
Automated suspect screening methodology for the identification of antibiotic transformation products generated during water treatment with microalgae

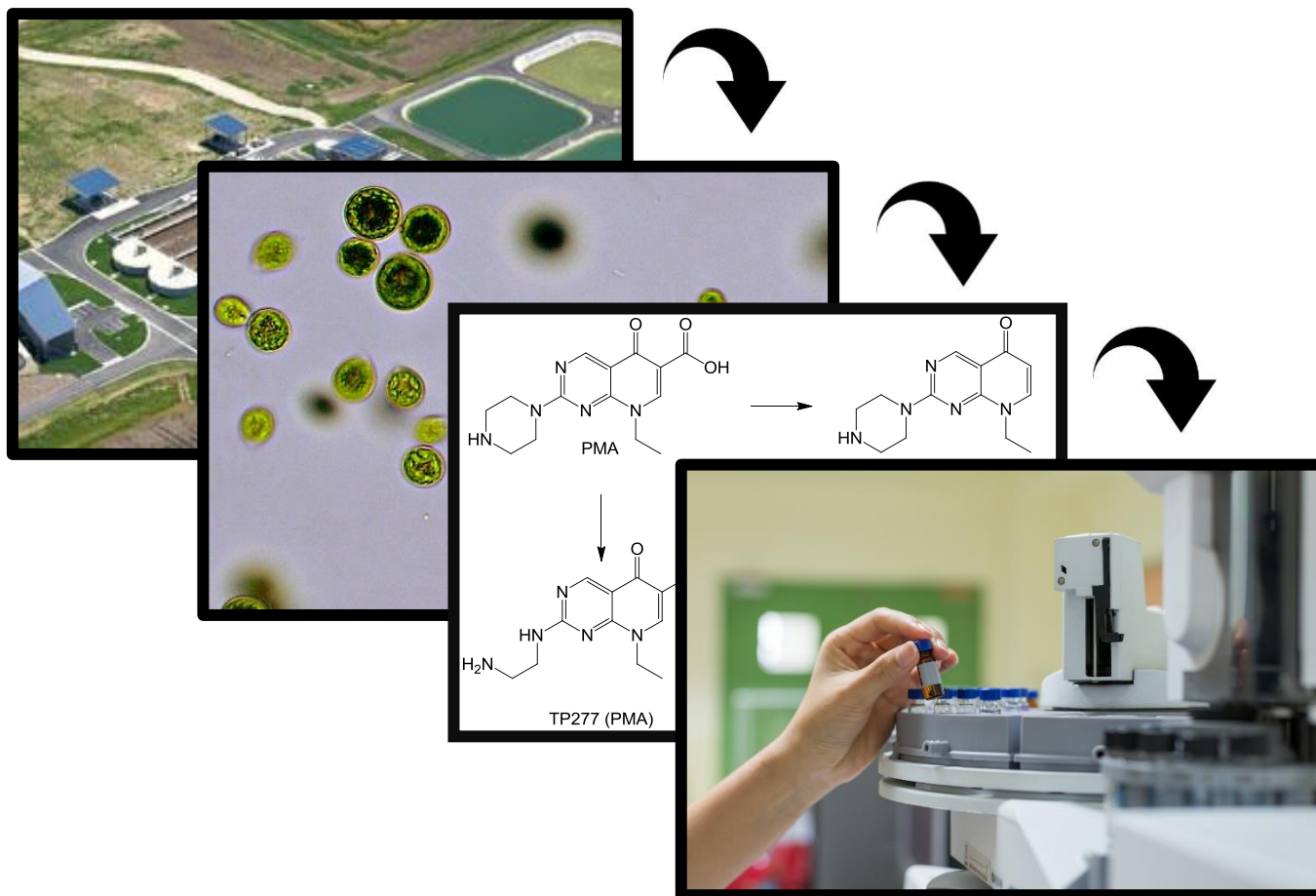
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Introduction



Objectives

- Optimize a novel on-line turbulent flow methodology (TFC-HPLC-LTQ-Orbitrap-MS/MS) combined with an automated suspect screening to easily identify a large number of transformation products in a single run.
- Use this methodology to identify transformation products from 9 antibiotics after microalgae water treatment.
- Evaluate the contribution of biotic and abiotic factors in the removal of contaminants and propose the corresponding degradation pathways.
- Assess the overall contribution of algae treatment for mitigation of water pollution.

Experimental set-up

Azithromycin (AZI)
Erythromycin (ERY)
Clarithromycin (CTM)



Ofloxacin (OFC)
Ciprofloxacin (CFC)
Norfloxacin (NFC)



Sulfapyridine (SPY)
Trimethoprim (TMP)
Pipemidic acid (PMA)

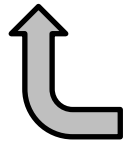


$C = 100 \text{ ug} \cdot \text{L}^{-1} / \text{comp.}$



After 15 days-treatment for:

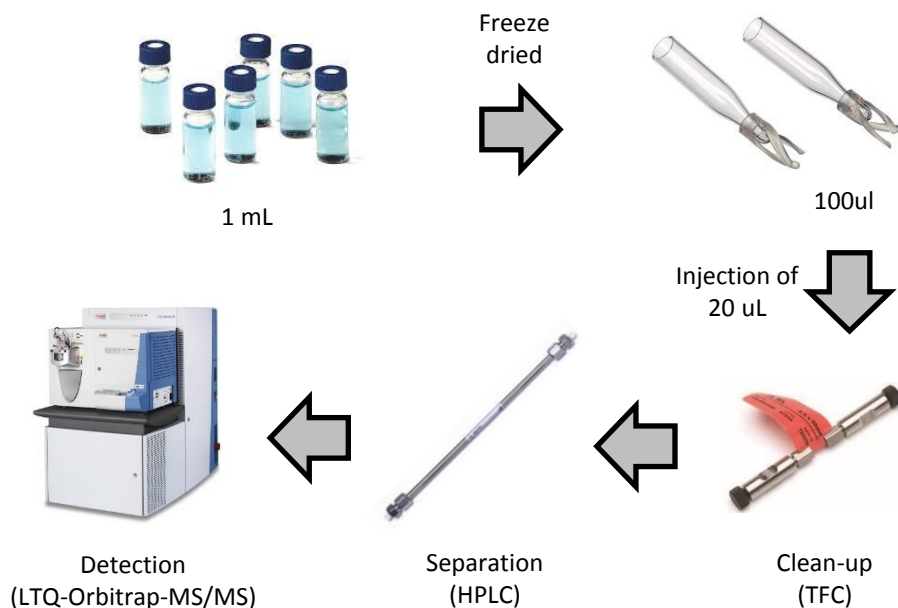
- *Chlamydomonas reinhardtii*
- *Chlorella sorokiniana*
- *Dunaliella tertiolecta*
- *Selenastrum capricornutum*



	Light Biomass (LB)	Light Abiotic (LA)	Dark abiotic (DA)
Biotransformation	✓	✗	✗
Phototransformation	✓	✓	✗
Other transformations	✓	✓	✓

Analysis

TFC-HPLC-LTQ-Orbitrap-MS/MS

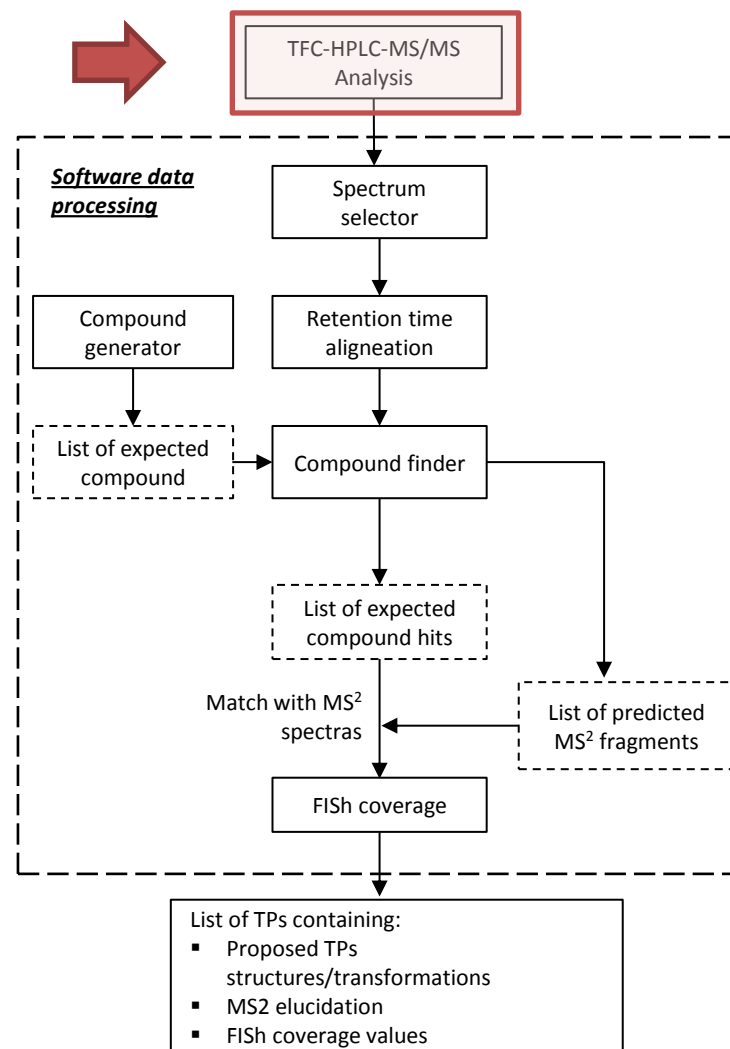


TFC: Cyclone (50 × 0.5 mm, 60 μm)

