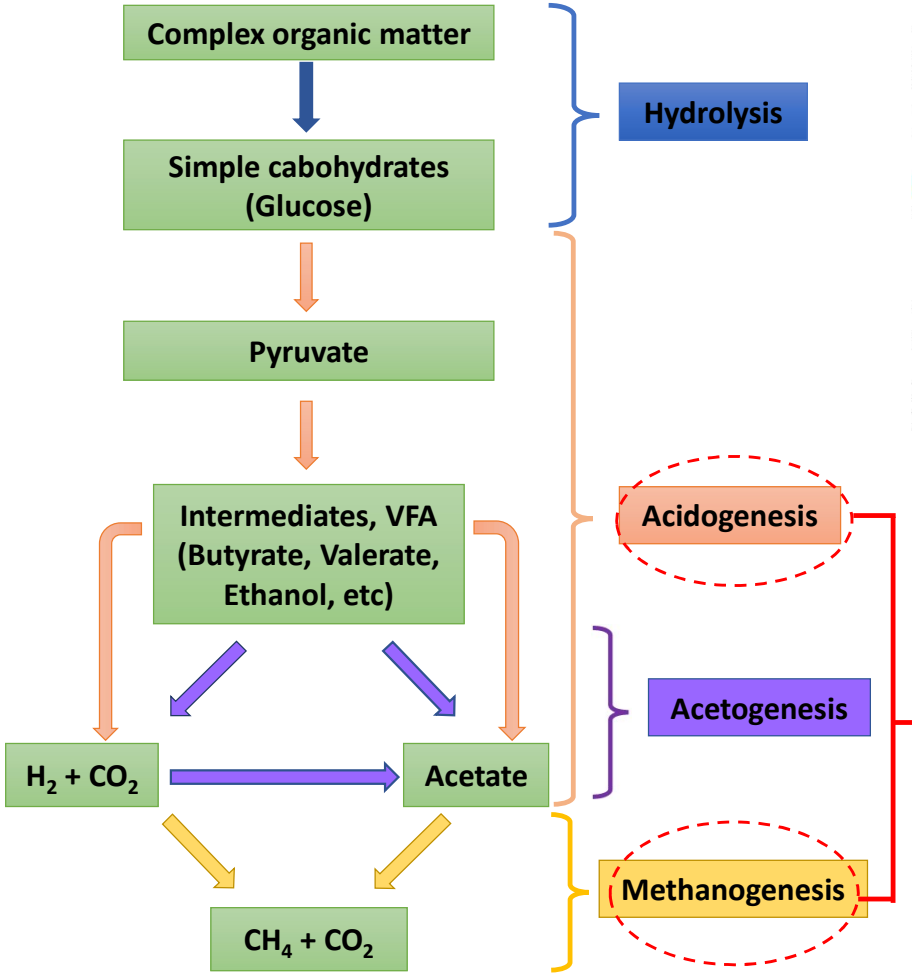
Anaerobic Fixed-film Reactors

Biofilm growth and adhesion in a support material



Anaerobic Digestion with phase separation

A novel strategy to boost the PhCs anaerobic removal in WWTPs



Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

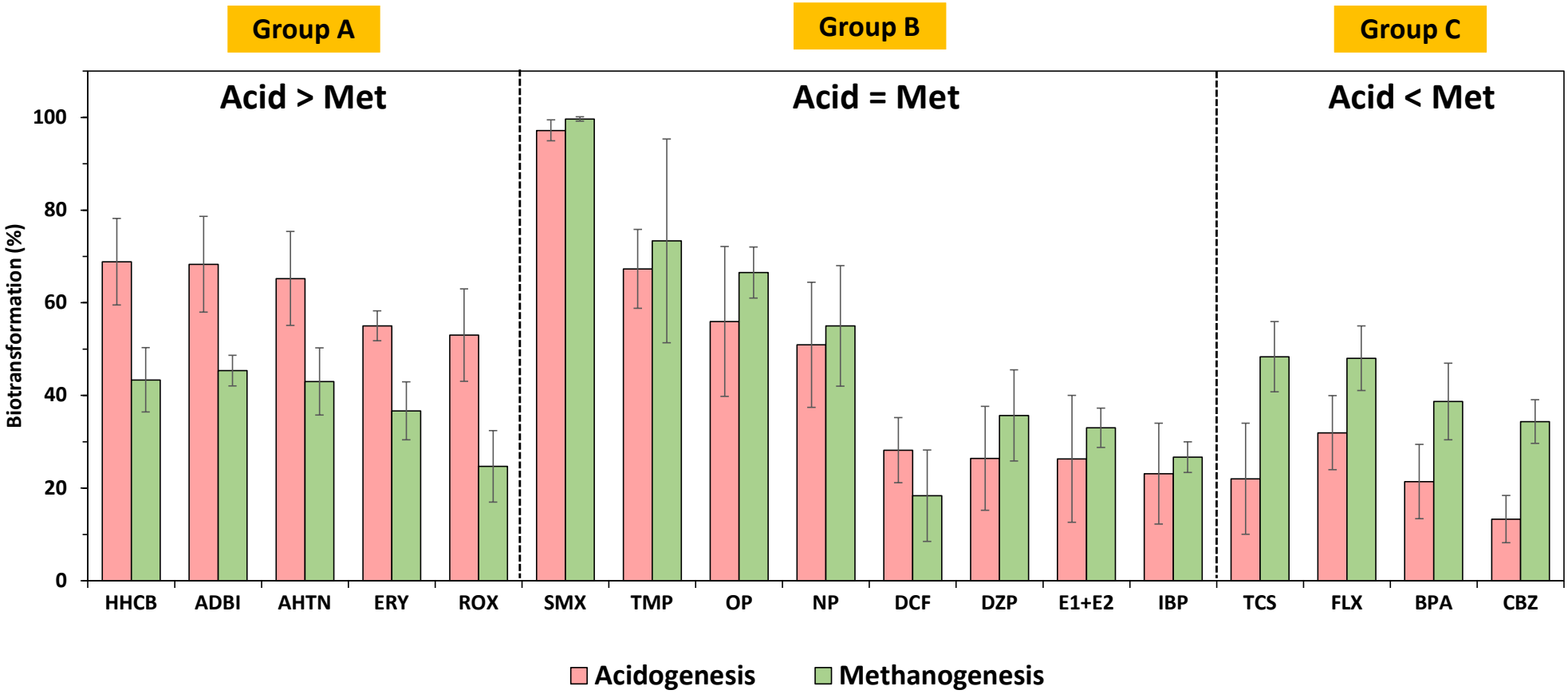
Acidogenesis is a key step in the anaerobic biotransformation of organic micropollutants

Rodrigo B. Carneiro[a,b,*,1], Lorena Gonzalez-Gil[a,1], Yudy Andrea Londoño[c], Marcelo Zaiat[b], Marta Carballa[a], Juan M. Lema[a]

Acidogenesis and methanogenesis act differently on the biotransformation of PhCs

These two phases can be easily distinguished ➔ organic acids and methane production, respectively

