

Enhancement of the activated treatment process for increased diclofenac degradation

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INTRODUCTION

Pharmaceutical micropollutants are increasingly detected in aquatic environments. Diclofenac (DCF), a nonsteroidal anti-inflammatory drug (NSAID), is widely used and poorly removed in conventional wastewater treatment making it a common micropollutant of concern. The carboxylic group in its chemical structure increases its mobility while the aromatic rings and chlorine molecules increase its stability and hydrophobicity (see Fig. 1). These characteristics contribute to persistence, limit biodegradation, and increase toxic effects to aquatic organisms.

In Switzerland, regulations now require selected wastewater treatment plants to remove ≥80% of micropollutants, including diclofenac. Although current removal technologies are effective, they are costly and energy intensive.

Thus, the search for scalable and more sustainable alternatives, such via microbial biodegradation, is essential for combatting DCF contamination in wastewater.

Objectives

- Identify microorganisms capable of diclofenac degradation in literature.
- Select the best candidates for wastewater treatment, evaluate their DCF degradation and propose survivability enhancements.
- Develop a lab-scale experimental strategy.

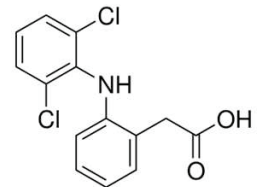


Figure 1: Chemical structure of Diclofenac (DCF).

LITERATURE REVIEW

Methodology

- From a collection of 41 papers, biodegradation of DCF was assessed by comparing the mechanisms of degradation and the reaction conditions.
- Ideal candidates were identified based on degradation efficiencies and their enzymatic activity was assessed using KBase (for bacteria) and literature data (for fungi) (see Table 1).

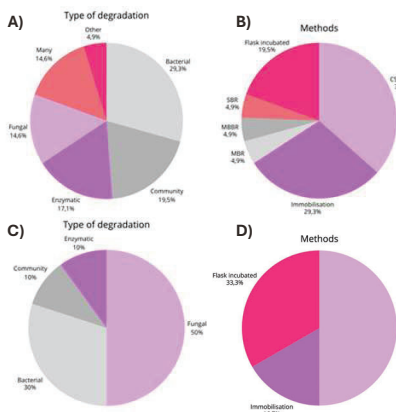


Figure 2: Literature review findings for the 41 papers examining biodegradation of diclofenac (A-B) and for the papers discussing the shortlisted candidates (C-D).

A) and C) summary of the main biodegradation types discussed in literature experiments.

B) and D) summary of treatment methods used for diclofenac degradation.

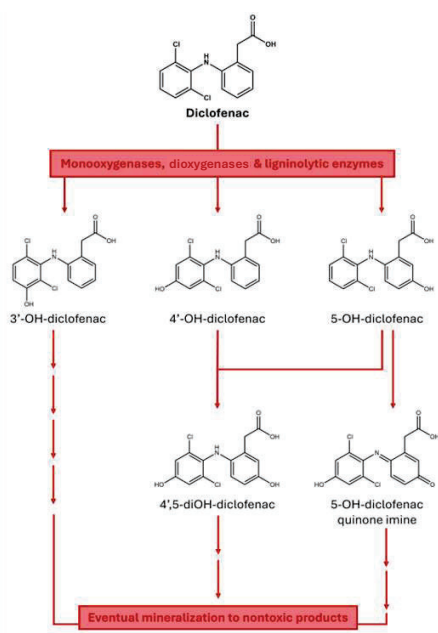
Results

Candidates for DCF degradation and their respective enzymatic activity

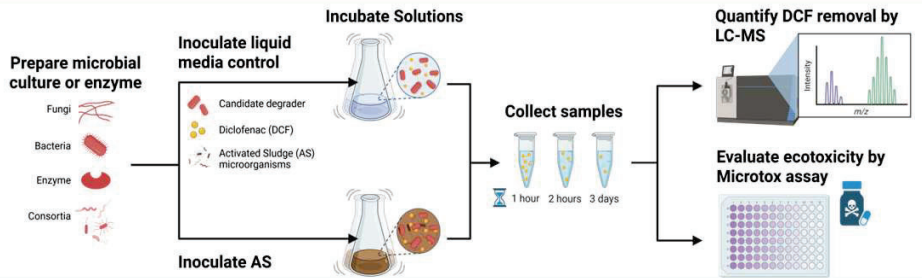
Organism	Degradation	Toxicity	Enzymatic Activity										
			Dioxygenase					Monoxygenase		Lignolytic Enzyme			
			1	2	3	4	5	6	7	8	9	10	
Bacteria	<i>R. ruber</i>	0.05 mg/L in 6 days	N/A										
	<i>L. portucalensis</i>	0.5 mg/L in 6 days	N/A										
	<i>P. moorei</i>	1 mg/L in 10 days	N/A										
Bacterial Consortia	<i>P. moorei</i>												
	<i>B. thuringiensis</i>	0.5 mg/L in 14 days	N/A										
	<i>S. maltophilia</i>												
Fungi	<i>R. microsporus</i>	1 mg/L in 1 day	N/A										
	<i>P. oxalicum</i>	34 mg/L in 36 hours	None by Microtox assay after 48 hours										
	<i>P. chrysosporium</i>	1 mg/L in 23 hours	N/A										
	<i>P. sordida</i>	30 mg/L in 6 days	None by EC ₅₀ test after 6 days										
	<i>T. versicolor</i>	10 mg/L in 1 hour	None by Microtox assay after 24 hours										
Enzyme	Laccase	5 mg/L in 8 hours	None by BLT-Screen after 48 hours										

Table 1: Selected candidates from the literature review based on complete DCF degradation and rate; and the ecotoxicity of transformed products if tested. Coloured columns indicate the presence (purple), absence (red) or lack of assessment (grey) of dioxygenases; monoxygenase** and lignolytic enzymes*** identified in Kbase (for bacteria) and in literature (Fungi). *1) catechol 1,2-dioxygenase, 2) catechol 2,3-dioxygenase, 3) benzoate 1,2-dioxygenase, 4) protocatechuete 3,4-dioxygenase, 5) homogentisate 1,2-dioxygenase, 6) gentisate 1,2-dioxygenase; **7) cytochrome P450; ***8) manganese peroxidase, 9) lignin peroxidase, 10) laccase.

DEGRADATION PATHWAYS



EXPERIMENTAL DESIGN



SURVIVABILITY

	Bacteria	Fungi
pH	6-9.5	4.5-5.5
Temperature	23°C - 28°C	25°C - 37°C
Co-metabolism	Add easily degradable carbon source such as glucose/acetic acid	
Starvation	Test over short term (~2 days) before inoculation	
Immobilization	Necessary for enzymes, requires further investigation for bacteria and fungi	

CONCLUSIONS

- Microbial degradation of micropollutants is cost effective, efficient and a green alternative to conventional methods.
- 10 microbial candidates for the degradation of diclofenac were identified via literature review.
- L. portucalensis* was found to be most promising thanks to AS compatibility, high degradation & enzymatic activity.
- Laboratory screening under complex activated sludge treatment conditions is required.



Sources