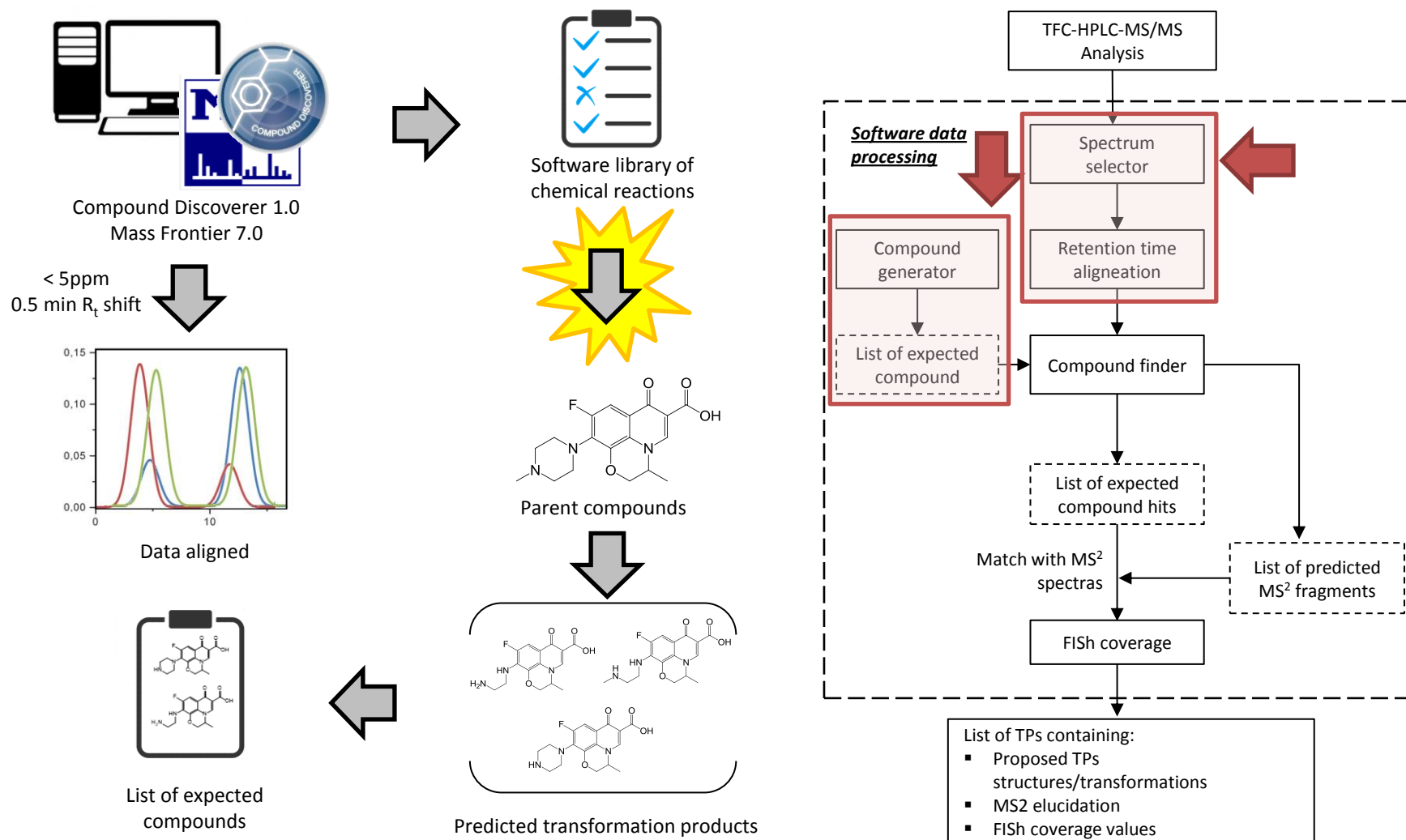
Analytical column: ZORBAX Eclipse XD-C18 (150 × 4.6mm; 5 μm)

Acquisition mode: Data Dependent Acquisition (DDA) fragmenting the 10th most intense ions:

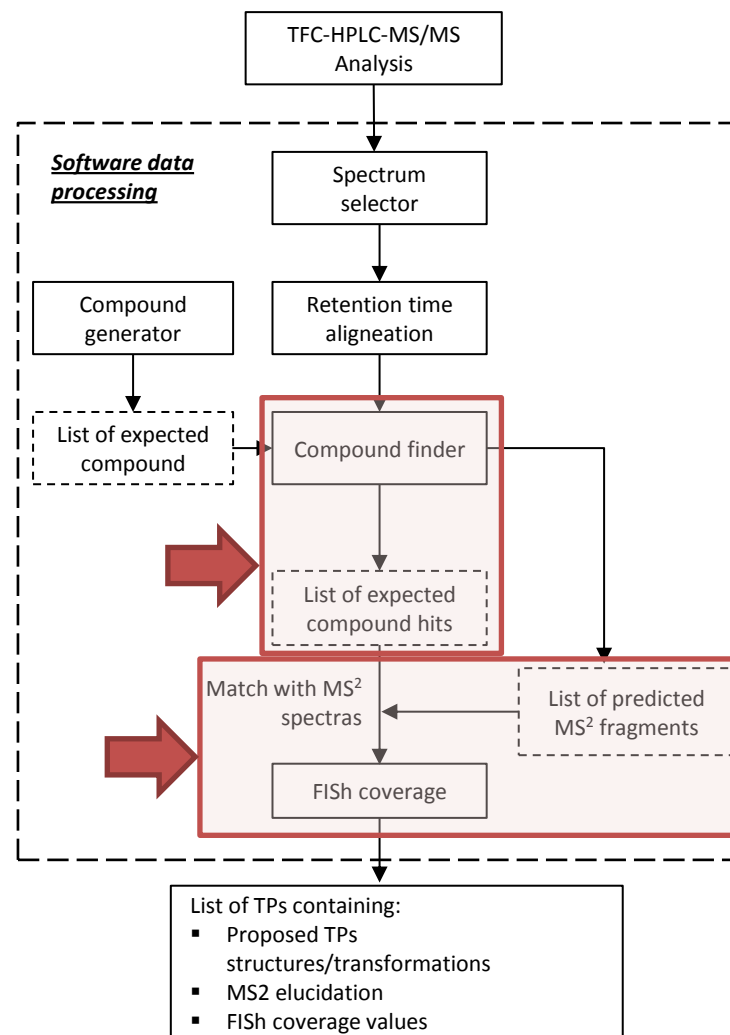
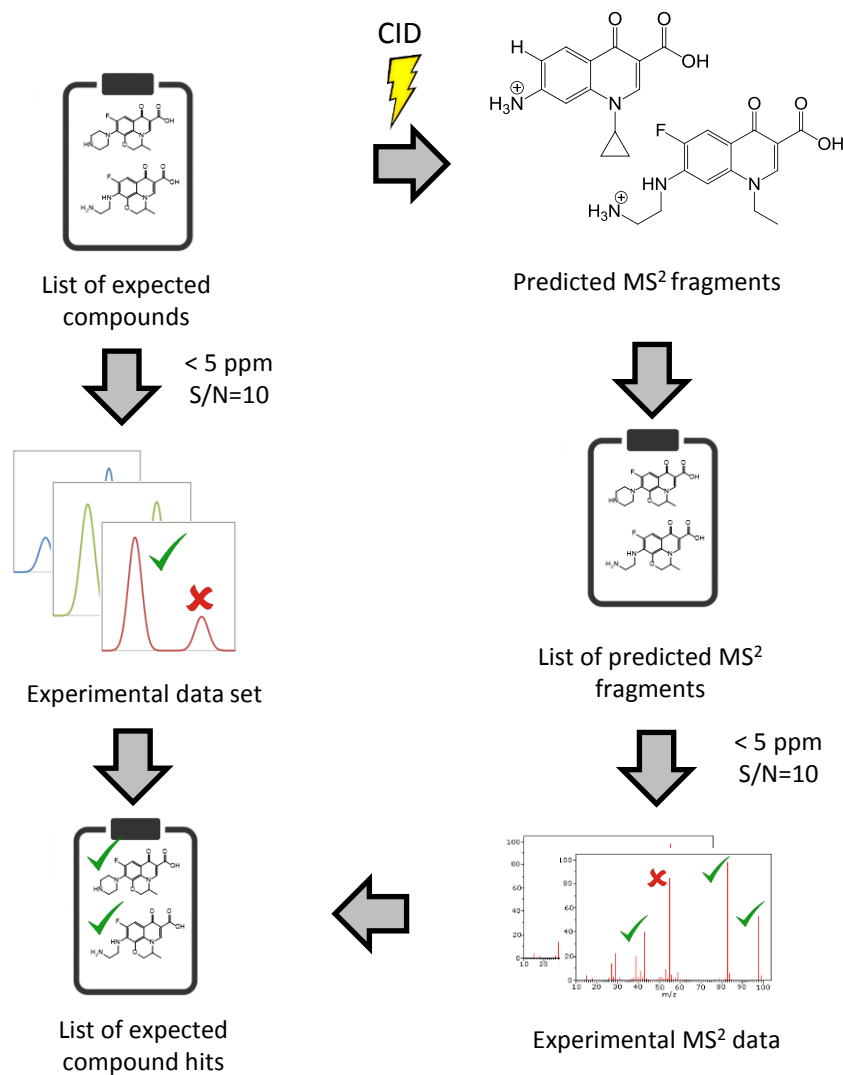
- ✓ Range of 100-800 m/z
- ✓ 60000 FWHM for MS¹ and 30000 FWHM for MS²