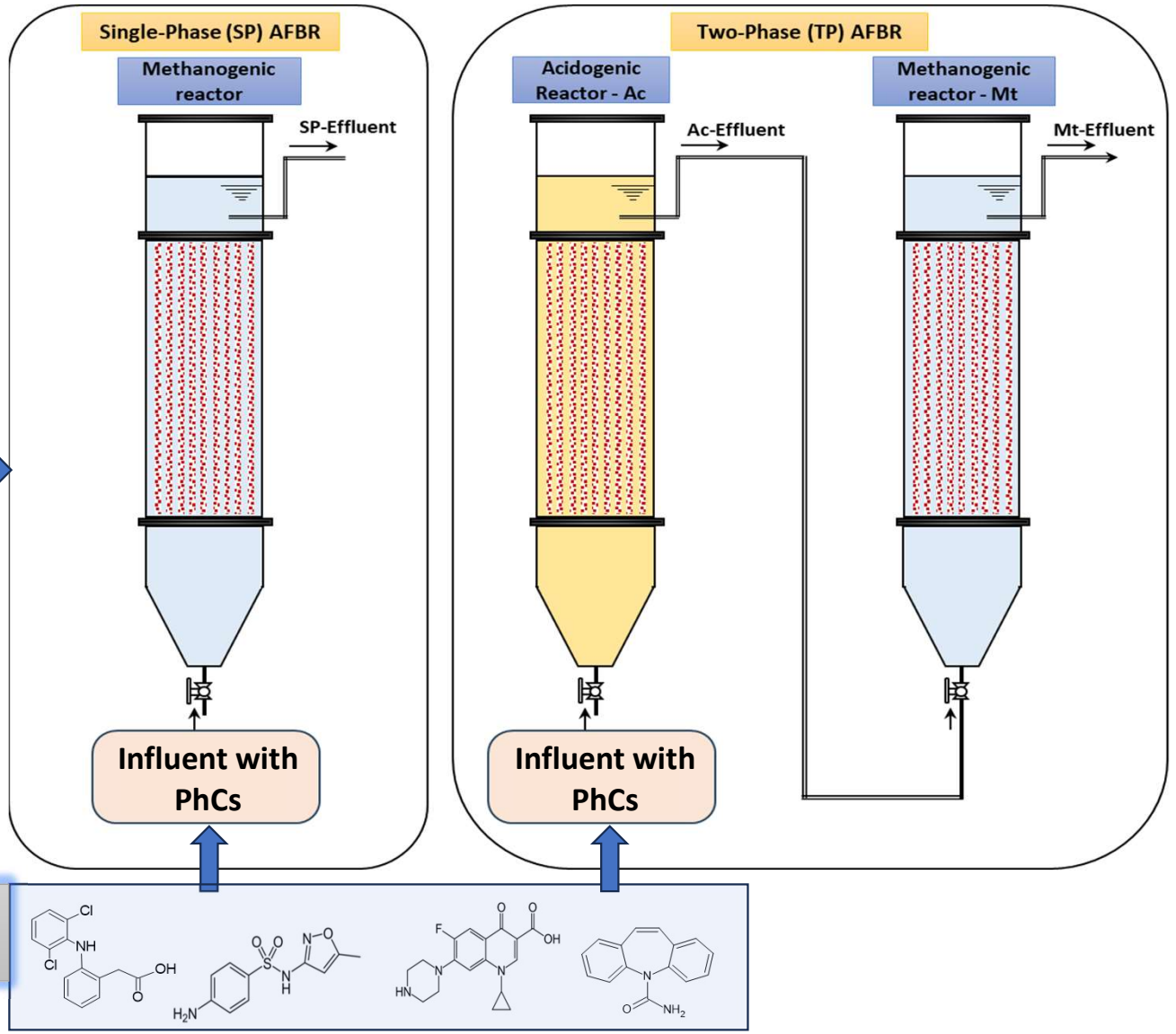
Acidogenesis *vs* methanogenesis in batch reactors - previous results



Different results in terms of biotransformation ➔ different BTPs?

Objective

Single-Phase *vs* Two-Phase continuous-flow anaerobic bioreactors



Varied Target PhCs

BIOTRANSFORMATION PRODUCTS (BTPs)

Elucidate BTPs from PhCs during AD, and their BT pathways during acidogenesis and methanogenesis

Methods – experimental apparatus

Contents lists available at ScienceDirect

Journal of Environmental Management

journal homepage: www.elsevier.com/locate/jenvman

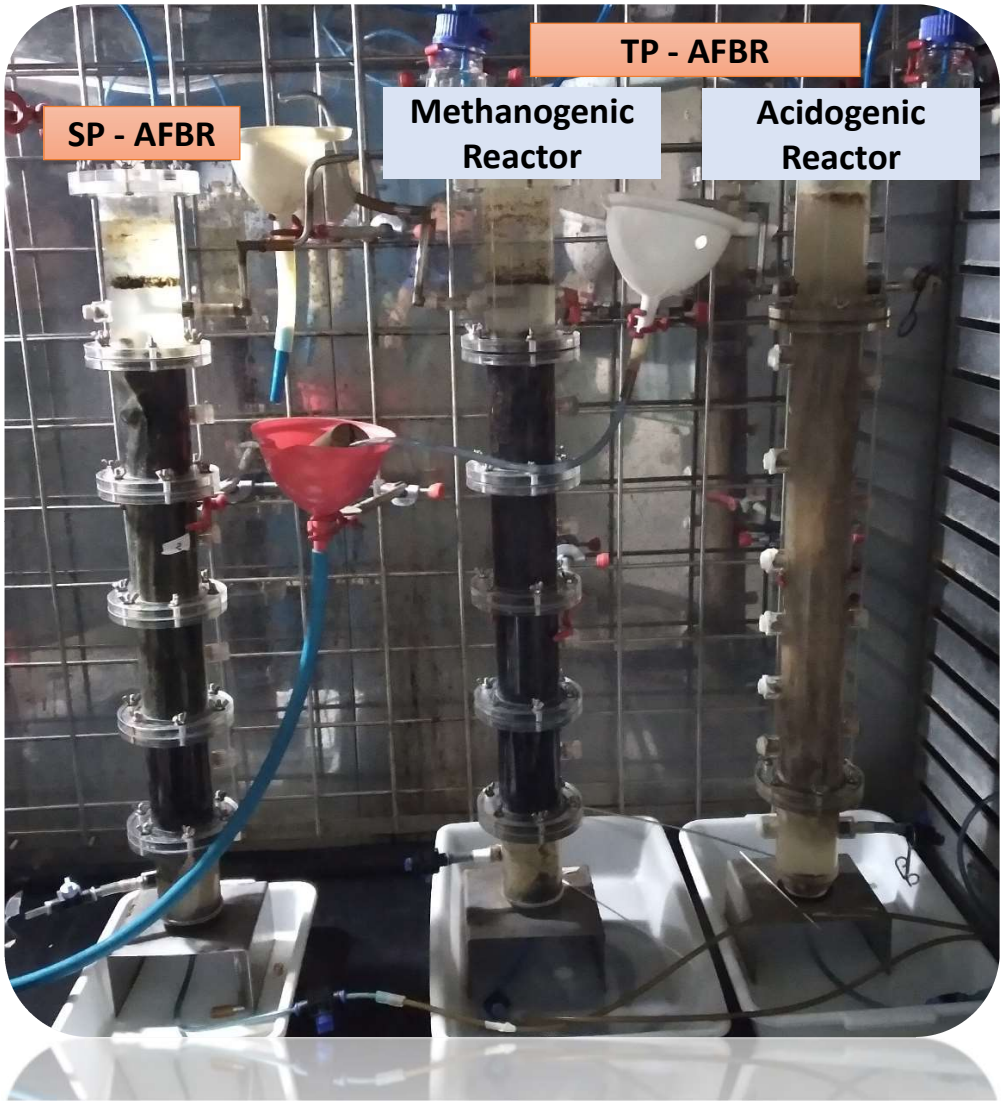
Research article

Two-phase (acidogenic-methanogenic) anaerobic fixed bed biofilm reactor enhances the biological domestic sewage treatment: Perspectives for recovering bioenergy and value-added by-products

