GRADUATE SEMINAR · ENVIRONMENTAL ENVIRONMENTAL MICROBIOLOGY

Bacterial Biodegradation of Pharmaceuticals, Hormones & Microplastics

Engineered and Natural Strains for Industrial-Scale Wastewater Treatment Treatment

UNIVERSITY SEMINAR · DEPARTMENT OF MICROBIOLOGY · 2026

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FIVE CHAPTERS

Presentation Roadmap

5 CHAPTERS ~25 MIN + Q&A

A guided tour from pollutant chemistry through microbial mechanisms, engineered engineered strains, and industrial deployment.

01 · Introduction & Background

Emerging pollutants, biodegradation, mechanisms overview.

02 · Pharmaceutical Pollutants

Antibiotics, NSAIDs, key strains & enzymes.

03 · Hormonal Pollutants

Estrogens (E1/E2/E3/EE2), natural & engineered degraders.

04 · Microplastics

PET/PE/PS, Ideonella, engineered PETase, industrial recycling.

05 · Industrial Scale & & Outlook

Bioreactor configs, scale-up, biosafety, future directions, references.

CHAPTER 01 OF 05

01

INTRODUCTION & BACKGROUND

Introduction & Background

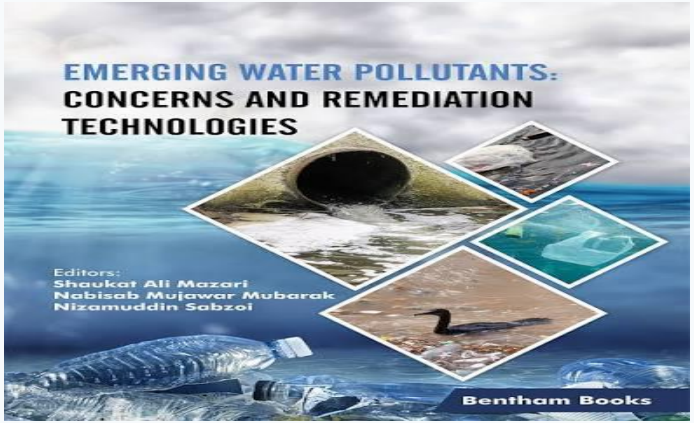
Why emerging pollutants demand biological solutions, and how microbes do the work.

THE PROBLEM

The Emerging Pollutant Crisis in Wastewater Wastewater

⚠ PRIORITY CONTAMINANTS

- WWTPs designed for BOD, N, P — not micropollutants
- Pharmaceuticals, hormones, microplastics escape treatment (ng/L–mg/L)
- Accumulate in receiving waters
- Concerns: persistence, bioaccumulation, sub-lethal effects



[1] Wilkinson et al., PNAS 2022 · [2] Adeel et al., Environ Int 2017 · [3] Boucher & Friot, IUCN 2017 · [4] Zhang et al., Water Res 2008 · Removal data: typical conventional activated sludge

ADVANTAGES OVER PHYSICO-CHEMICAL TREATMENT

INDUSTRIAL RATIONALE

Why Biological Degradation? Degradation?

1. **Cost-effective at scale**
2. **Mineralization potential**
3. **Selectivity for trace pollutants**
4. **Compatibility with existing WWTP infrastructure**

BIOLOGICAL VS PHYSICO-CHEMICAL

CONSTRAINTS TO PLAN FOR

Limitations

- ❄Kinetic lag at low temperatures temperatures (<10°C) slows microbial activity in winter operation.
- ◔Acclimation period of 7–30 d is required before steady-state removal is reached.
- ⛨Biosafety containment required for engineered strains released into open systems.

→ See Chapter 05 for scale-up

Adapted from Shahid et al., Chem Eng J 2021 [5]; Castillo-Carvajal et al., Water Air Soil Pollut 2024 [6]

MECHANISTIC OVERVIEW

Microbial Degradation Mechanisms — A Unified View

✦ 4 STRATEGIES · 3 CLASSES

Catabolic biodegradation 01

- Pollutant = sole carbon/energy source
- Complete mineralization via TCA cycle
- Dedicated catabolic operons evolve for simple substrates

Cometabolic degradation 02

- Cometabolism = fortuitous pollutant transformation
- Enzymes induced by primary substrates (methane, ammonia, phenol)
- Important when pollutant levels are too low for growth

Enzymatic hydrolysis (plastics) 03

- Secreted hydrolases (PETase, MHETase, lipases) cleave ester bonds
- Resulting monomers enter central metabolism
- Applicable only to hydrolysable polymers (PET, PUR)

Synergistic consortia 04

- Multiple strains → one step each
- Needed for complex polymer degradation
- Prevents toxic intermediate buildup

[7] Chen et al., Microbiology 2017 · [8] Shi et al., Water Res 2004 · [9] Yoshida et al., Science 2016 · [10] Ru et al., Sci Total Environ 2020

PHARMACEUTICAL POLLUTANTS

Pharmaceutical Pollutants

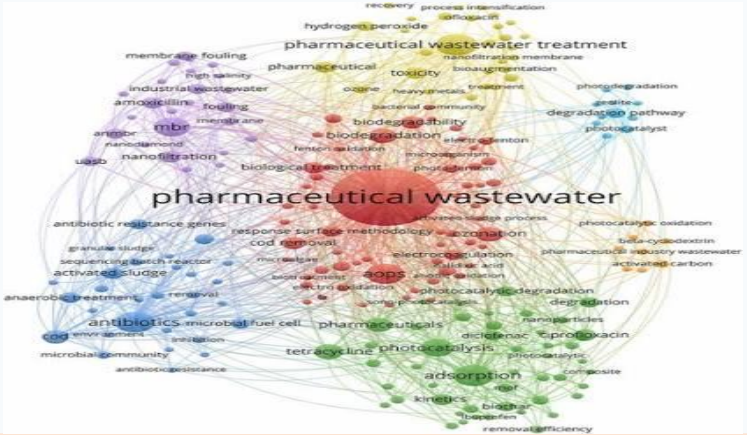
Antibiotics, NSAIDs, psychiatric drugs — and the bacteria that bacteria that break them down.