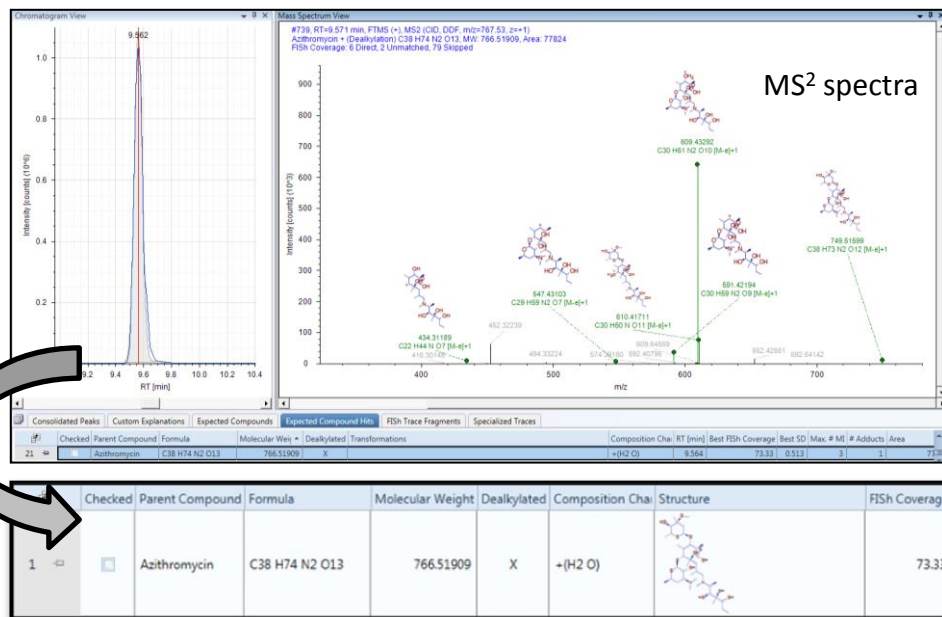
Data processing



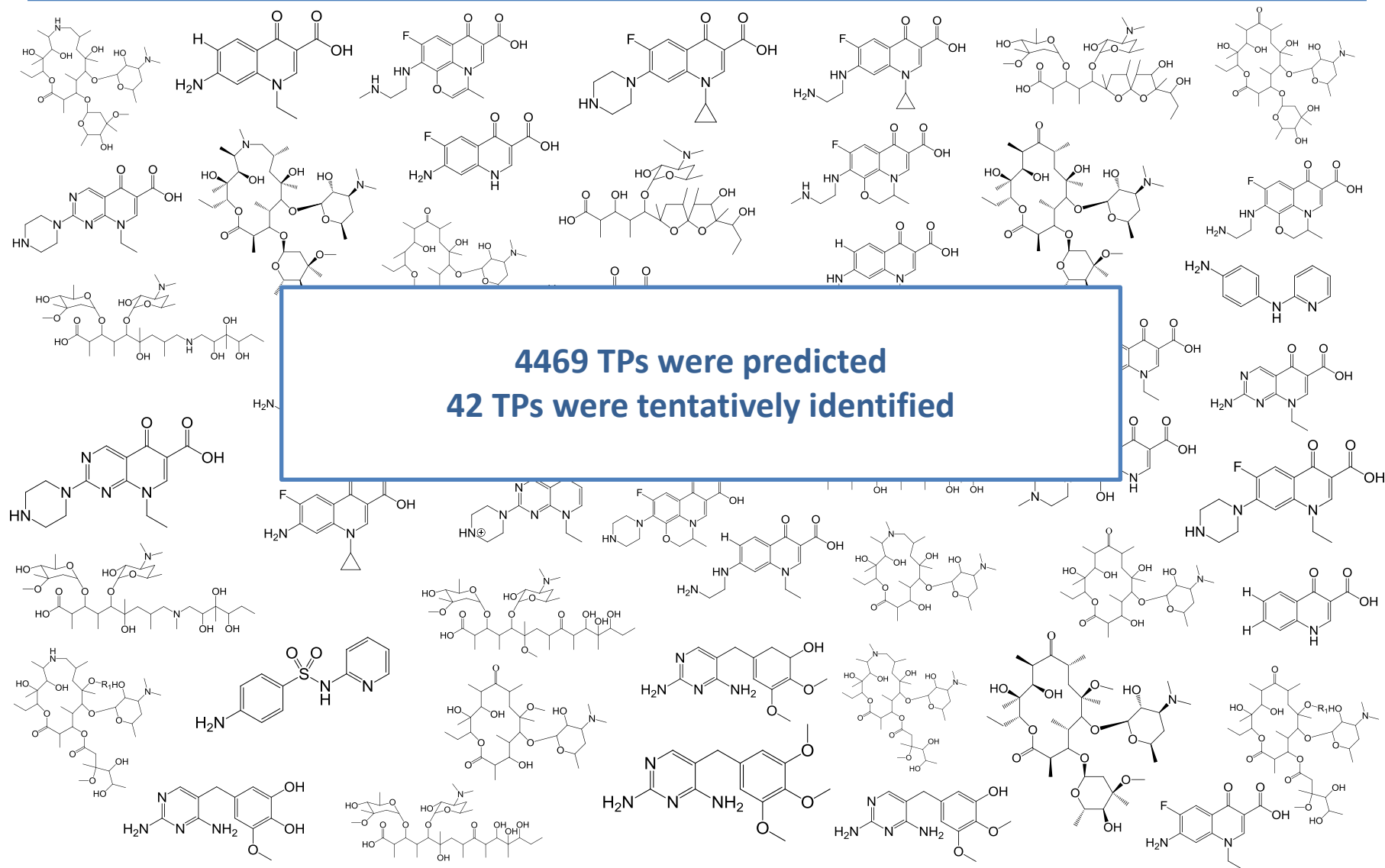
Data processing



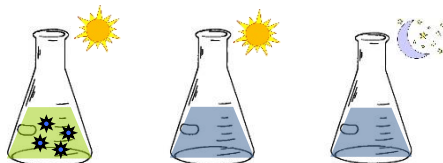
Data processing



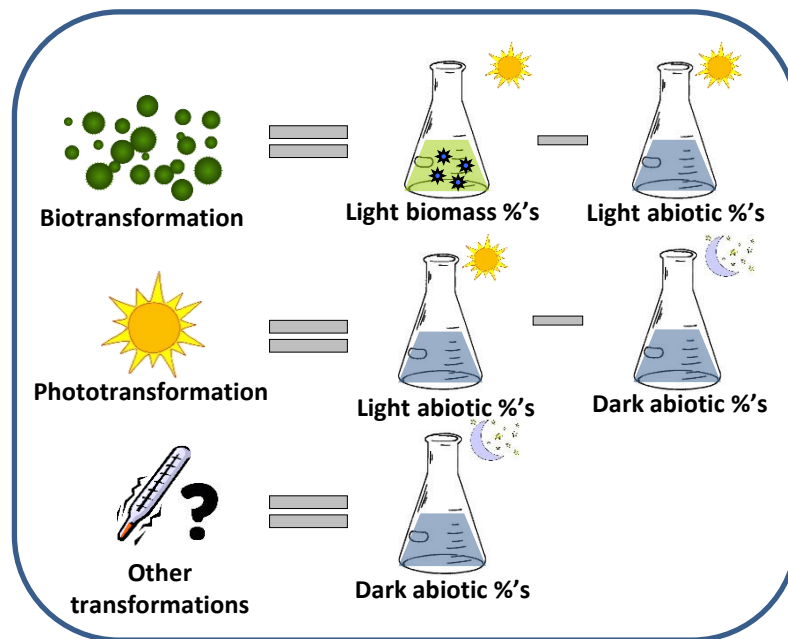
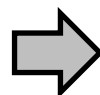
Tentative transformation products



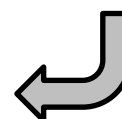
Factor contributions for azithromycin transformation products



AZI TPs	Light biomass	Light abiotic	Dark abiotic
TP766	18.5%	2.1%	1.6%
TP764	4.7%	0.9%	0.5%
TP752	0.9%	0.1%	0.1%
TP750	1.6%	0.4%	0.0%
TP734	8.0%	0.8%	0.6%
TP608	2.4%	0.2%	0.2%
TP590	12.8%	24.2%	18.9%



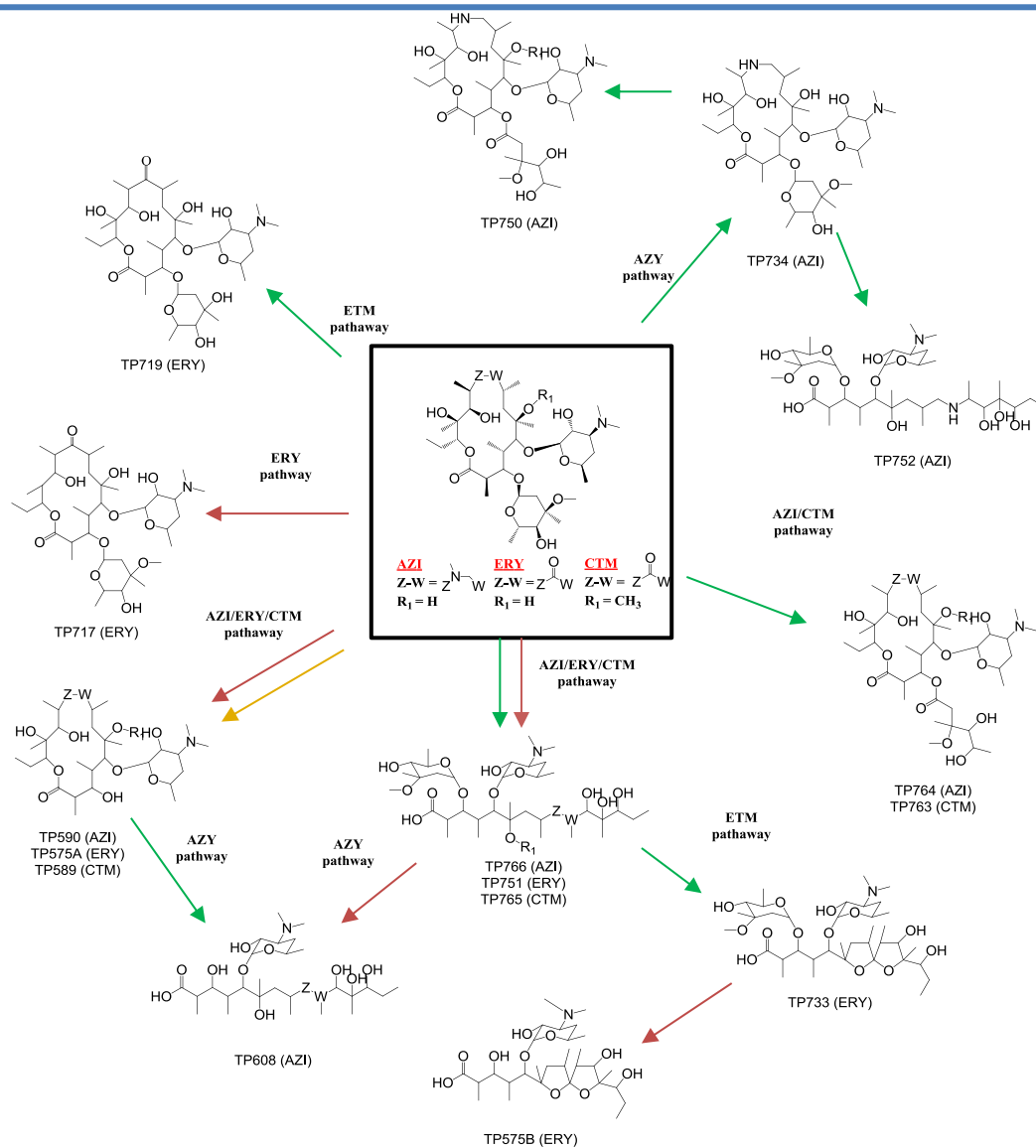
AZI TPs	Biotransformation	Phototransformation	Other transformations	CV
TP766	16.4%	0.6%	1.6%	1.4
TP764	3.8%	0.4%	0.5%	1.2
TP752	0.8%	0.0%	0.1%	1.5
TP750	1.2%	0.4%	0.0%	1.1
TP734	7.1%	0.2%	0.6%	1.5
TP608	2.2%	0.0%	0.2%	1.5
TP590	-11.4%	5.3%	18.9%	1.2