Rodrigo B. Carneiro [a,b,*], Gisele M. Gomes [b], Marcelo Zaiat [b], Álvaro J. Santos-Neto [a]

AFBR – hydraulic tests

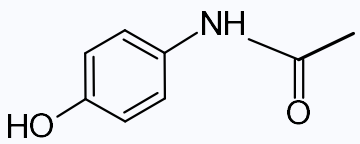
Support material ➔ Poliurethane Foam



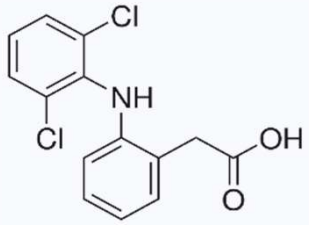
Selection of target PhCs and operating conditions

PhCs frequently detected in sewage, effluent and sludge of WWTPs

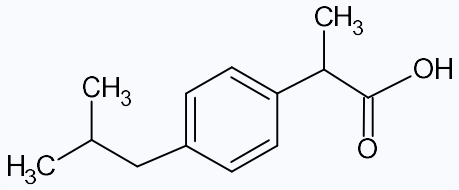
ACT - Acetaminophen



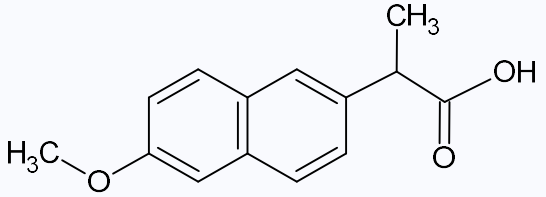
DCF - Diclofenac



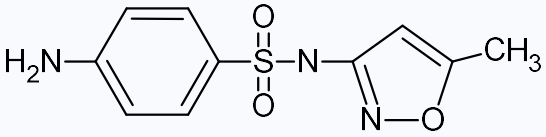
IBP - Ibuprofen



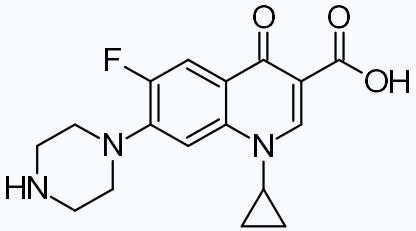
NPX - Naproxen



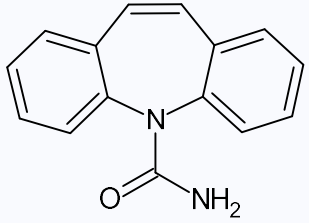
SMX - Sulfamethoxazole



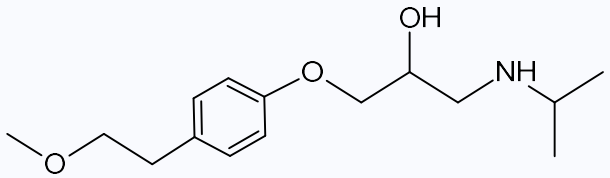
CIP - Ciprofloxacin



CBZ - Carbamazepine



MTP - Metoprolol



Operating conditions

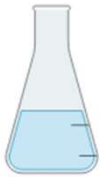
- T = 30ºC; HRT = 12 h;
- Feed substrate ➔ Lab-made sewage*
  OLR = 2.3 kgCOD m^{-3} d^{-1}
- PhCs ➔ 10 ug/L

*** Influent sewage composition**

Component	mg L^{-1}
Sucrose	211
Starch	221
Meat Extract	530
$NaHCO_3$	600
KH_2PO_4	30
NaCl	125
$CaCl_2.2H_2O$	18
$MgCl_2.6H_2O$	28

1st Phase – without PhCs
2nd Phase – with PhCs

BTPs analysis strategy



Liquid Samples
(100 mL)

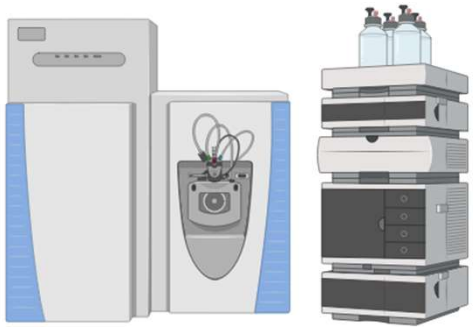
Filtration 0.45 um
and adjust pH at 6.5

SPE HLB
Cartridges

Elution - MOH

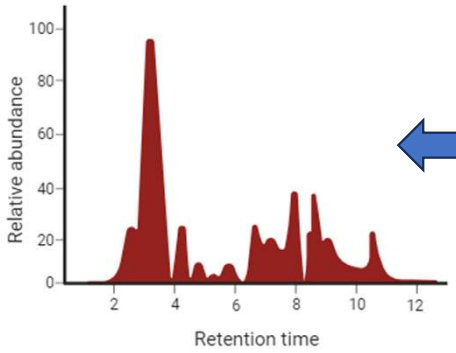
Dryness – N2

Final extract

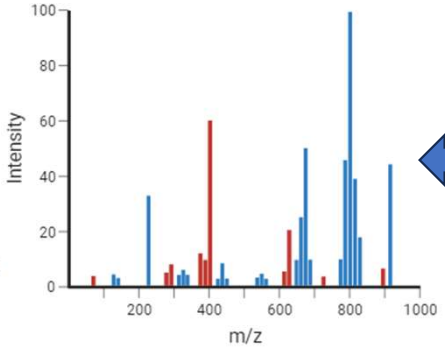


LC-MS/MS

Chromatogram



Mass Spectra – MS2



Target, Suspect
and Non-target
analysis

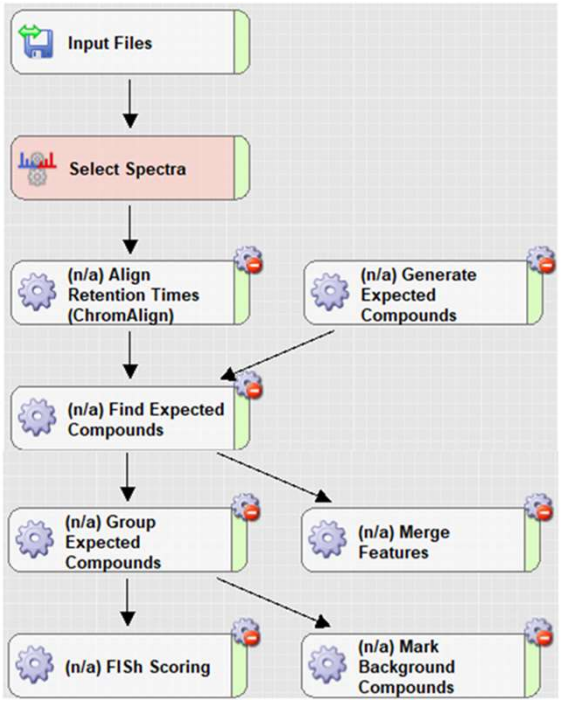
Results – Identification of PhCs and BTPs: different strategies