SCOPE OF THE PROBLEM

Pharmaceuticals in Wastewater — Sources & Concentrations

200+ APIS IN SURFACE WATER

- Pharmaceuticals enter via excretion, disposal, and hospital/manufacturing effluents
- 200 APIs detected in surface waters worldwide
- Antibiotics, NSAIDs, and antidepressants dominate mass flux
- Hospital effluents drive extreme local concentrations and resistance hotspots



Carbamazepine & diclofenac are recalcitrant — EU Watch List substances 2022.

Data: aus der Beek et al., Environ Toxicol Chem 2016 [11]; WHO 2022 report on AMR [12]; EU Watch List Decision 2022/1307

CONCENTRATION RANGES IN WWTP INFLUENT (MG/L)

Class	Example compound	Influent (µg/L)	Effluent (µg/L)	Removal %
Antibiotics	Ciprofloxacin	5–65	1–12	70–85
Antibiotics	Sulfamethoxazole	1–10	0.3–4	50–75
NSAIDs	Ibuprofen	5–85	0.5–8	80–95
NSAIDs	Diclofenac	1–7	0.5–4	20–50
NSAIDs	Naproxen	2–20	1–8	40–65
Psychiatric	Carbamazepine	1–6	0.8–5	5–25

DUAL HAZARD

Antibiotic Pollution — Resistance Co-Selection Risk

AMR CO-POLLUTANT

- Antibiotic residues select for resistance and HGT
- Even µg/L sub-inhibitory levels trigger resistance mechanisms
- Biodegradation must destroy the antibiotic scaffold to remove selective pressure
- Diverse enzymatic pathways target different antibiotic classes
- **β-lactams:** β-lactamase hydrolysis + dioxygenase ring fission
- **Fluoroquinolones:** Flavin-dependent reductive dehalogenation
- **Sulfonamides:** Cytochrome P450 monooxygenase de-ethylation

WORLD HEALTH AMR BENCHMARK

Antimicrobial Resistance — Global Burden

4.95M deaths
Associated with bacterial AMR in 2019
2019 (Global burden, Lancet 2022).

1.27M deaths
Directly attributable to AMR.

10M / yr by 2050
Projected annual deaths if unchecked.

Murray et al., Lancet 2022

✓ Bacterial degradation ≠ selection for resistance

Murray et al., Lancet 2022 [13]; Bengtsson-Palme et al., Environ Int 2023 [14]; WHO Global Antimicrobial Resistance Surveillance 2022

NSAID PROFILE

NSAIDs — Structural Diversity & Recalcitrance

Recalcitrance

+ 100 KT/YEAR CONSUMED

- NSAIDs: >100 kt/year, globally high-volume
- Diverse structures → need specific catabolic pathways
- Diclofenac = most resistant to degradation

NSAID BIODEGRADABILITY PROFILE

4 compounds · WWTP removal + environmental half-life

Compound	Structure class	WWTP removal %	Half-life in river water (d)	Biodegradability
Ibuprofen	Arylpropionic acid	80–95	6–20	✓Readily biodegradable
Naproxen	Arylpropionic acid	40–65	15–40	→Moderately biodegradable
Diclofenac	Phenylacetic acid	20–50	20–60	⊘Recalcitrant
Ketoprofen	Arylpropionic acid	30–60	20–50	→Moderately biodegradable

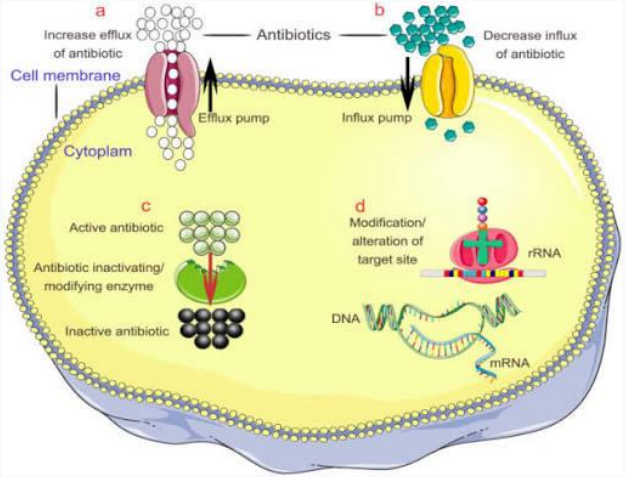
Kosma et al., Sci Total Environ 2014 [15]; Langenhoff et al., Rev Environ Sci Biotechnol 2013 [16]; Bouju et al., Biodegradation 2016 [17]