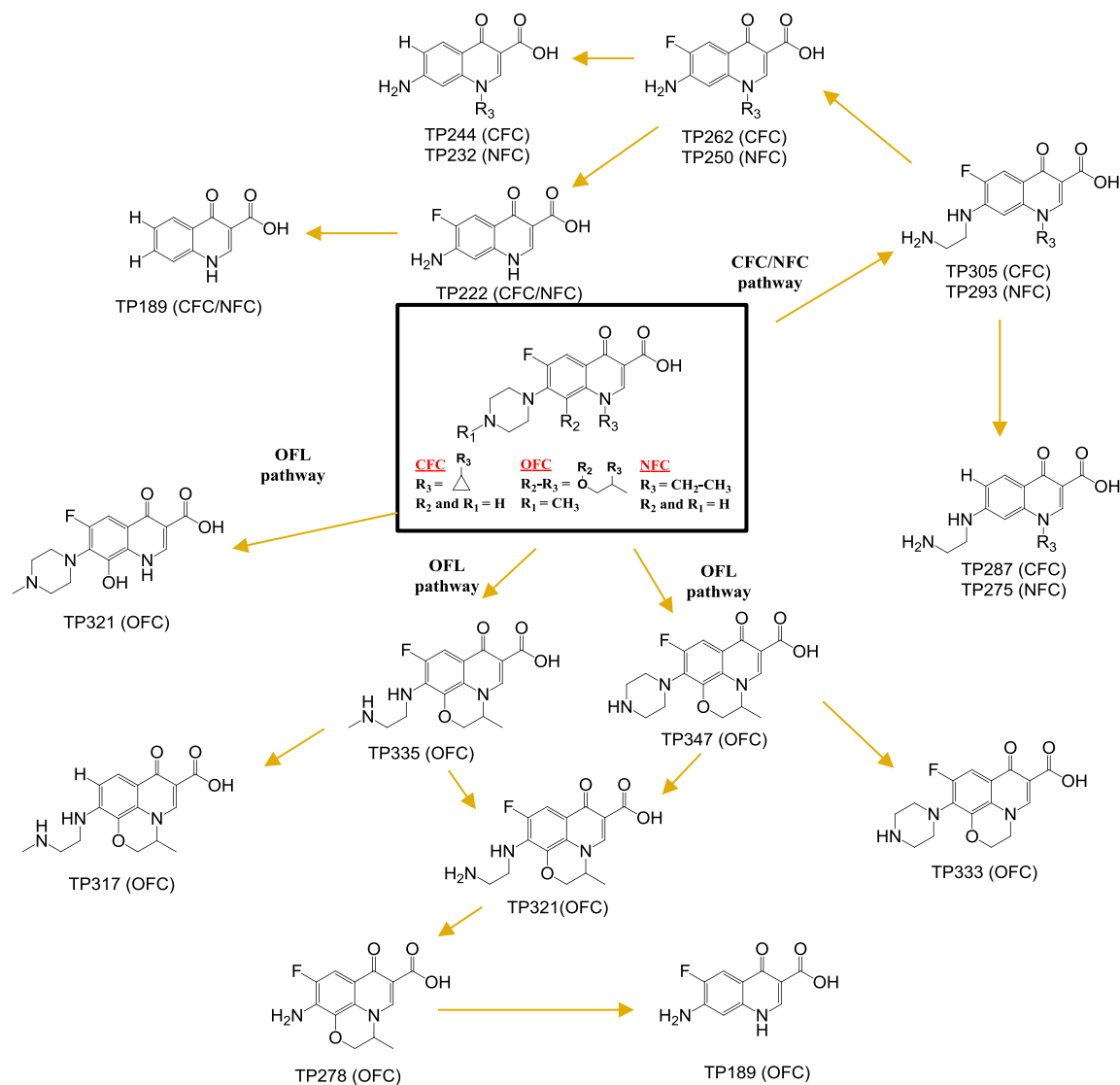
CV > 1

High-variance between individual factors

Macrolide transformation pathway

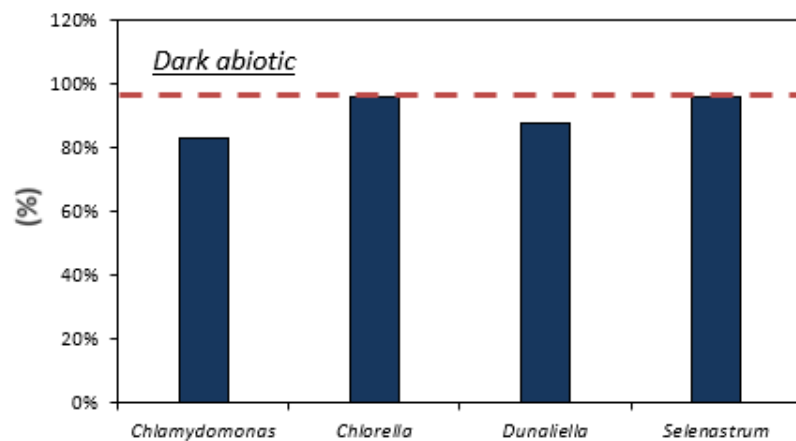


Fluoroquinolones transformation pathway



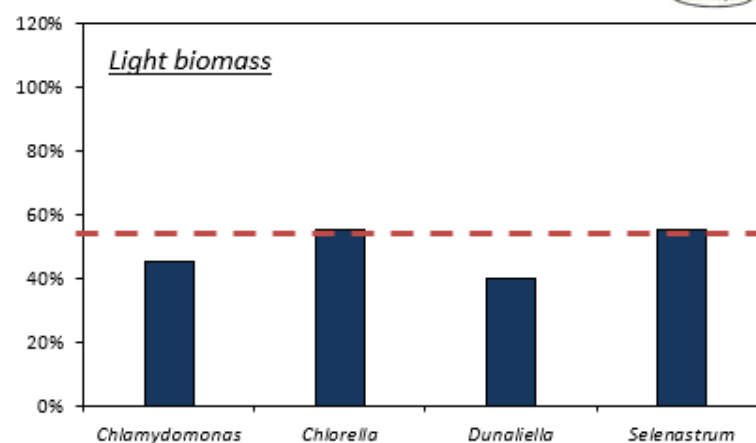
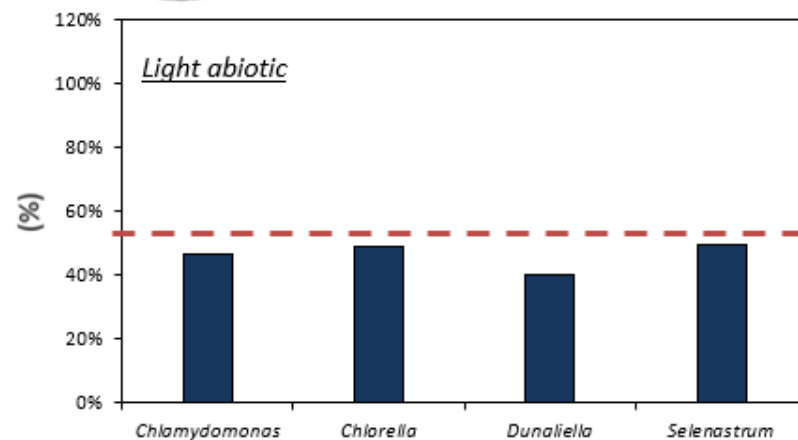
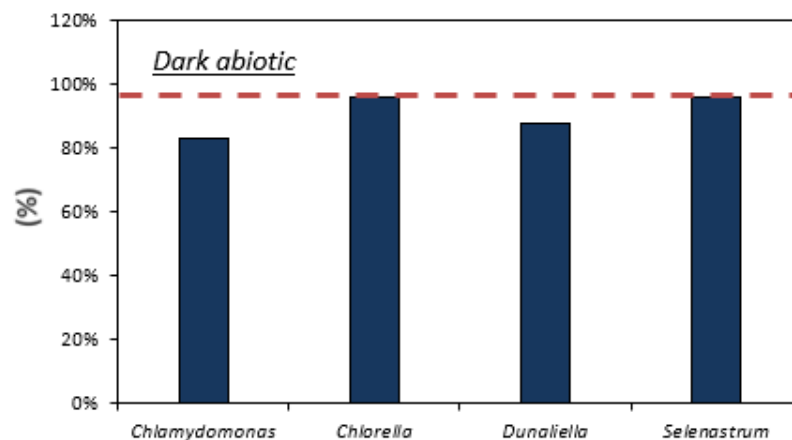
Microalgae ability for transformation

■ Presence of parent compounds



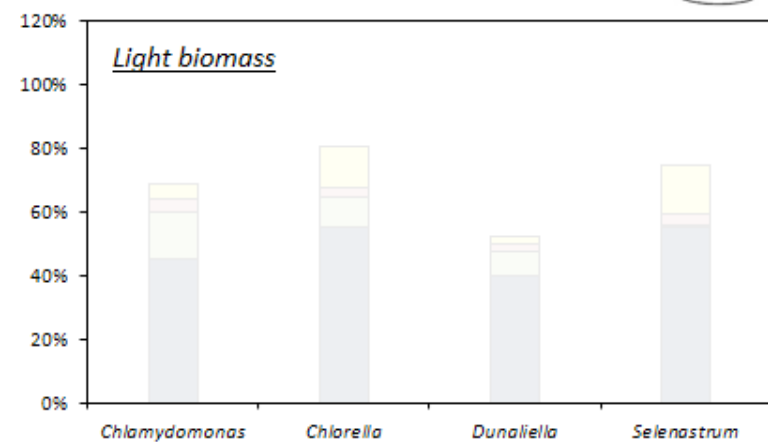
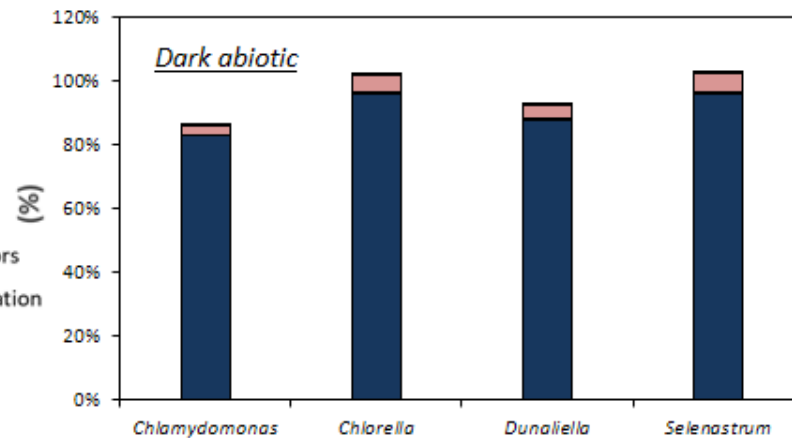
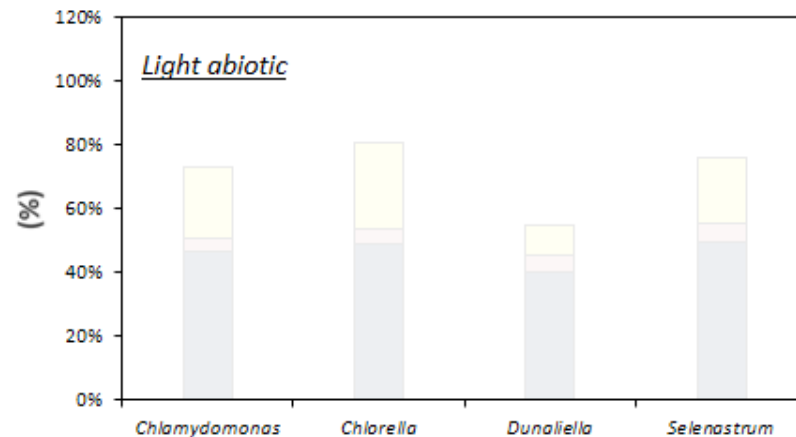
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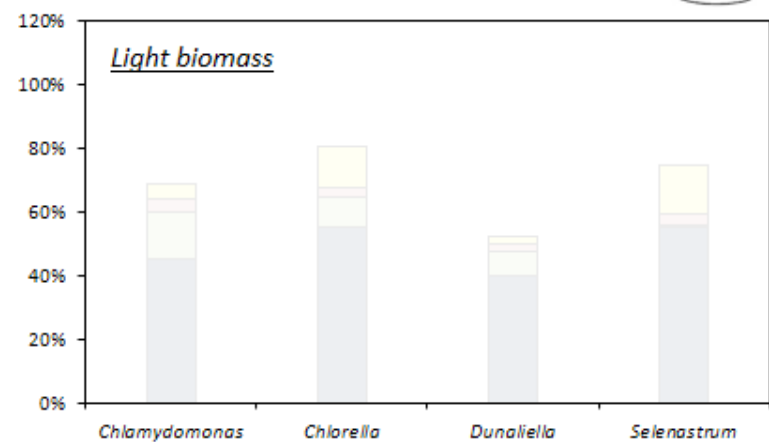
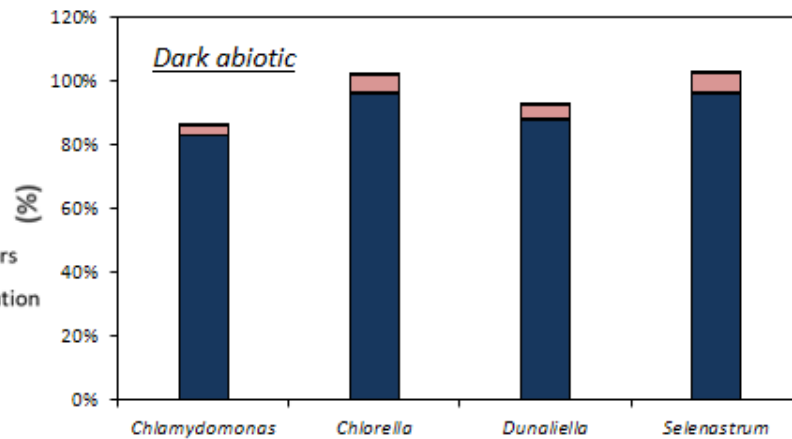
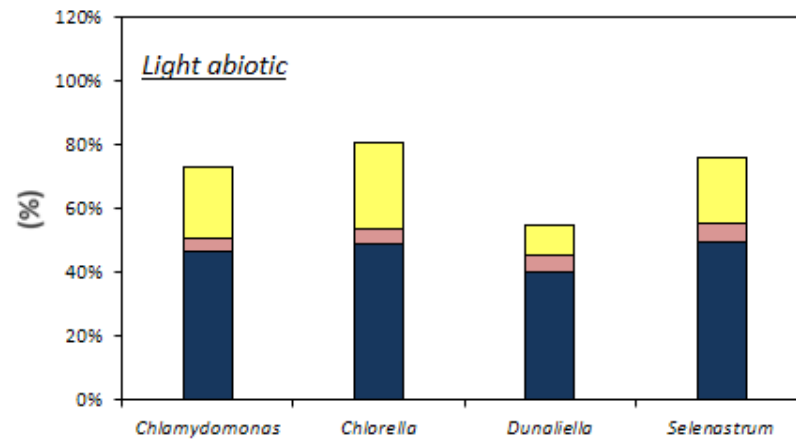
Microalgae assessment for transformation