1. Target strategy: Confirm the presence of PhCs – MSMS patterns already known

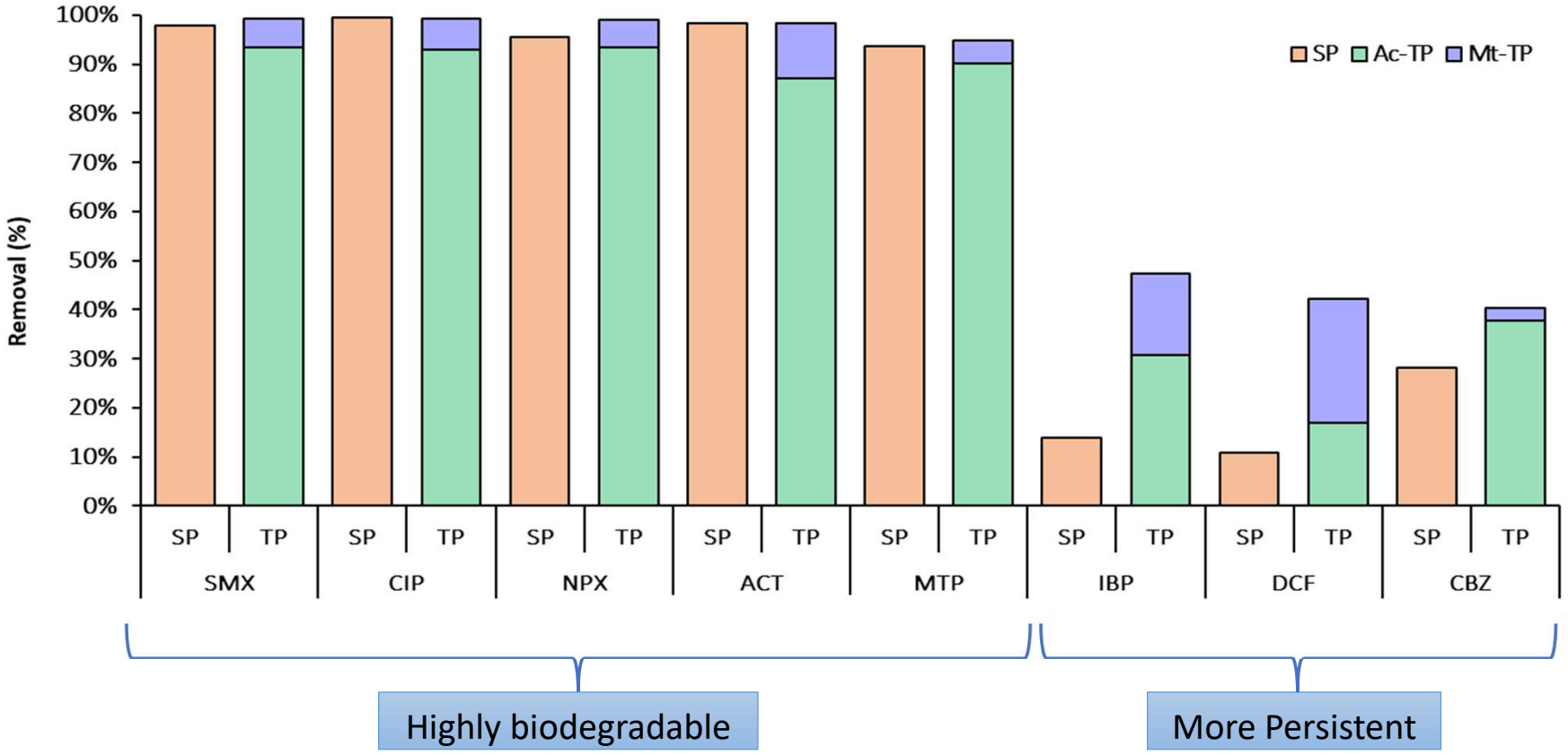
2. Suspect screening strategy: Manually search for masses of suspected BTPs (~ 160 compounds) based on previous literature studies and databases

3. Non-target strategy: Compound Discoverer software

- Minimum mass - 67 Da
- Adduct ions - $[M+H]^+$ / $[M-H]^-$
- Mass tolerance - 3 ppm
- Intensity tolerance - 50%,
- Minimum isotopes - 2,
- Minimum peak intensity at 10^5
- RT tolerance - 0.1 min
- S/N threshold - 3



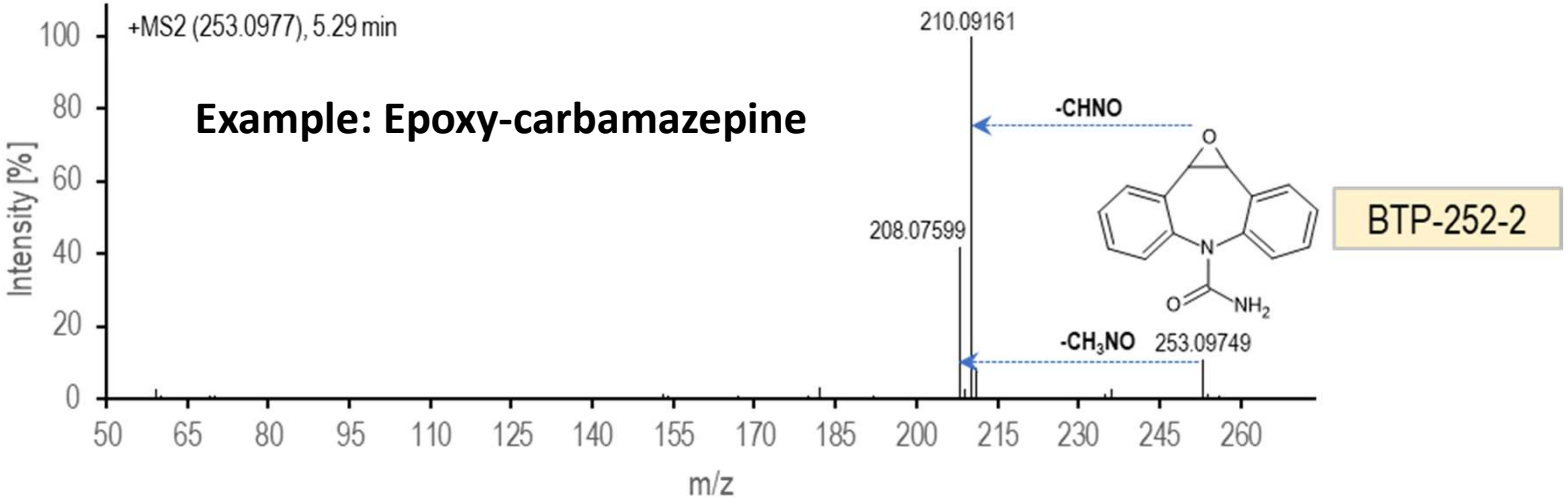
Results – Target PhCs removal

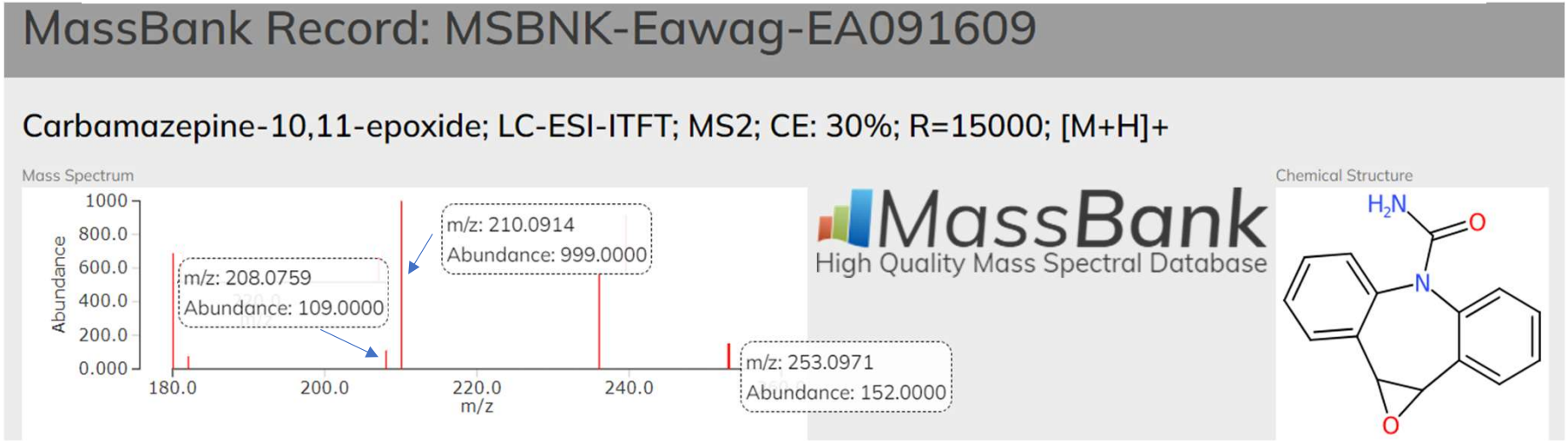


- Acidogenic phase ➔ fundamental role in the total removal of PhCs
- Phase separation enhances the PhCs biodegradation, and is significant for the most persistent PhCs