STRAIN SHOWCASE · ANTIBIOTICS

Bacterial Strains for Antibiotic Degradation Degradation

6 ISOLATES

- Multiple bacterial genera degrade antibiotic scaffolds
- Key genera: *Pseudomonas*, *Bacillus*, *Stenotrophomonas*, white-rot-associated
- Degradation performance is strain-dependent
- Optimal conditions: 28–37°C, pH 6.5–8.0, shaking aeration



[18] Cui et al., Environ Sci Pollut Res 2021 · [19] Majewsky et al., Water Res 2015 · [20] Wang et al., Ecotoxicology 2018 · [21] Liu et al., Appl Microbiol Biotechnol 2018 · [22] Liu et al., Int 2018 · [22] Liu et al., Int Biodeter Biodeg 2021 · [23] Pan et al., Chemosphere 2015

STRAIN 01

Fluoroquinolone

Pseudomonas aeruginosa

PAO1

Ciprofloxacin

78% in 7 d

30°C, pH 7.0

Glucose co-substrate

Via C-7 piperazine dealkylation [18]

STRAIN 02

Sulfonamide

Stenotrophomonas maltophilia

KB2

Sulfamethoxazole

92% in 6 d

28°C, phenol primary

Cometabolic

Via ipso-hydroxylation [19]

STRAIN 03

Tetracycline

Bacillus subtilis

1220

Tetracycline

86% in 5 d

35°C, pH 7.2

tetA-like efflux reversal

Enzymatic [20]

STRAIN 04

Macrolide

Microbacterium sp.

4N3-2

Erythromycin

90% in 4 d

30°C

Erythromycin esterase

Cleaves macrolide lactone ring [21]

STRAIN 05

β-lactam

Enterobacter sp.

CE5

Cefalexin

82% in 3 d

30°C

β-lactamase + ring cleavage

β-lactamase + ring cleavage [22]

STRAIN 06

β-lactam

Klebsiella sp.

KAY1036

Amoxicillin

95% in 24 h

37°C

High-rate β-lactamase

High-rate β-lactamase producer [23]

STRAIN SHOWCASE · NSAIDS

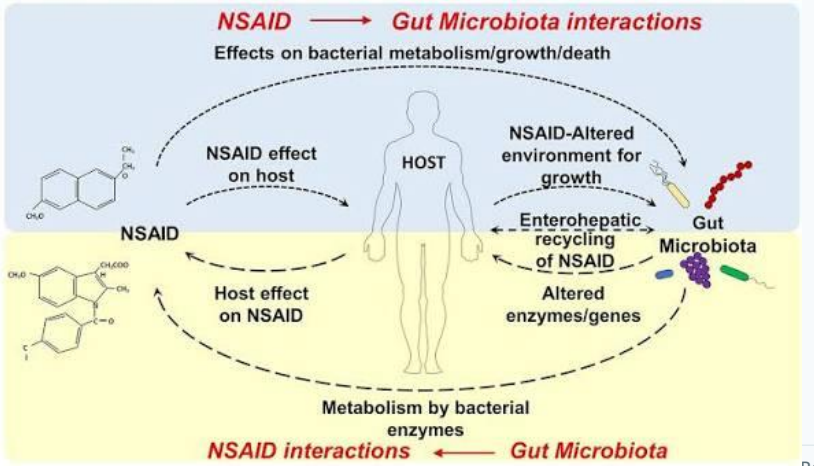
Bacterial Strains for NSAID Degradation Degradation

6 ISOLATES

•Ibuprofen: readily degraded, can serve as sole carbon source

•Diclofenac: highly recalcitrant, >50% removal is rare

.



[24] Murdoch & Hay, Appl Environ Microbiol 2005 · [25] Almeida et al., Res Microbiol 2013 · [26] Domaradzka et al., Int Biodeter Biodeg 2016 · [27] Webb et al., Mar Pollut Bull 2017 · Mar Pollut Bull 2017 · [28] Grandclément et al., Water Res 2020 · [29] Bartels et al., Eng Life Sci 2022

STRAIN 01

100% Ibuprofen

Sphingomonas sp. Ibu-2

+ Ibuprofen

100% in 7 d · **mineralization**

28°C

Ibuprofen as sole C source

Pathway via catechol meta-cleavage [24]

STRAIN 02

95% Ibuprofen

Rhodococcus sp. BK6

+ Ibuprofen

95% in 5 d · co-metabolic

30°C

Phenol co-substrate

Produces 2-hydroxyibuprofen intermediate [25]

STRAIN 03

70% Naproxen

Bacillus thuringiensis B1

+ Naproxen

70% in 8 d · **cometabolic**

30°C, pH 7.0

Glucose co-substrate

Demethylation pathway [26]

STRAIN 04

65% Naproxen

Vibrio gazogenes PHP4

+ Naproxen

65% in 10 d · halotolerant

28°C

Marine sediment isolate

Halotolerant marine strain [27]

STRAIN 05

55% Diclofenac

Brevibacterium sp. D4

+ Diclofenac

55% in 14 d · **recalcitrant**

30°C

Reductive dechlorination

Dechlorination precedes ring cleavage [28]

STRAIN 06

45% Diclofenac

Raoultella sp. KDF8

+ Diclofenac

45% in 10 d · **consortium**

30°C, pH 7.2

Side-chain cleavage only

Needs consortium for further degradation [29]