- Presence of parent compounds
- Sum of TPs (%) generated by light
- Sum of TPs (%) generated by other factors
- Sum of TPs (%) generated by biodegradation



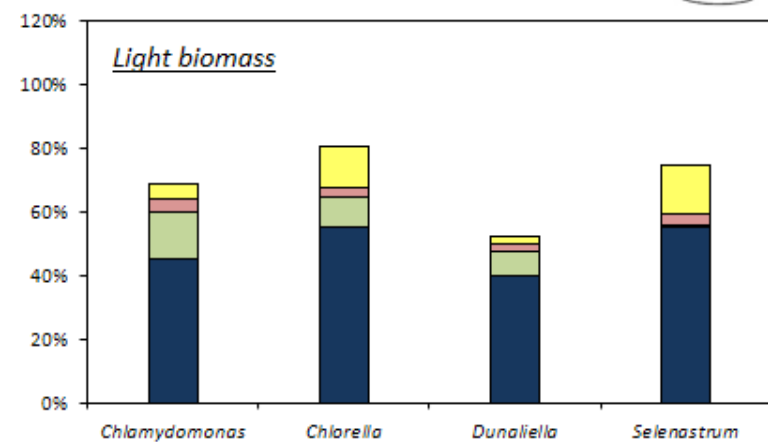
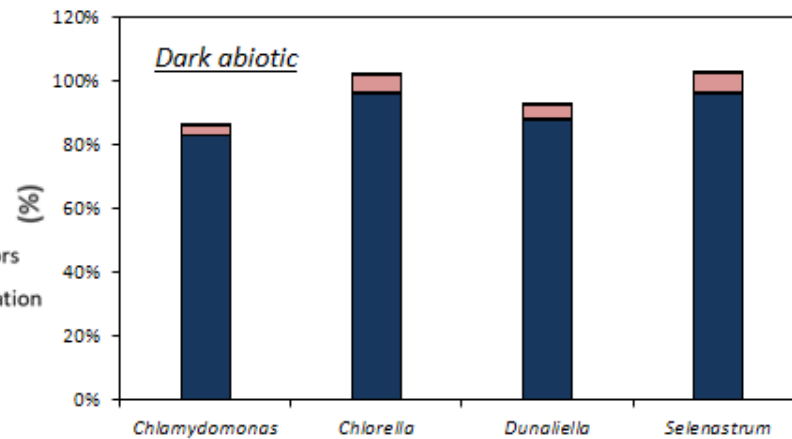
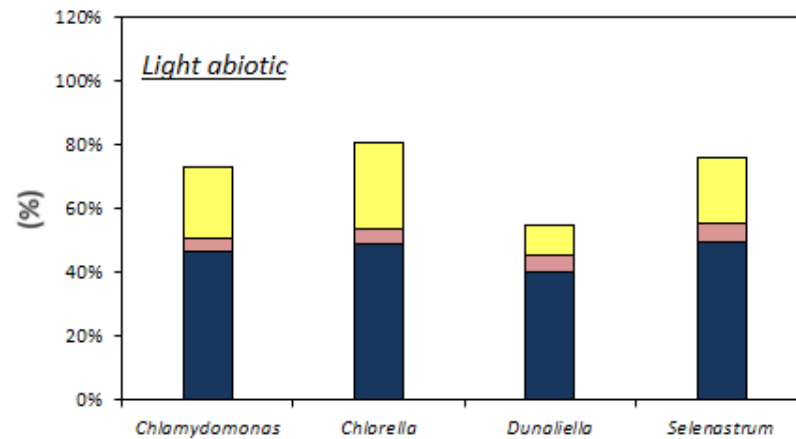
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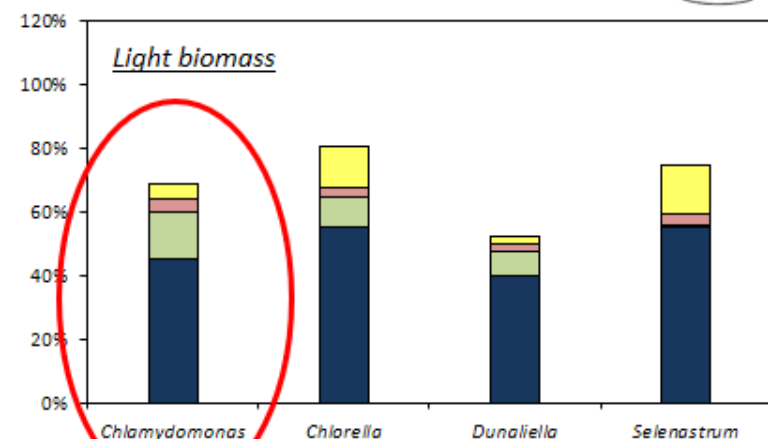
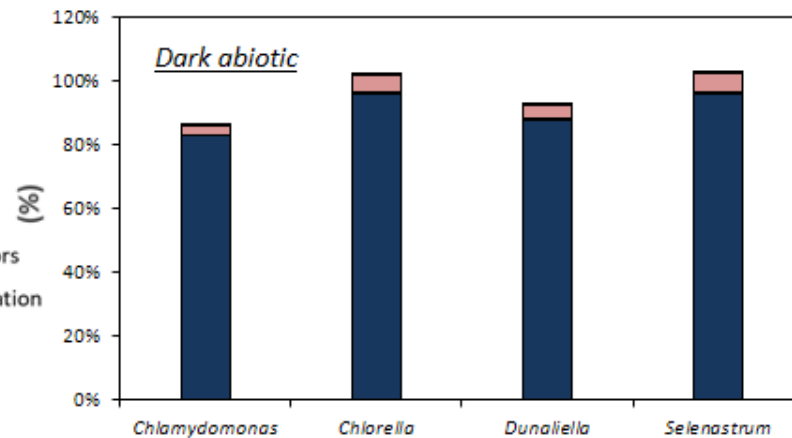
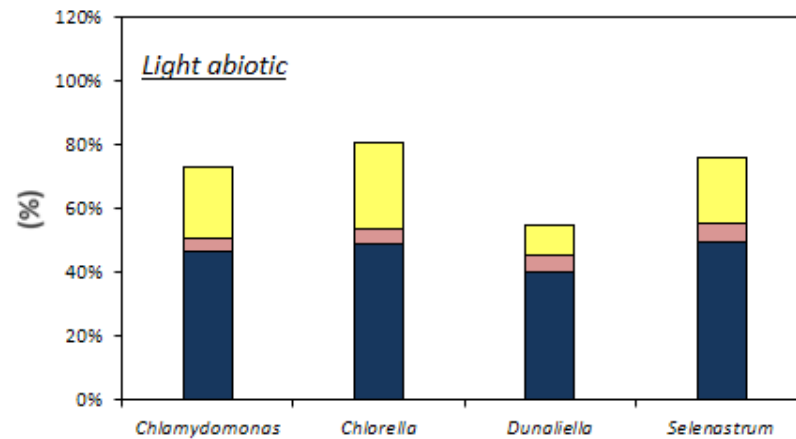
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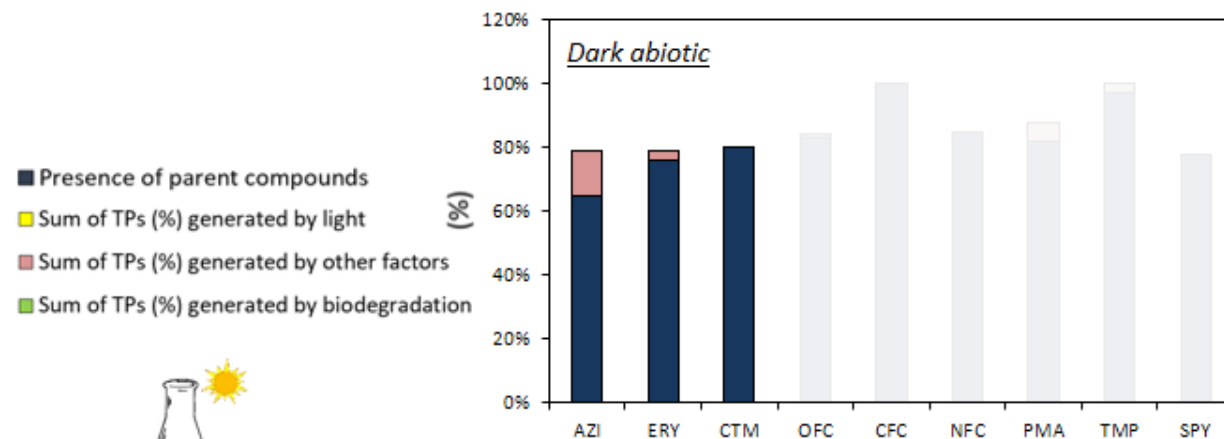