Results – Identification of BTPs: Suspect screening strategy

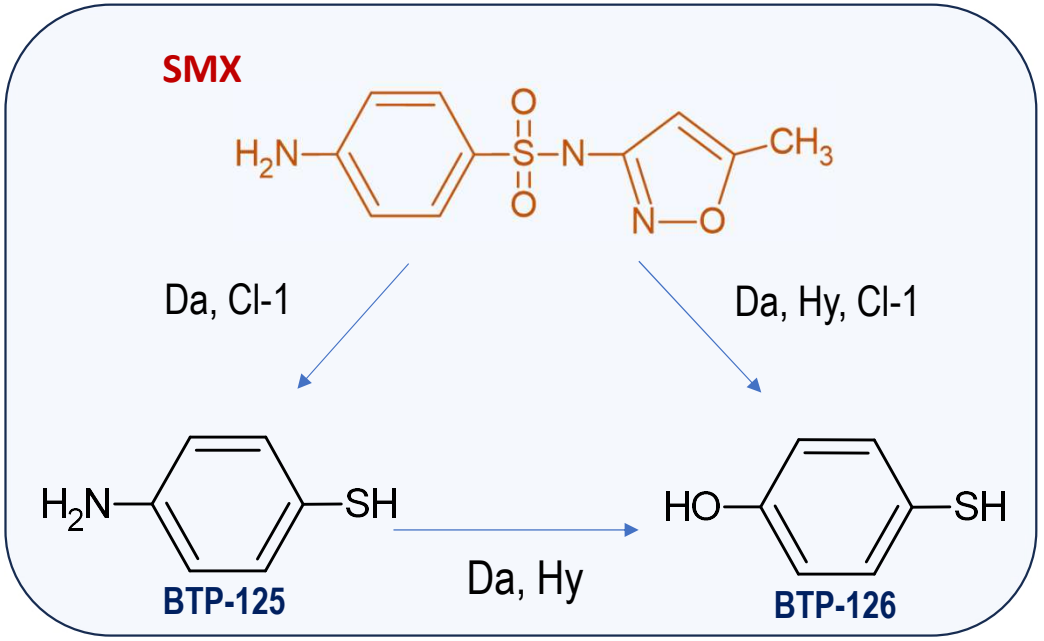
Confirmation with database

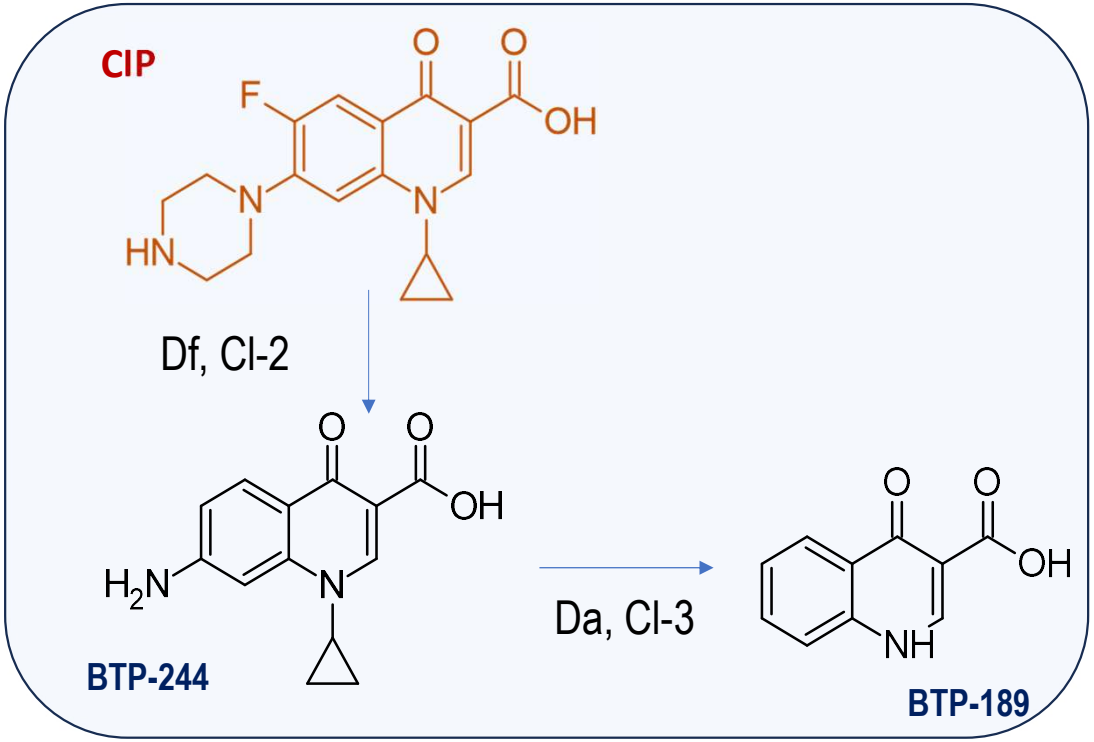




Results – Proposed biotransformation pathways

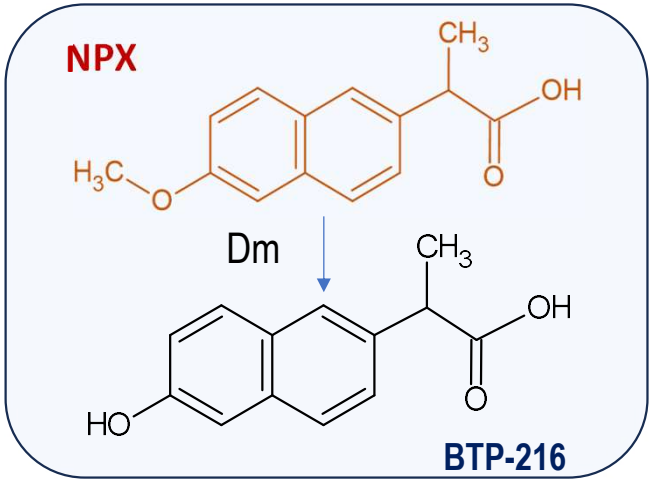
13 BTPs - confidently identified from the 8 PhCs

