Swiss Federal Institute of Technology (EPFL), Lausanne
Ecological Engineering Laboratory (ECOL)
Laboratory for Environmental Biotechnology (LBE)

ÉCOLE POLYTECHNIQUE
FÉDÉRALE DE LAUSANNE

Treatment of micropollutants in municipal wastewater using white-rot fungi



Jonas Margot

Micaela Vargas, Andreu Contijoch, Andrew Barry, Christof Holliger

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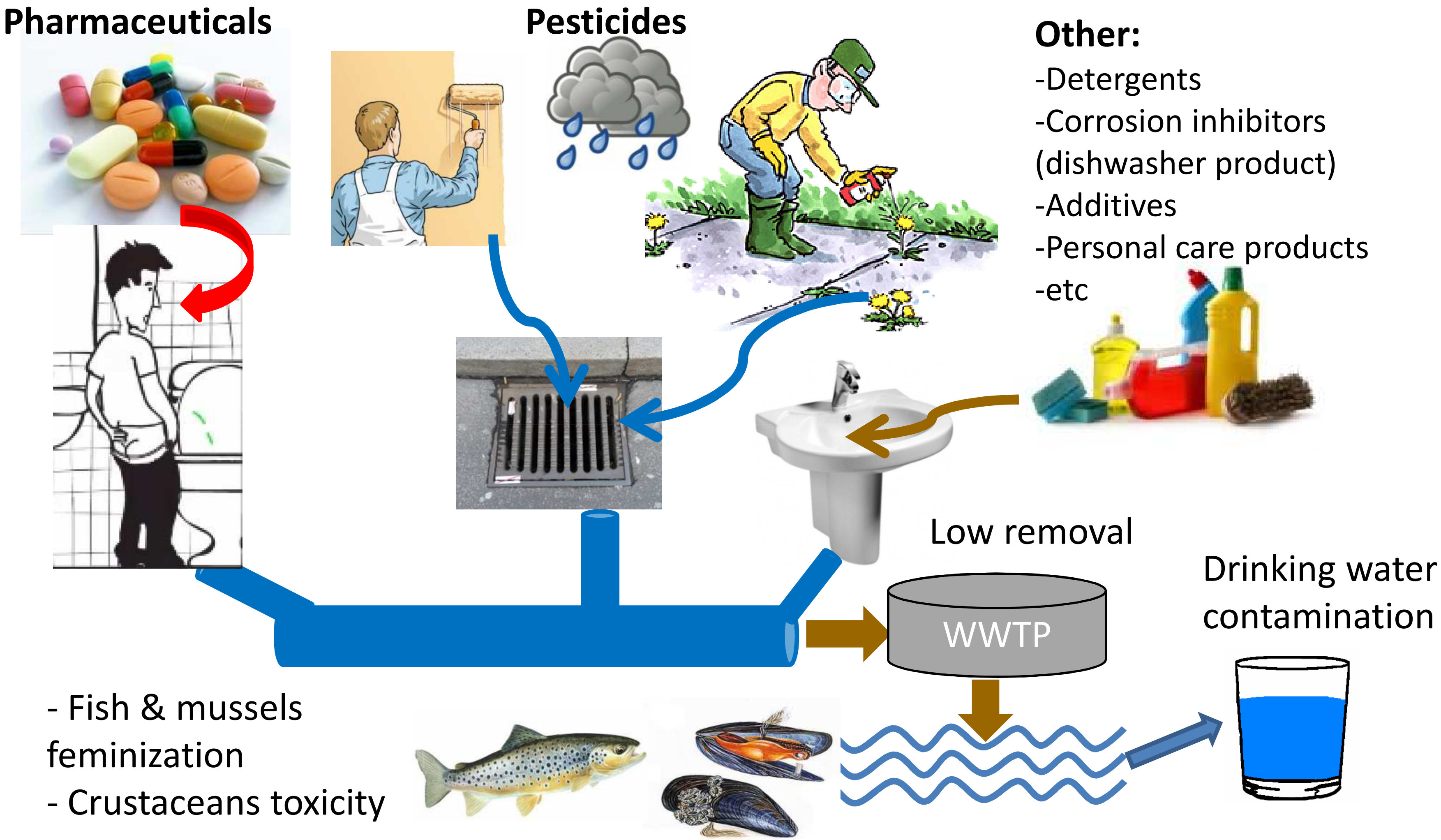
Micropollutants in wastewater