Microalgae assessment for transformation

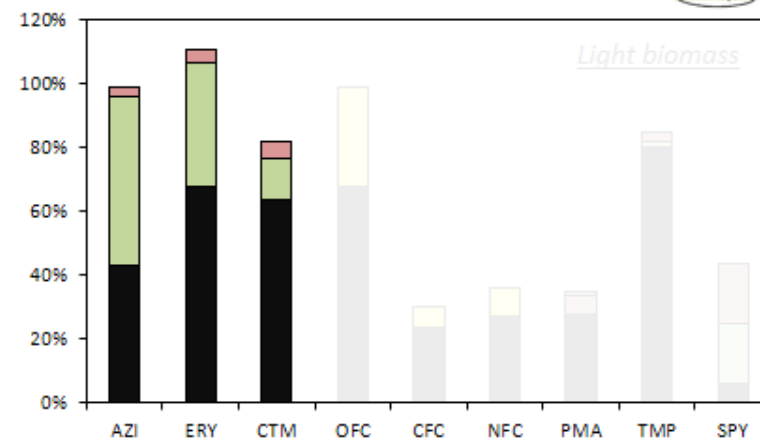
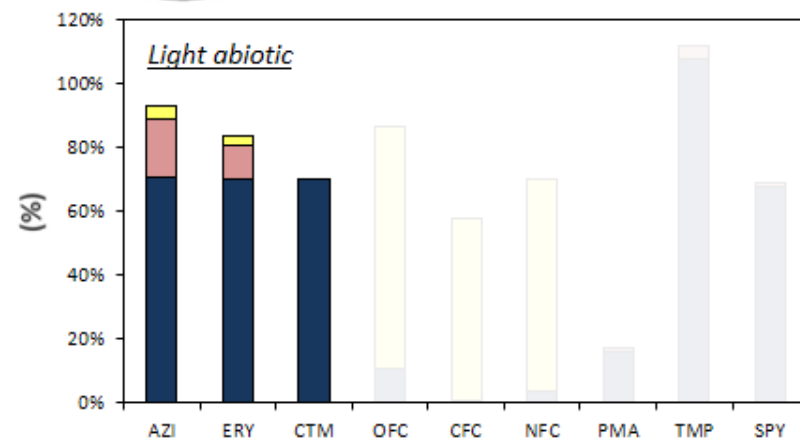
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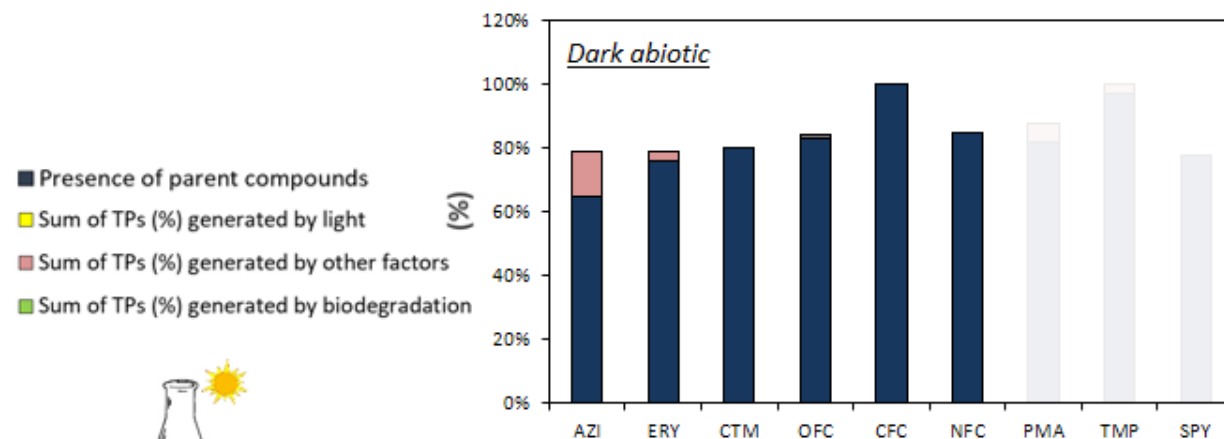
Compound transformation in *Chlamydomonas reinhardtii*



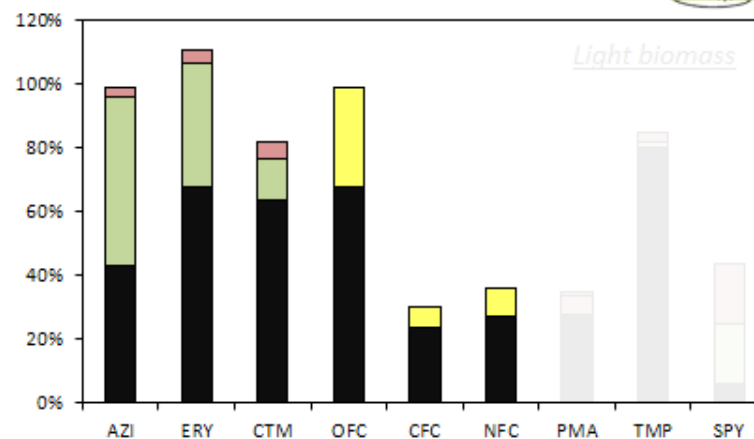
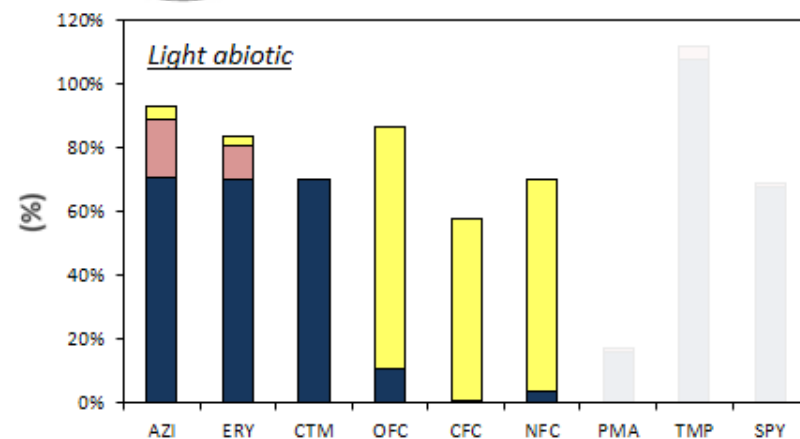
Macrolides



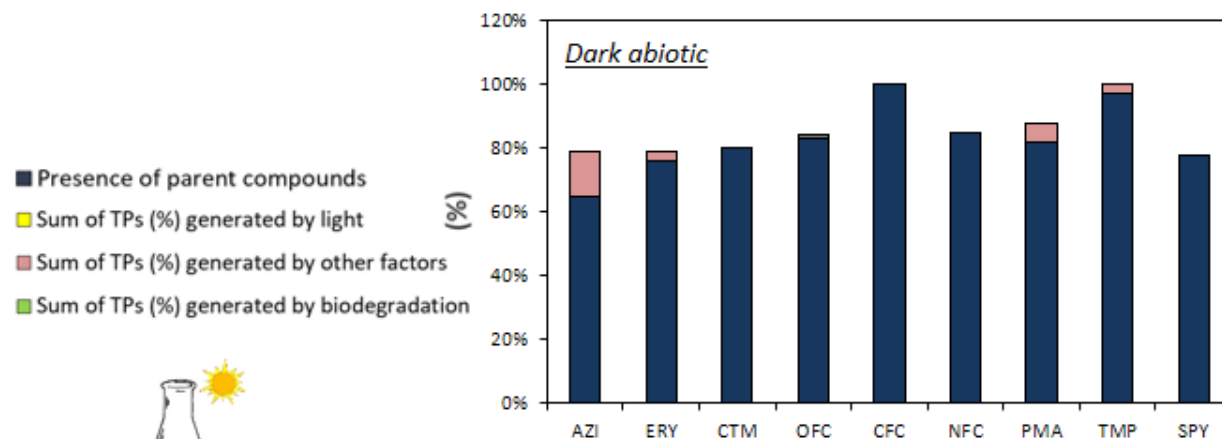
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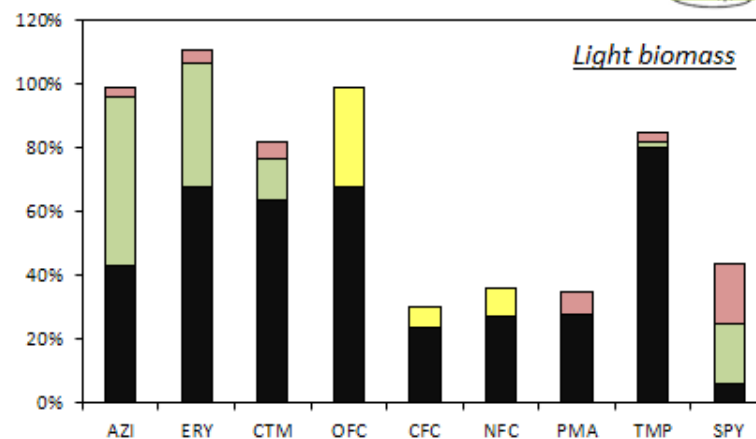
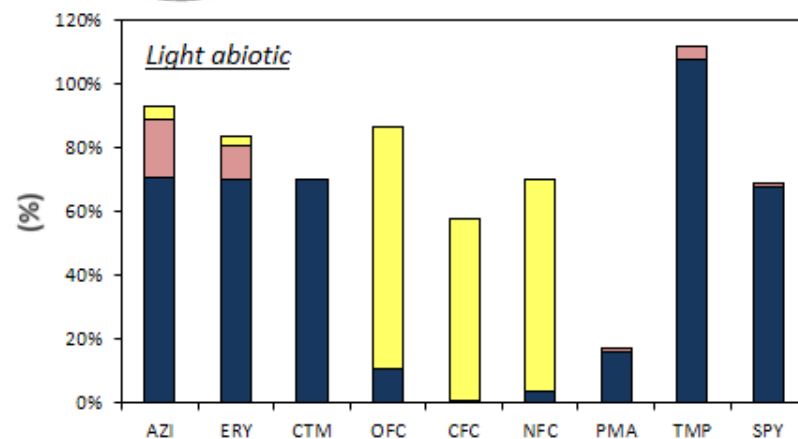
Fluoroquinolones