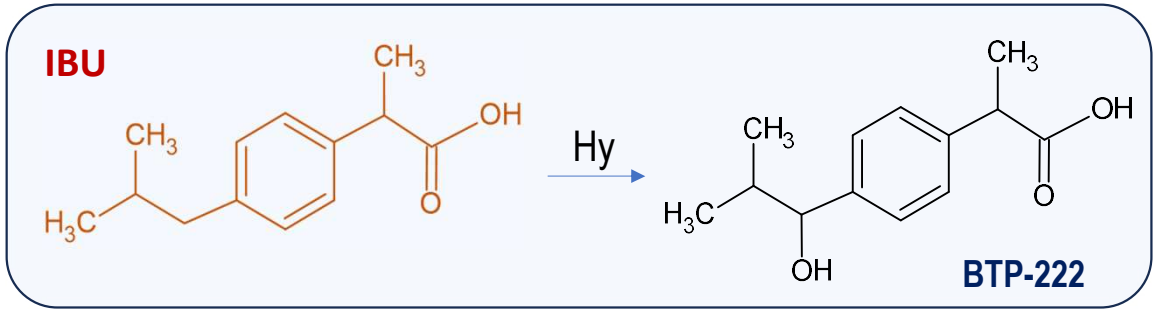


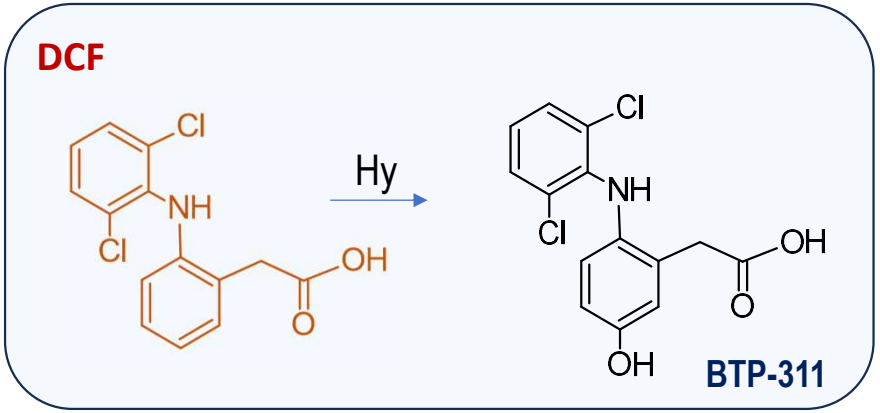


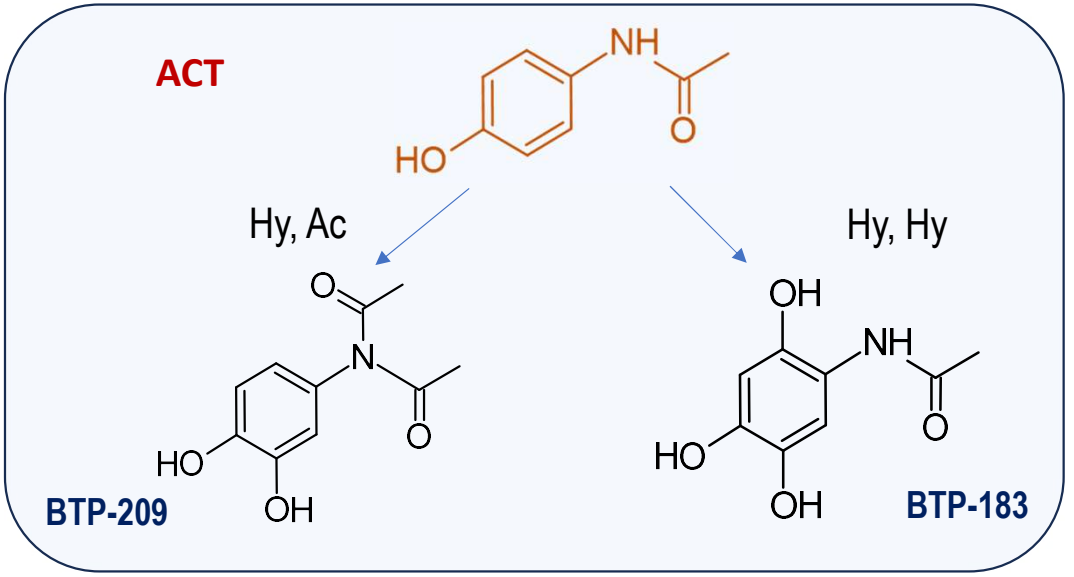
Cl-1 - cleavage of isoxazole ring; Cl-2 - cleavage of piperazine ring; Cl-3 - cleavage of cyclopropyl ring; Da – deamination; Df – defluorination; Hy - hydroxylation