Key Enzymes & Catabolic Pathways for Pharmaceuticals

ENZYME PORTFOLIO

- **β-Lactamases:** Hydrolyze β-lactam ring (penicillins/cephalosporins)
- **Cytochrome P450s:** Dealkylation & hydroxylation (sulfonamides, erythromycin)
- **Laccases:** Radical coupling oxidation (diclofenac, tetracycline)
- **Catechol Dioxygenases:** Aromatic ring cleavage (2-hydroxyibuprofen)



CASE STUDY · PILOT SCALE

Pilot Membrane Bioreactor for Pharmaceutical Effluent

- extreme antibiotic pollution hotspot (31 mg/L)
- MBR + bio-augmentation (PAO1 + KB2) operated for 180 days
- Achieved >85% removal of ciprofloxacin and sulfamethoxazole
- Reduced antibiotic resistance genes (ARGs) by 2-log
- Demonstrates effective biological treatment for high-strength pharmaceutical wastewater



DAY 0–7

Inoculation & Acclimation

Seed sludge spiked with 10^8 CFU/L CFU/L of each strain; HRT 24 h; SRT 30 d.

DAY 7–30

Biofilm Formation

Carriers (K3 AnoxKaldnes) colonized; MBR flux 15 LMH.

DAY 30–90

Steady-State Operation

Influent ciprofloxacin 5–31 mg/L; removal 87±6%.

DAY 90–150

ARG Monitoring

intI1 abundance decreased from 10^8 10^8 to 10^6 copies/mL.

Stress Test

Spike load 2× baseline; removal held > 80%.

Based on Larsson et al., PLoS ONE 2007 [33]; Marathe et al., Sci Total Environ 2021 [34]; cost data from Bengtsson-Palme & Larsson, 2022 [32]

CHAPTER 03 OF 05

Hormonal Pollutants

Endocrine-disrupting compounds at trace concentrations, biological biological effects at parts-per-trillion.

POLLUTANT CLASSES

E1 · E2 · E3 · EE2

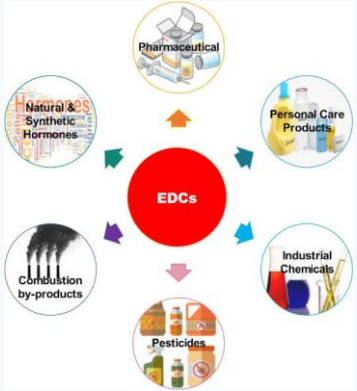
POTENCY FLOOR

~1 ng/L bioactivity

ENDOCRINE DISRUPTORS

Endocrine-Disrupting Compounds (EDCs) — Classes & Effects

- Active at very low concentrations (ng/L)
- Key steroids: E1, E2, E3, EE2
- EE2 is potent at sub-ng/L in fish
- Steroid hormones are the main drivers of estrogenic activity in WWTP effluent



0.1 ng/L

EE2 concentration inducing vitellogenin (VTG) in male fish. male fish.

4–8 ng/L

Threshold for complete feminization of fish populations populations

EE2 ADDED TO THE EU WATCH LIST IN 2013 (DECISION 2015/495) — REMAINS ON THE 2022 LIST.

[35] Purdom et al., Chem Ecol 1994 · [36] Kidd et al., PNAS 2007 · [2] Adeel et al., Environ Int 2017 · [37] Hamilton et al., Environ Sci Technol 2016

WWTP ESTROGEN CONCENTRATIONS & REMOVAL

Compound	Influent (ng/L)	Effluent (ng/L)	Conv. removal
Estrone (E1)	5–80	1–20	60–85%
17β-Estradiol (E2)	5–50	0.5–5	80–95%
Estriol (E3)	10–100	2–15	80–90%
17α-Ethinylestradiol (EE2)	0.5–10	0.3–5	40–70%

Concentrations: Johnson & Sumpter, Sci Total Environ 2015 [38]; chemistry review: Cajthaml, Chemosphere 2015 [39]

Natural Bacterial Strains for Estrogen Degradation

- Estrogen degraders are phylogenetically narrow (*Sphingomonadaceae* dominant)
- E1 and E2: used as sole carbon sources
- EE2: only partially degraded

Sphingomonas sp. KC8 01
Substrate: E2, E1

100% E2 in 24 h · 92% EE2 in 7 d · 30°C

First isolated estrogen degrader; A-ring 4,5-ring 4,5-dioxygenase (*edcB*).

Novosphingobium sp. S2 02
Substrate: E2, E1

100% in 3 d · 30°C

Produces 4-hydroxyestrone intermediate.

Sphingobium sp. JLU-2 03
Substrate: EE2

85% in 5 d · 30°C, pH 7.0

Fastest natural EE2 degrader reported.

Rhodococcus equi K2 04
Substrate: E2

95% in 5 d · 30°C

Gram-positive alternative to sphingomonads. sphingomonads.

Pseudomonas fluorescens S-5 05
Substrate: E1, E2

90% in 6 d · 28°C

Soil isolate.

Achromobacter xylosoxidans E1 06
Substrate: E1, E2

88% in 5 d · 30°C

Isolated from activated sludge.

[40] Yu et al., Appl Environ Microbiol 2007 · [41] Hai et al., Sci Rep 2018 · [42] Chen et al., Environ Microbiol 2018 · [43] Ying et al., J Chem Technol Biotechnol 2017 · [44] Shi et al., Can J Microbiol 2019 · [45] Xu et al., Chemosphere 2017

Engineered Strains & Synthetic Biology Approaches

Recombinant expression of catabolic enzymes in robust hosts (*E. coli*, *Pseudomonas putida*) aims to (i) to (i) extend substrate range to recalcitrant EE2, (ii) raise reaction rates, and (iii) combine depolymerization depolymerization and catabolism in one chassis. The field has shifted from single-gene knock-ins to ins to refactored catabolic operons and biosafety-locked kill switches.