Compound transformation in *Chlamydomonas reinhardtii*

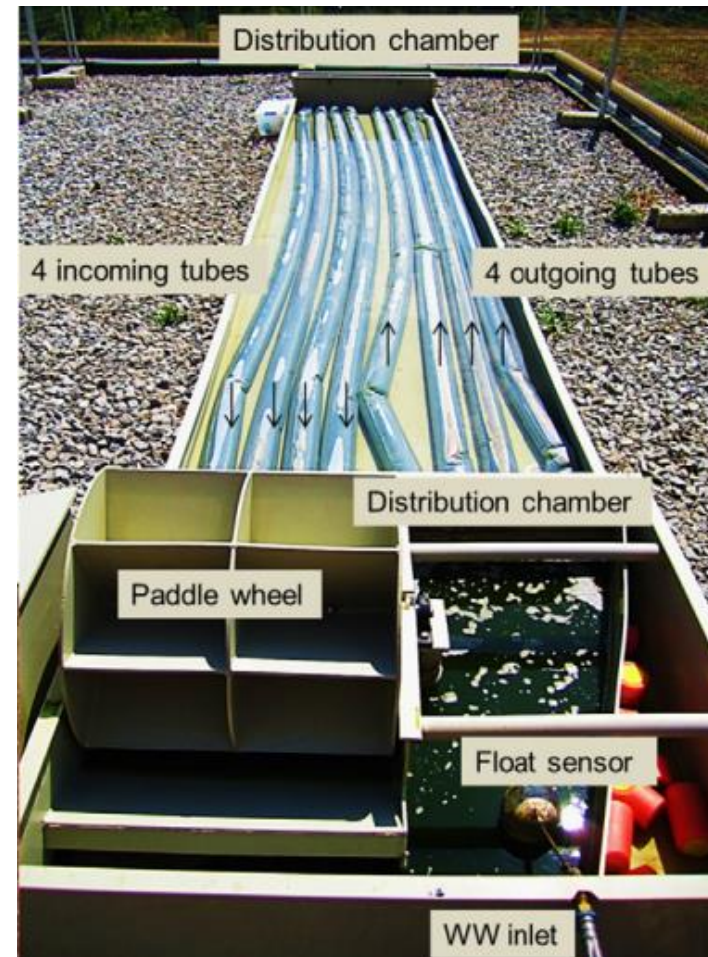
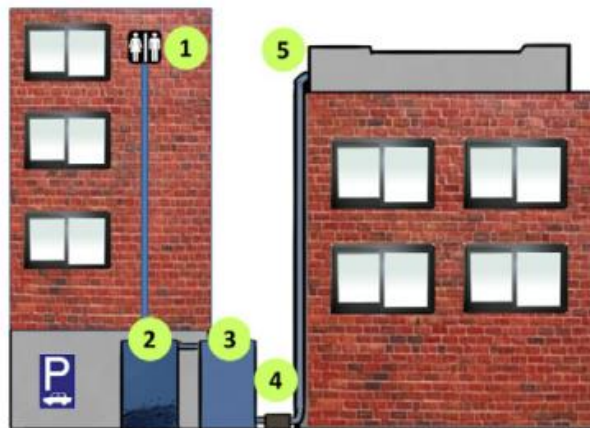


Other antibiotics



Microalgal photobioreactor treating urban wastewater

Urban wastewater was collected from the toilets drainage of the Chemical, Biological and Environmental Engineering Department (Universitat Autònoma de Barcelona, Barcelona, Spain) and pumped to an enclosed 1200 L multitubular microalgal photobioreactor (PBR).

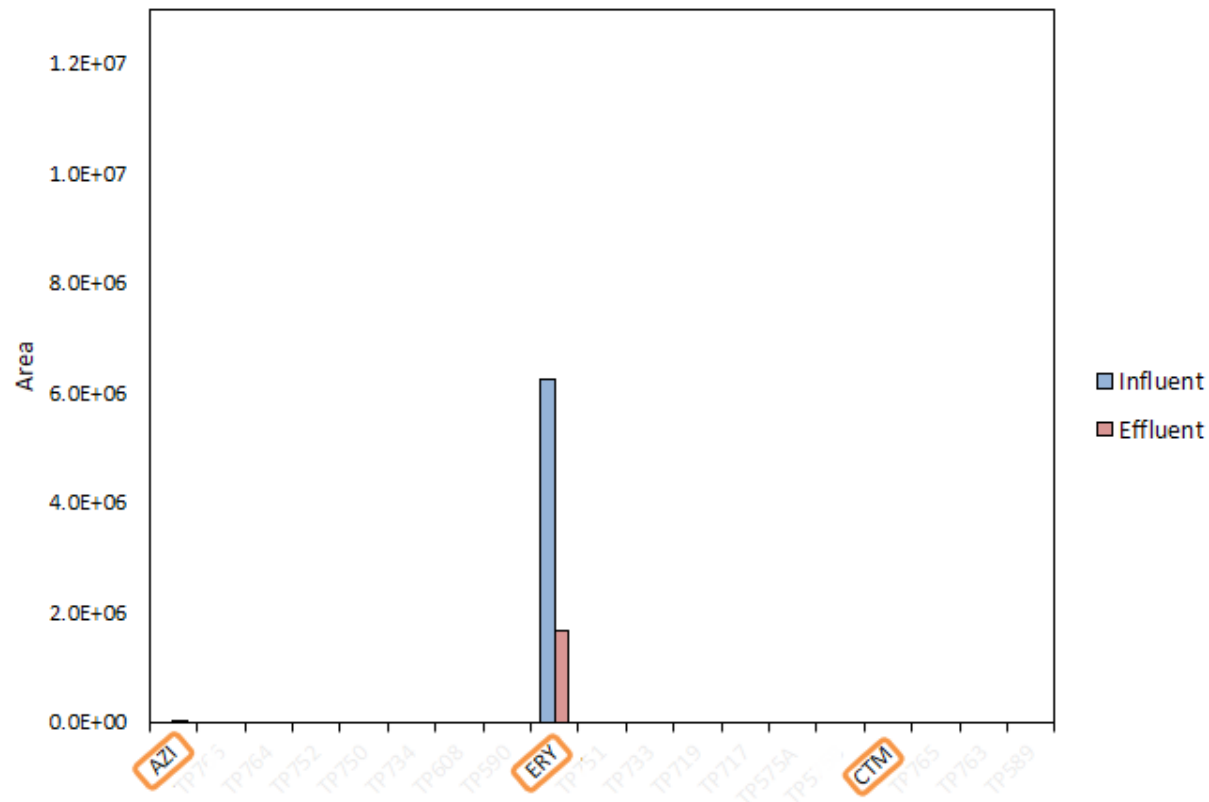


Hom-Díaz, A., Jaén-Gil, A., Bello-Laserna, I., Rodríguez-Mozaz, S., Vicent, T., Barceló, D., Blánquez, P., 2017. Performance of a microalgal photobioreactor treating toilet wastewater: Pharmaceutically active compound removal and biomass harvesting. *Sci. Total Environ.* 592, 1–11. doi:10.1016/j.scitotenv.2017.02.224

Microalgae photobioreactor treating urban wastewater

The PBR performance was monitored by taking samples from influent and PBR effluent at 12 days hydraulic retention time (HRT):