Results – Proposed biotransformation pathways



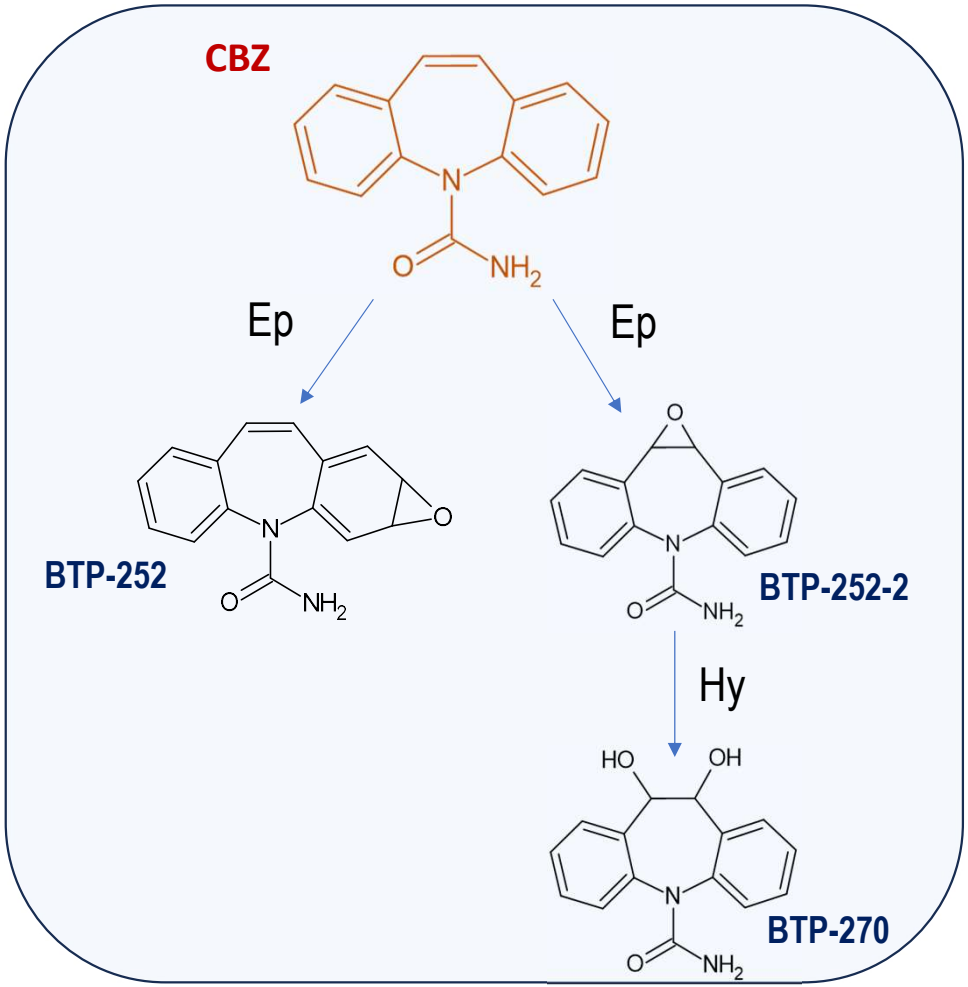


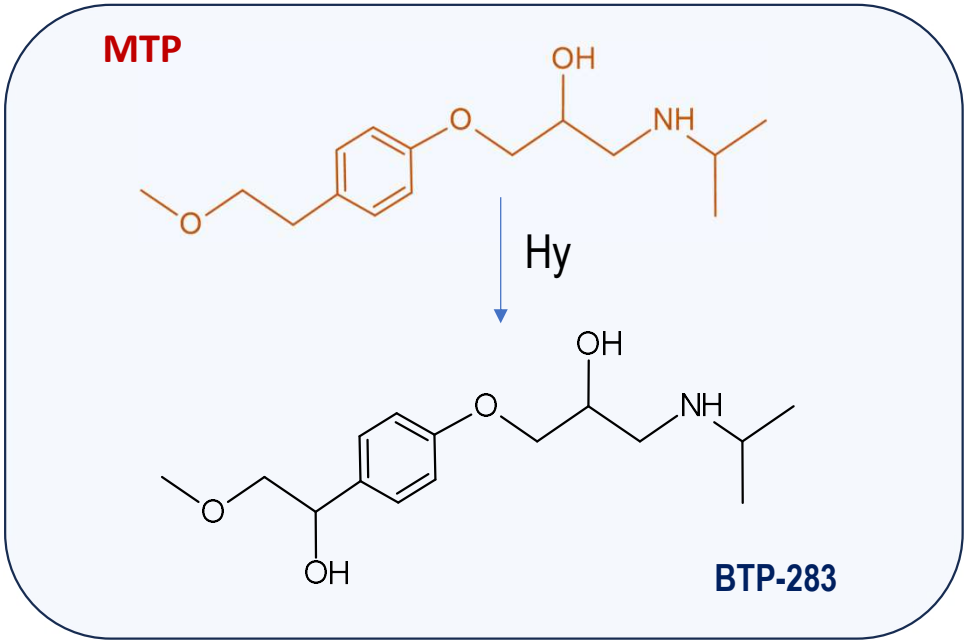




Ac: acetylation; Dm - demetylation; Hy - hydroxylation

Results – Proposed biotransformation pathways



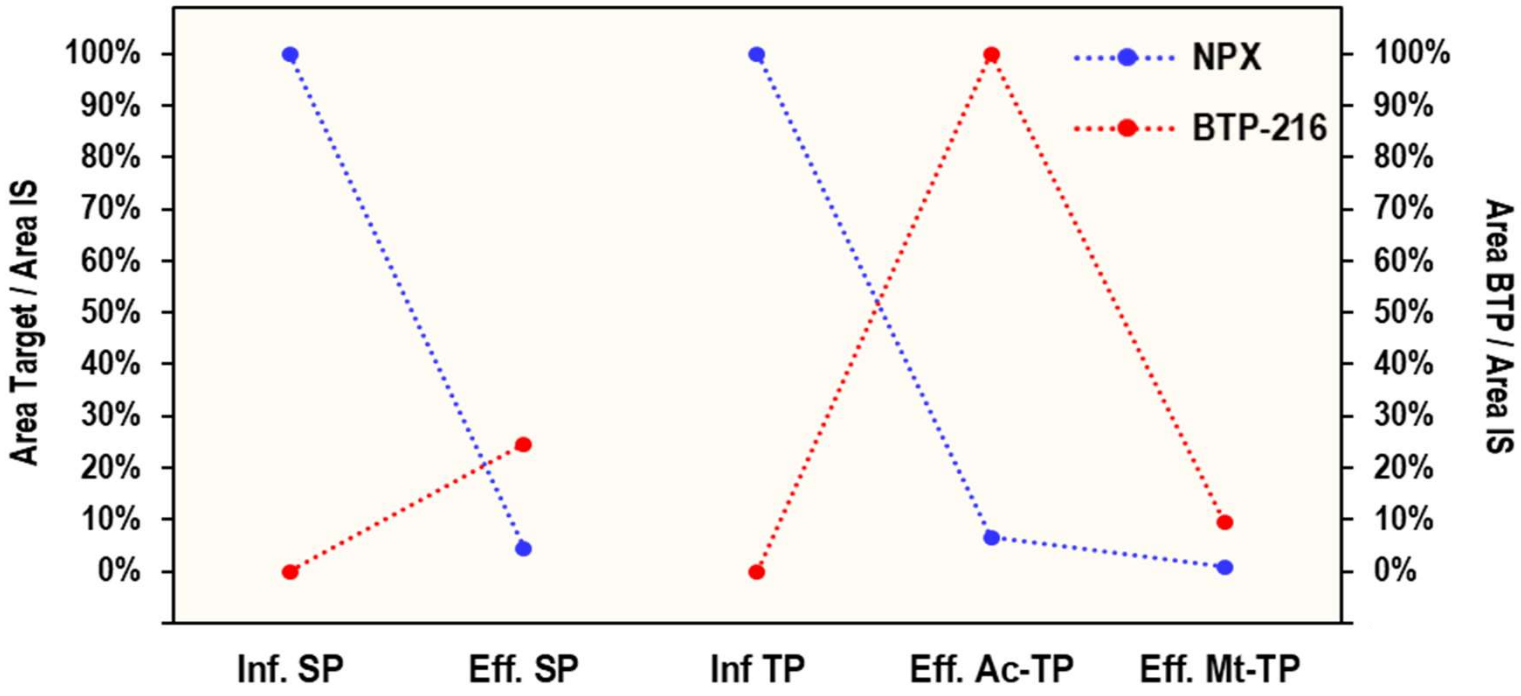


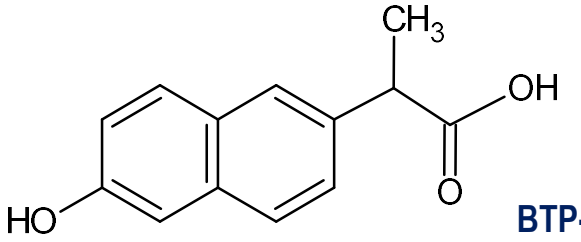
Ep - epoxydation; Hy - hydroxylation

BTPs – Acidogenic *vs* methanogenic phases

1st group of BTPs:

Generated in acidogenic condition, and subsequently consumed during methanogenesis