⚡ Heterologous laccase expression

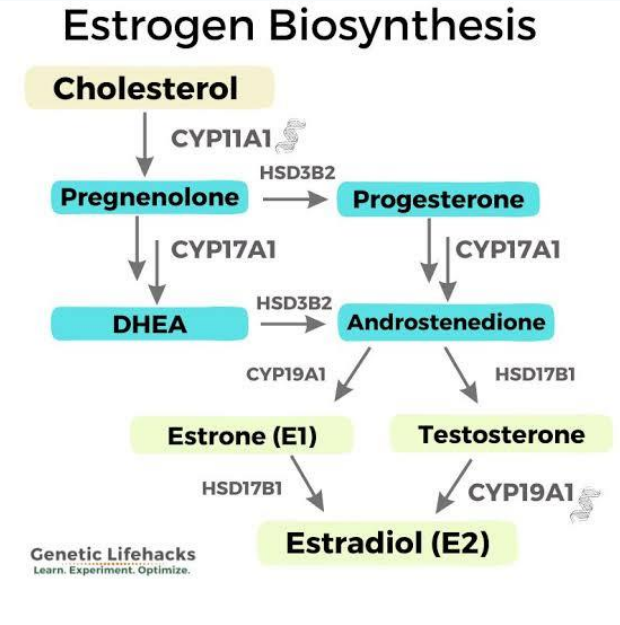


Kill-switch containment

Key Enzymes & Pathways for Estrogen Degradation

ENZYME PORTFOLIO

- **Estrone 4-hydroxylase (*edcA*):** Initiates degradation via A-ring hydroxylation
- **4-Hydroxyestrone 4,5-dioxygenase (*edcB*):** Cleaves A-ring, destroying receptor binding
- **Laccase:** Oxidizes phenolic estrogens via radical coupling
- **17β-HSD:** Interconverts E2/E1, limited by EE2's ethinyl group



[50] Bertin et al., Appl Environ Microbiol 2015

CASE STUDY · FULL-SCALE MBR

Full-Scale MBR with Bio-Augmentation at Ulu Pandan WRP

- 5,000 m³/day MBR + *Sphingobium* JLU-2 bio-augmentation
- EE2 removal improved from **67% → 96%**



Adapted from Lim et al., Water Res 2023 [51]; PUB Singapore Annual Report 2022 [52]; Yi et al., Chemosphere 2022 [53]

CHAPTER 04 OF 05

Microplastics

From polyethylene terephthalate bottles to bacterial monomers — monomers — closing the plastic loop.

ANNUAL PRODUCTION
400 Mt · 2022

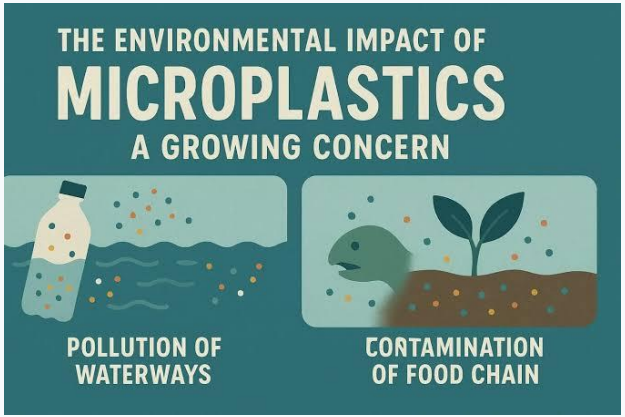
OCEAN INFLUX
~10 Mt / yr

GLOBAL SCALE

Microplastic Pollution — Scale & Wastewater Pathway

- Plastic production: 10 Mt reaches oceans annually
- WWTPs are a major microplastic pathway
- Removal is high (90–99%), but residual effluent and sludge loads remain an environmental concern

- Global plastic production is massive (**400 Mt/year**)
- **4–8 trillion** MP particles enter surface waters yearly from WWTP effluent
- Conventional WWTPs remove **90–99%** of MPs, but residual loads remain significant



SLUDGE RETAINS THE REMAINING MPS → LAND APPLICATION TRANSFERS THEM TO SOILS (NIZZETTO ET AL., 2016).
AL., 2016).

[54] PlasticsEurope 2023 · [3] Boucher & Friot, IUCN 2017 · [55] Mason et al., Environ Sci Technol 2016 · [56] Murphy et al., Environ Sci Technol 2016

Plastic Types — Biodegradability Spectrum

- Polymer chemistry dictates biodegradability
- Hydrolysable backbones (esters, urethanes) = easily degraded
- C–C backbone polyolefins = highly resistant

.