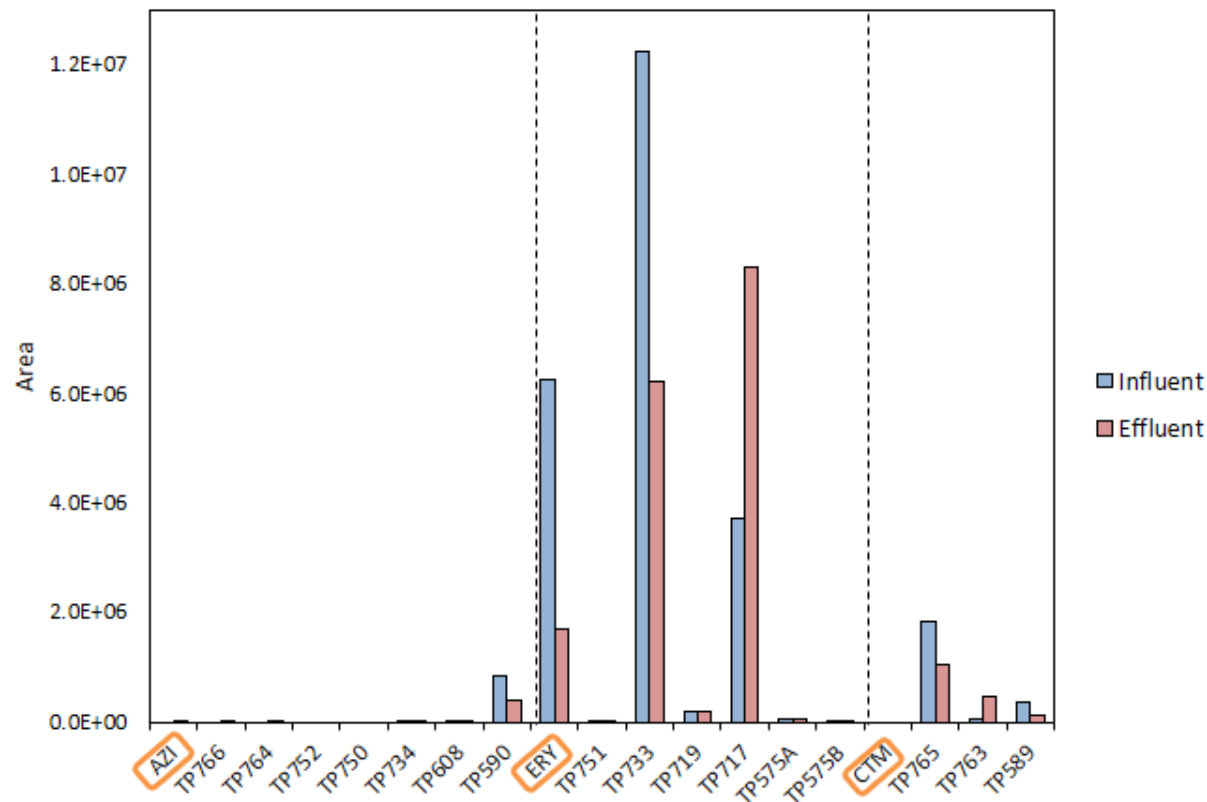
HRT= 12 days



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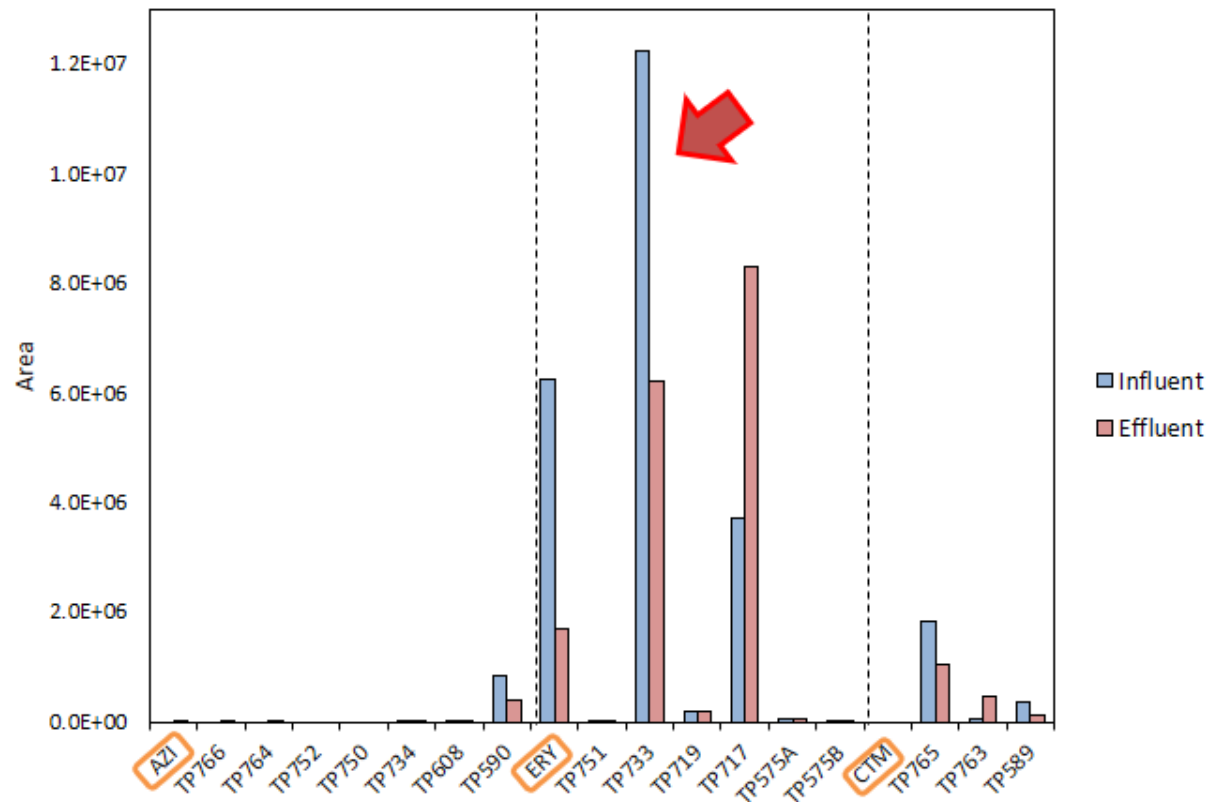
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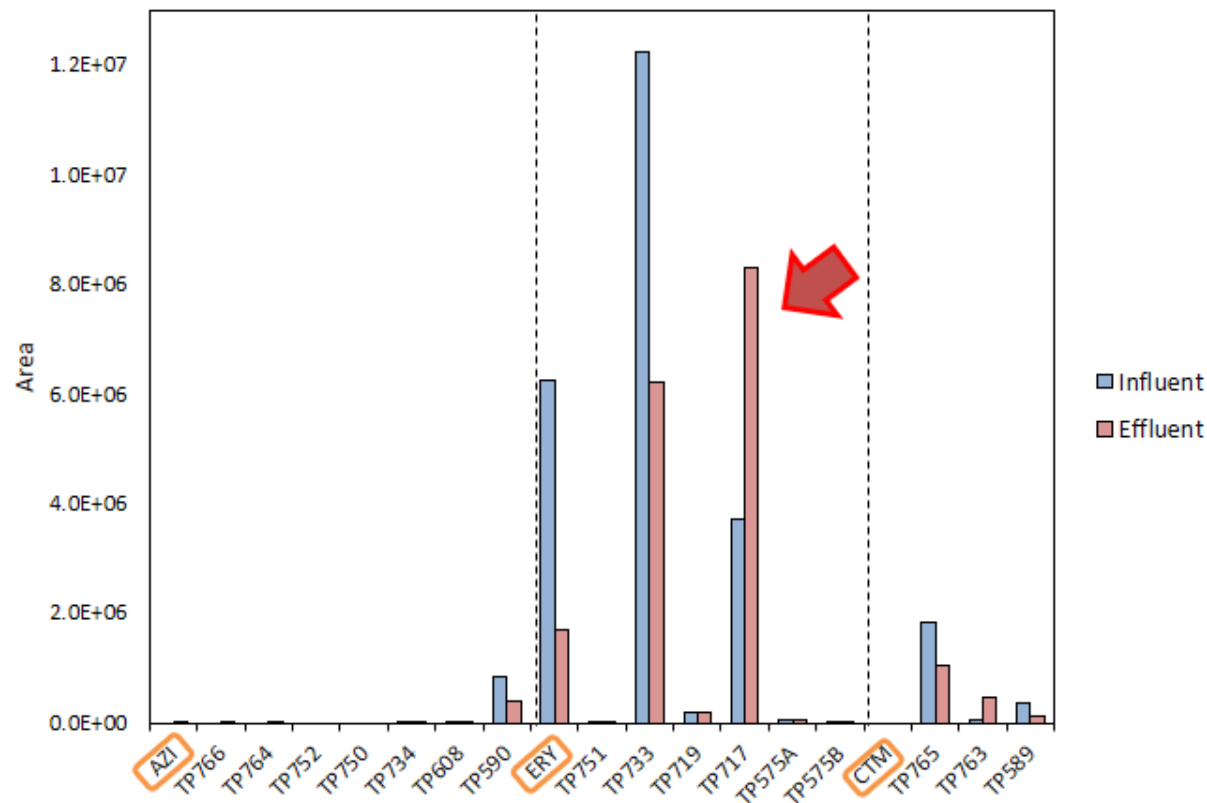
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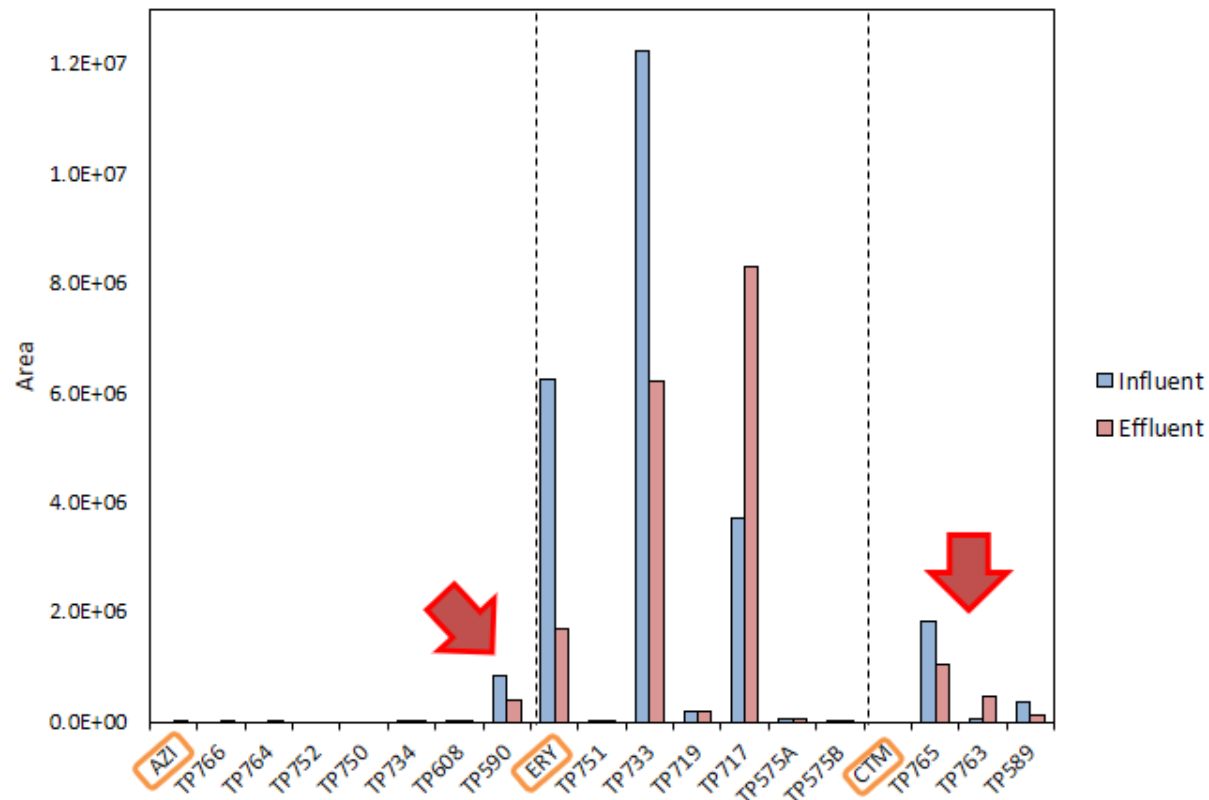
HRT= 12 days



Microalgae photobioreactor treating urban wastewater

The PBR performance was monitored by taking samples from influent and PBR effluent at 12 days hydraulic retention time (HRT):

HRT= 12 days



Conclusions

- A promising on-line HPLC methodology combined with an automated suspect screening was used for the identification of transformation products of contaminants reducing manual processing and time investment.
- 42 transformation products and their degradation pathways were tentatively proposed for the 9 antibiotic in algae water treatment.
- Biodegradation by algae led to the highest elimination of macrolide antibiotics producing a high amount of TPs.
- Photodegradation led to the highest elimination of fluoroquinolones but also producing a high amount of TPs.
- Comprehensive studies based on transformation products are required for total water assessment of water treatment.

Acknowledgements

Thank you for listening

- Spanish Ministry of Economy and Competitiveness (CTM2013-48548-C2), partly supported by the European Union through the European Regional Development Fund (ERDF) and supported by the Generalitat de Catalunya (Consolidate Research Group 2014SGR476, 2014-SGR-599 and 2014-SGR-291).
- Adrián Jaén-Gil acknowledges her PhD scholarship from AGAUR (2017FI_B 00771)
- Andrea Hom-Diaz acknowledges her PhD scholarship from AGAUR (2013FI_B 00302)
- Sara Rodriguez-Mozaz acknowledges the Ramon y Cajal program (RYC-2014-16707)