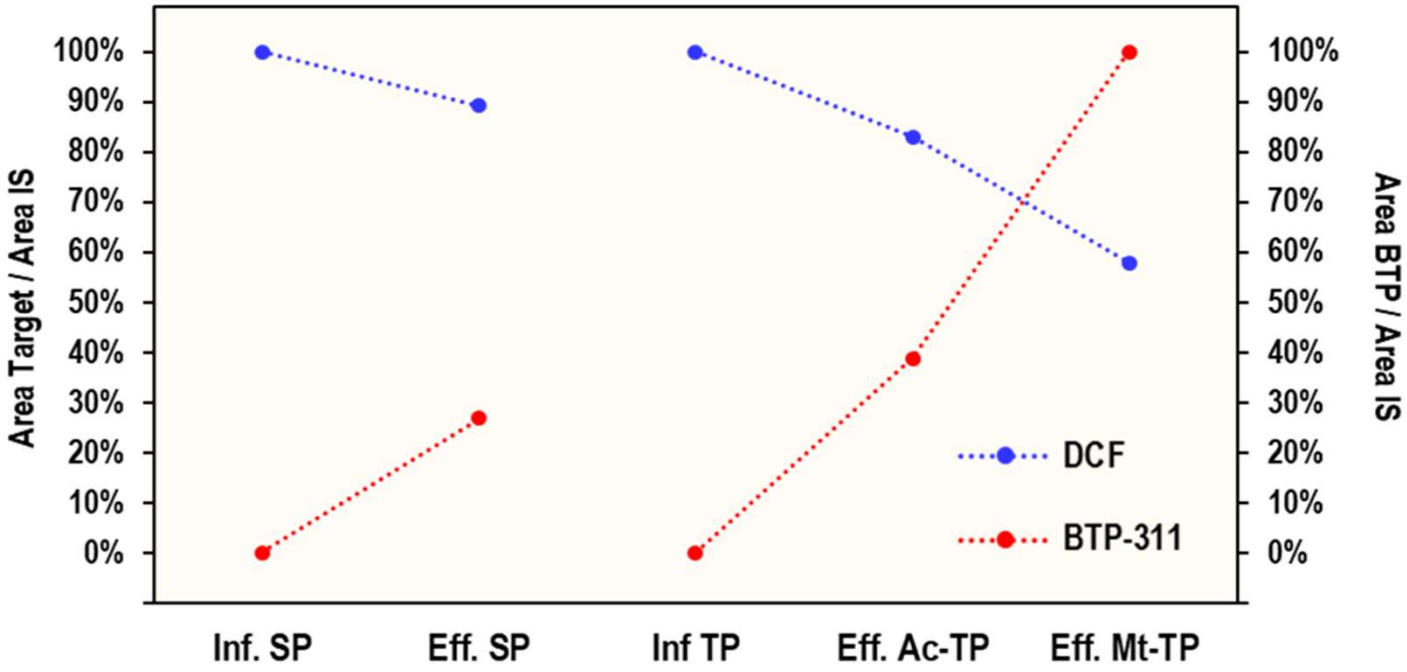


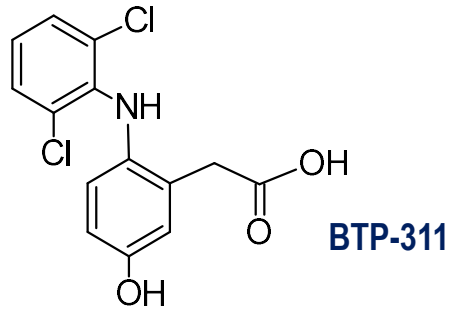
BTP-216

BTPs – Acidogenic *vs* methanogenic phases

2nd group of BTPs:

Generated in acidogenic condition, and favored by phase separation in the TP-reactor, increasing their concentration in Mt-TP



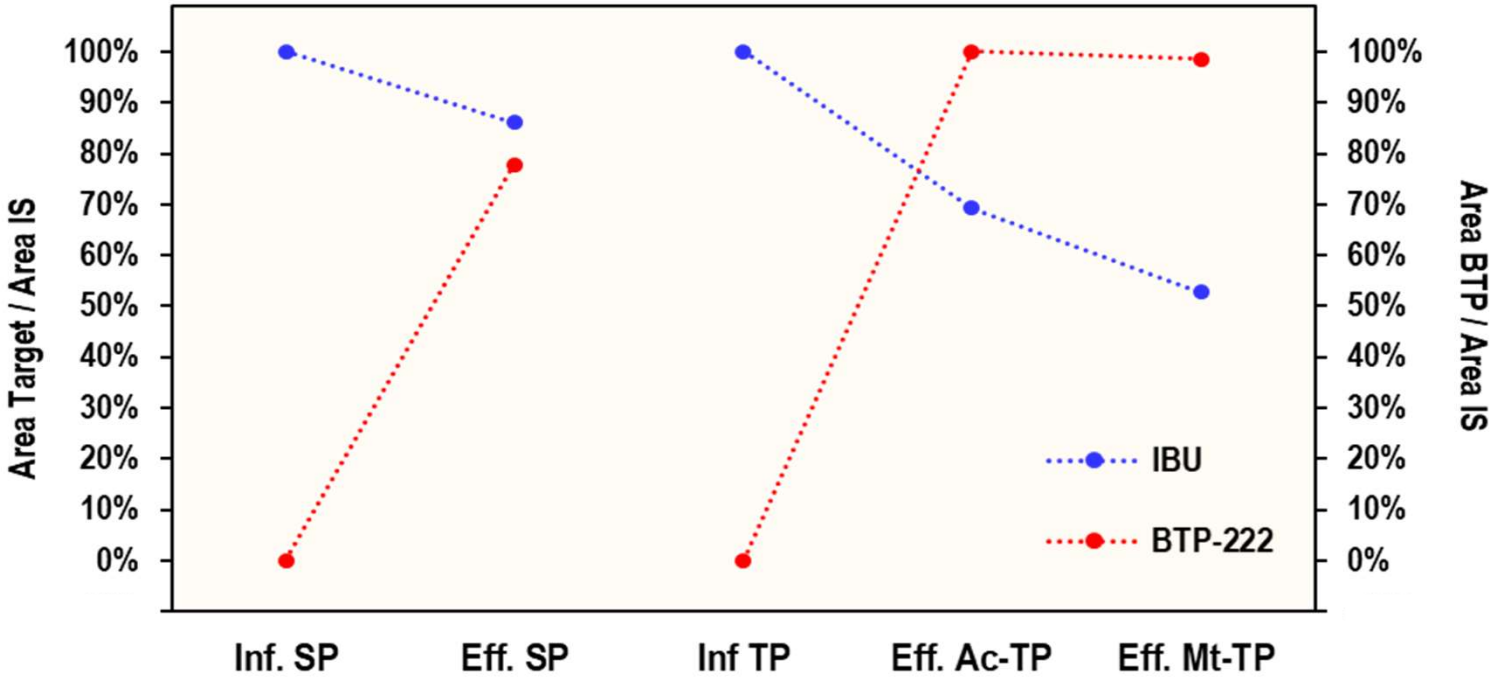


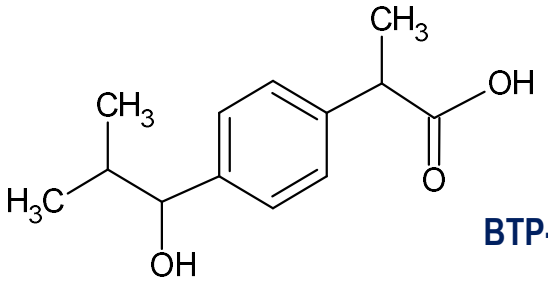
BTP-311

BTPs – Acidogenic *vs* methanogenic phases

3rd group of BTPs:

Generated in acidogenic condition, but not affected by the methanogenesis





BTP-222

Final Remarks and conclusions

- Compounds **highly biodegradable** in the two systems – **SMX, CIP, ACT, NPX, MTP**
- More **persistent compounds** – **DCF, IBU, CBZ** < 50% removal
- **Crucial role of the acidogenic phase** on the total removal of PhCs ➔ largest fraction removed and all BTPs generated during Acidogenesis
- Phase separation into Ac / Mt ➔ selection and **boost fermentative and methanogenic microbial enzymes - PhCs cometabolism**
- For the more recalcitrant compounds (CBZ, IBU, DCF) ➔ **TP-reactor promoted higher BT**.
- **Next steps ➔**
  - Quantify BTPs with standards;
  - Predict risks of these BTPs to the environment and human health

ICRAPHE

4th INTERNATIONAL CONFERENCE ON RISK ASSESSMENT OF PHARMACEUTICALS IN THE ENVIRONMENT

Barcelona, 9 - 10 October 2023

Thank you for your attention!

Rodrigo Carneiro - rodrigocarneiro@sc.usp.br

idaea

CSIC

LPB LABORATÓRIO DE PROCESSOS BIOLÓGICOS EESC/USP

CROMA

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CAPES

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