Wilkes & Aristilde, ACS EST Eng 2017 [57]; Danso et al., Microb Biotechnol 2019 [58]; plastic production: PlasticsEurope 2023 [54]

PLASTIC POLYMER BIODEGRADABILITY RANKING

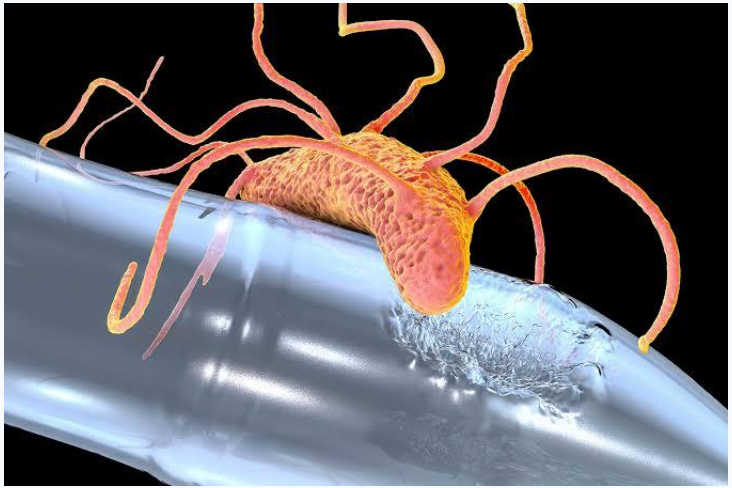
	Backbone	Global production (Mt)	Biodegradation half-life	Hydrolysable?	Known microbial degraders
PET	Ester	82	6–24 months*	✓Yes	Ideonella sakaiensis, Thermobifida fusca
PUR (polyurethane)	Urethane	26	6–18 months	Partial	Pseudomonas fluorescens, Bacillus subtilis
PLA (polylactic acid)	Ester	0.8	3–6 months (industrial composting)	✓Yes	Amycolatopsis sp., Tritirachium album
PS (polystyrene)	C–C	33	10–50 years	✕No	Pseudomonas putida, Bacillus cereus (slow)
PVC	C–C	41	> 50 years	✕No	White-rot fungi (limited bacteria)
PE (polyethylene)	C–C	120	10–100 years	✕No	Rhodococcus rhodochrous, Bacillus gottheilii
PP (polypropylene)	C–C	84	20–100 years	✕No	Bacillus sp. (very slow)

NATURAL ISOLATES

Natural Plastic-Degrading Bacteria

50+ CONFIRMED SPECIES

- *Ideonella sakaiensis* discovered in 2016 (Sakai, Japan)
- 50 plastic-degrading bacterial species now known
- Most are slow-growing environmental isolates
- Natural degradation rates are too low for industry



[9] Yoshida et al., Science 2016 · [59] Kawai et al., Appl Microbiol Biotechnol 2014 · [60] Howard, Int Biodeter Biodeg 2002 · [61] Tribedi & Sil, Environ Sci Pollut Res 2017 · [62] Auta et al., 2017 · [62] Auta et al., Environ Sci Pollut Res 2017 · [63] Palm et al., Microb Biotechnol 2019

01

Ideonella sakaiensis 201-F6

✦ PET

75% weight loss · 6 wk · 30°C

Produces PETase + MHETase; first complete PET catabolism.

Foundational isolate — Yoshida et al., 2016. 2016.

02

Thermobifida fusca

✦ PET · PET FILM

50% weight loss · 4 wk · 50°C

Thermophilic actinomycete; thermostable hydrolase.

Industrially relevant due to thermophilicity.

03

Pseudomonas fluorescens B-22

✦ PUR

80% weight loss · 30 d · 30°C

Secretes polyurethanase esterases.

Polyurethane backbone hydrolysis.

04

Rhodococcus rhodochrous

✦ PE (UV-PRETREATED)

30% weight loss · 30 d · 30°C

Requires photo-oxidation pretreatment.

C–C backbone needs oxidative pre-step. step.

05

Bacillus cereus CH6

✦ PE · PS

25% PE **18%** PS · 40 d

Broad substrate range, slow rate.

Versatile but kinetically limited.

06

Comamonas testosteroni E6

✦ PET INTERMEDIATES (MHET, BHET)

Mineralizes monomers

Completes PET catabolic loop.

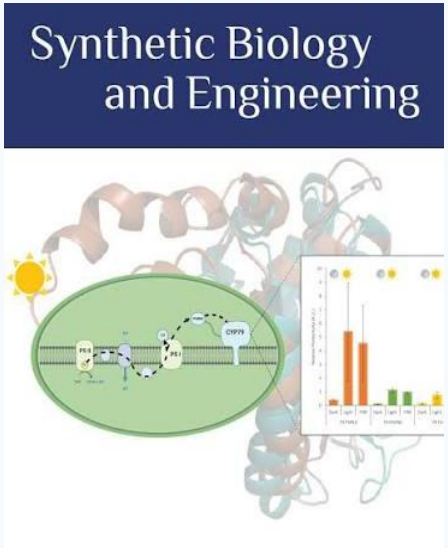
Closes the loop after

PROTEIN ENGINEERING · DIRECTED EVOLUTION

Engineered Strains & Enzyme Variants for Plastic Depolymerization

↗ 100–10,000× ACTIVITY

- **Natural PETase limitation**: too slow and thermolabile for industrial use
- **Optimal temperature**: ~30°C
- **Industrial challenge**: PET crystallinity requires **≥65°C** for polymer chain mobility
- **Solution**: protein engineering + directed evolution
- **Engineered variants**:
  - 100–10,000× higher activity
  - Improved stability at industrial temperatures
- **Deployment**: integrated into robust chassis strains

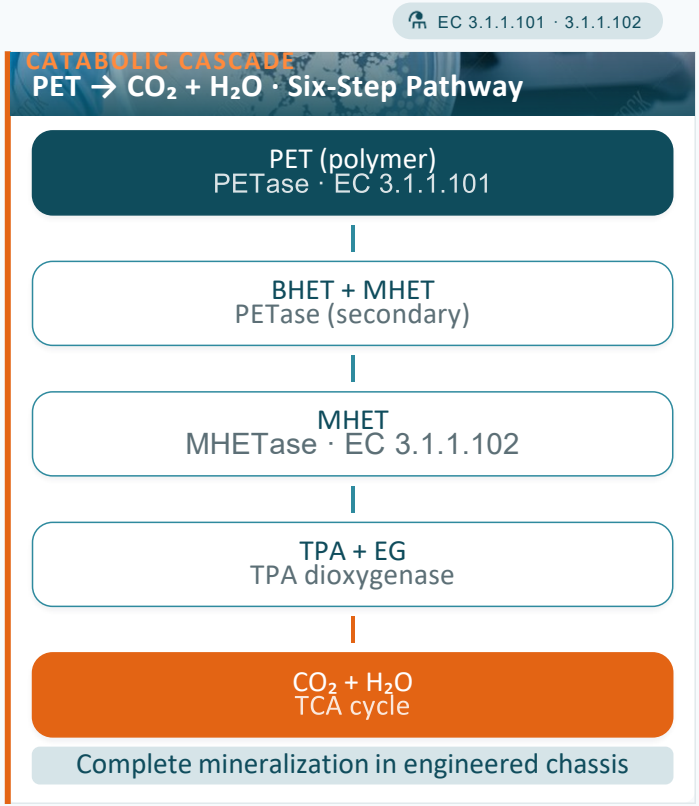


ENZYMOLOGY

PETase & MHETase — Mechanism & Engineering

ENZYMATIC CASCADE

- **Step 1:** PETase → cleaves ester bonds → BHET + MHET
- **Step 2:** MHETase → hydrolyzes MHET → TPA + EG
- **Step 3:** TPA + EG → central metabolism in engineered chassis
- Enables complete PET depolymerization to bioavailable monomers



[64] Austin et al., Nature 2022 · [66] Knott et al., ChemBioChem 2020 · [68] Son et al., ACS Catal 2019 · [69] Huang et al., Microb Cell Fact 2018

SYNTHETIC COMMUNITIES

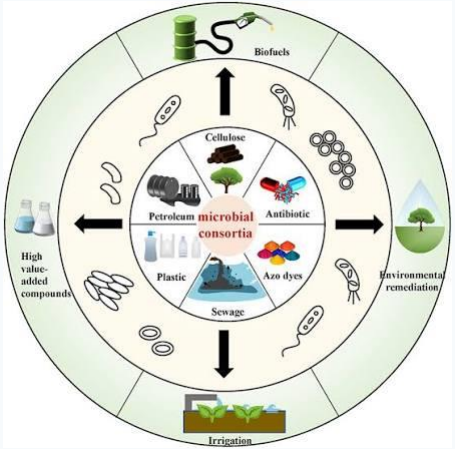
Microbial Consortia for Microplastic Degradation

↑ DIVISION OF LABOR

•Single strains: poor performance on mixed polymers, biofilms, intermediate toxicity

•Engineered consortia: divide labor across specialized functions

•Consortia are more stable and resilient than monocultures



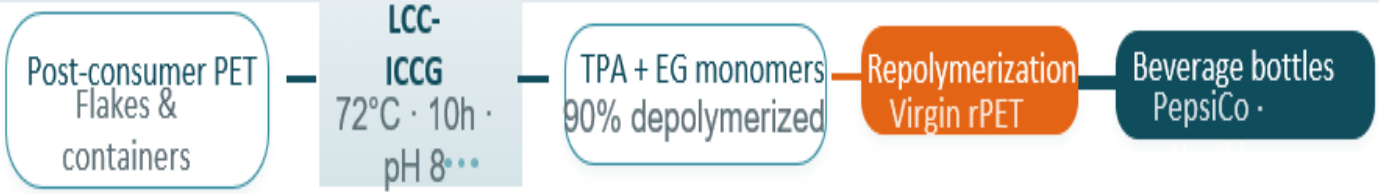
[63] Palm et al., Microb Biotechnol 2019 · [70] Ru et al., Sci Total Environ 2020 · [71] Moharir & Kumar, Environ Sci Pollut Res 2019 · [72] Gambarini et al., Water Res 2021 · [73] Zhang et al., 2021 · [73] Zhang et al., Sci Total Environ 2022 · [74] Montazer et al., Heliyon 2020

Carbios Enzymatic PET Recycling Plant — Longlaville, France, 2024

✦ 50,000 T/YEAR

- **First commercial-scale biological plastic recycling facility**
- **Capacity:** 50,000 t/year
- **Location:** Longlaville
- **Feedstock:** post-consumer PET flakes
- **Enzyme:** engineered LCC-ICCG cutinase (Tm 90°C)
- **Process:** PET → TPA + EG in 10 hours
- **Output:** monomers repolymerized to virgin-quality PET
- **End use:** beverage bottles
- **Offtakes:** PepsiCo, Nestlé Waters
- **Status:** reached nameplate capacity in Q2 2024

Based on Tournier et al., Nature 2020 [65]; Carbios Annual Report 2024 [75]; LCA: Carbios & Quantis, 2023 [76]



✦ INDUSTRIAL PROCESS TRAIN

2011–2017

Discovery phase
LCC isolated from leaf-branch compost metagenome; directed evolution at TBI Toulouse.

2018–2020

Variant optimization
LCC-ICCG (F243I/D238C/S283C/Y127G) achieves 90% depolymerization in 10 h at 72°C.

2020–2022

Pilot demonstration
1 t/day pilot at Clermont-Ferrand validates enzyme immobilization, reactor heat integration.

2021–2023

Plant construction
€230 M Longlaville plant; Carbios IPO; PepsiCo & Nestlé offtakes signed.

2024 Q2

Commercial production
Nameplate capacity 50,000 t/year; virgin-quality rPET for beverage bottles.

Industrial Scale & Future Outlook

From bench strains to 50,000-ton plants — what it takes to scale biology.

SCALE-UP BARRIERS

Scale-up Challenges

⚠ 4 RECURRING CLASSES

Translating laboratory biodegradation to industrial deployment faces **four recurring challenge classes**, regardless of pollutant. Each of pollutant. Each demands engineering — biological or process — beyond the initial isolate.

- **Substrate accessibility**
- **Low kinetics**
- **Stability**
- **Cost**

Adapted from Castillo-Carvajal et al., Water Air Soil Pollut 2024 [6]; Shahid et al., Chem Eng J 2021 [5]; Singh et al., Bioresour Technol 2021 [77]

POLICY · BIOSAFETY

Regulatory & Biosafety Considerations for Engineered Microbes

REGULATORY FRAMEWORK

- Engineered strains are governed by GMO regulations globally
- **EU**: Directives 2001/18/EC and 2009/41/EC
- **US**: EPA TSCA Biotechnology Rule (40 CFR 725)
- **China**: MoST Biosafety Law (2021)
- Must prove safety regarding persistence, dispersal, and HGT before release



Conclusions & Future Outlook

- Bacterial biodegradation has matured to industrial deployment
- Commercial viability proven across pharmaceuticals, hormones, and microplastics
- Key examples: Carbios, Ulu Pandan, Patancheru
- Next decade driven by AI enzyme design, synthetic consortia, and WWTP integration

Outlook based on Singh et al., Bioresour Technol 2021 [77]; Water JPI Strategic Research Agenda 2023 [83]; OECD Biotechnology Regulatory Policy 2024 [84]

Conclusions & Future Outlook

- **AI & Protein Design:** Achieving a 100-fold increase in enzyme activity by 2030.
- **Synthetic Consortia:** Designing 3–5 member teams for toxic intermediate removal, with field-readiness in 5 years.
- **Talent:** A demand for 25,000 full-time equivalent (FTE) hybrid experts per year by 2030.

Antibiotic resistance profiling and valorization of food waste streams to starter culture biomass and exopolysaccharides through fed-batch fermentations

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Microbial degradation and transformation of PPCPs in aquatic environment: A review

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ABSTRACT

The Pharmaceuticals and Personal Care Products (PPCPs) presence at harmful levels has been identified in aquatic ecosystems all over the world. Currently, PPCPs are more common in aquatic regions and have been discovered to be extremely harmful to aquatic creatures. Waste-water treatment facilities are the primary cause of PPCPs pollution in aquatic systems due to their limited treatment as well as the following the release of PPCPs. The degree of PPCPs elimination is primarily determined by the method applied for the remediation. It must be addressed in an eco-friendly manner in order to significantly improve the environmental quality or, at the very least, to prevent the spread as well as effects of toxic pollutants. However, when compared to other methods, environmentally friendly strategies (biological methods) are less expensive and require less energy. Most biological methods under aerobic conditions have been shown to degrade PPCPs effectively. Furthermore, the scientific literature indicates that with the exception of a few extremely hydrophobic substances, biological degradation by microbes is the primary process for the majority of PPCPs compounds. Hence, this review discusses about the optimistic role of microbe concerned in the degradation or transformation of PPCPs into non/less toxic form in the polluted environment. Accordingly, more number of microbial strains has been implicated in the biodegradation/transformation of harmful PPCPs through a process termed as bioremediation and their limitations.

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Review

Engineering of Synthetic Microbial Consortia for Sustainable Management of Wastewater and Polyethylene Terephthalate: A Comprehensive Review

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Abstract

Plastic pollution and wastewater have become the leading environmental concerns due to their harmful effects on human health and pose a severe threat to the biosphere. Polyethylene terephthalate (PET) is one of the most widely used plastics worldwide, but it is resistant to natural degradation. Additionally, the complex pollutants in wastewater demand advanced remediation strategies. Although physicochemical methods are commonly used for PET degradation and wastewater treatment, bioremediation with microorganisms offers a greener and more eco-friendly alternative. This review focuses on the molecular mechanisms and engineering of synthetic microbial consortia (SMC) for the bioremediation of wastewater and PET plastics. It examines the rational design of SMCs, utilizing both bottom-up and top-down methods, and emphasizes the importance of quorum sensing and metabolite cross-feeding in maintaining the stability and functionality of the consortium. Furthermore, the review critically assesses how CRISPR-Cas9 enables precise genome editing for robust pathway engineering and stress resilience, while Machine Learning provides predictive models to optimize consortium composition and function, thereby advancing SMC capabilities for both applications. These developments highlight SMC as a promising, eco-friendly, and efficient biological platform to tackle wastewater challenges and plastic pollution simultaneously.

Keywords: synthetic microbial consortia; wastewater treatment; polyethylene terephthalate

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