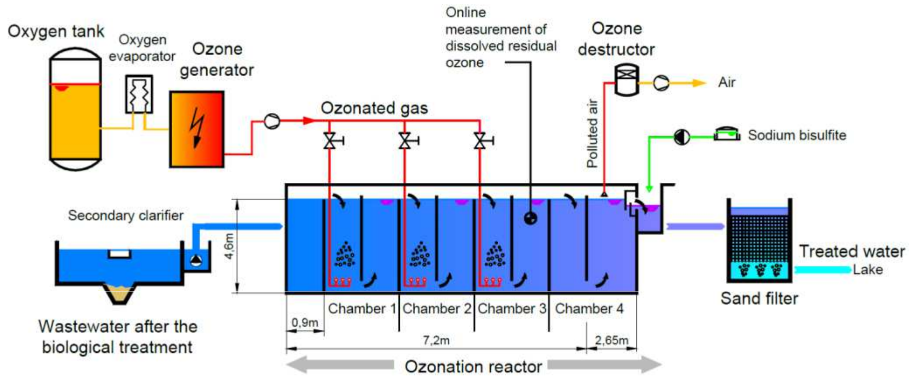


Complementary treatments necessary!

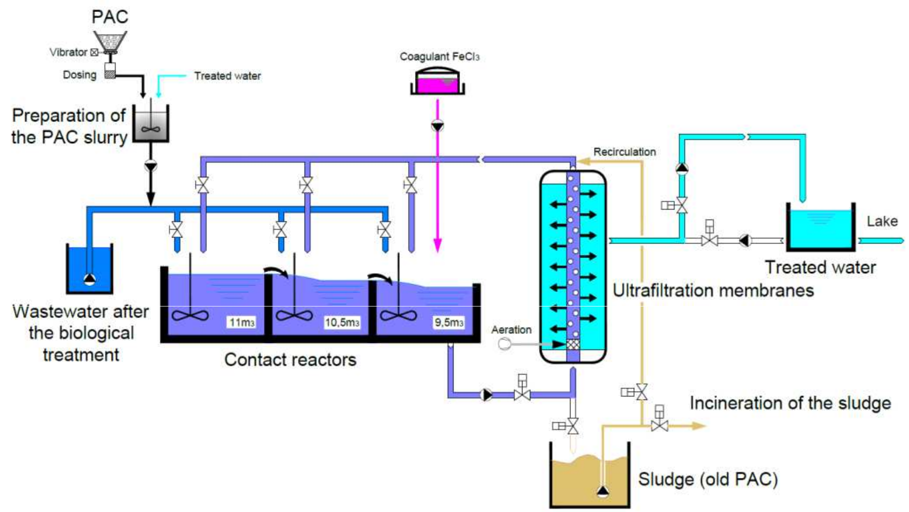
Treatment of micropollutants in wastewater

Solutions already exist!

Ozonation



Activated carbon adsorption



Efficient for a wide range of pollutants but:
- **costly**
- **energy intensive**
- **skill required**

(Margot et al. 2013, Science of the total environment)

Motivations:
Development of a treatment affordable for **small WWTPs** or low income countries, with:
- Low equipment needs
- Low skills and energy requirements

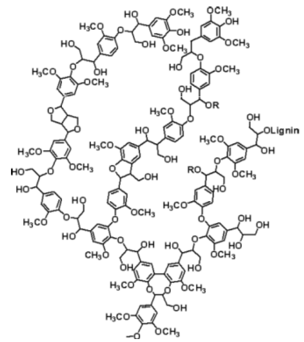
White-rot fungi for micropollutant removal

- Unique capacity to **degrade lignin**, a complex and highly resistant natural molecule
- Very **powerful oxidative exoenzymes**, such as laccase, lignin and manganese peroxidases
- Able to **oxidize several micropollutants** recalcitrant to bacterial degradation

Pleurotus ostreatus

Trametes versicolor

Laccase, peroxidases



Pesticides

Pharmaceuticals

PCBs

PAHs

Synthetic dyes

Munition waste

(Pointing et al. 2001, Yang et al. 2013)

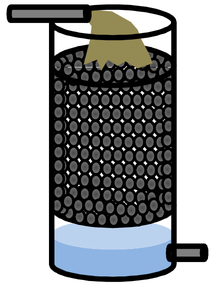
Goals

To **improve biodegradation** of micropollutants in municipal wastewater by **using white-rot fungi**

1st objective:

➢ **Ability** of white-rot fungi to **degrade micropollutants** in WWTP effluents?

Main challenge:
Treatment conditions far from the ideal habitat of the fungus: *how to maintain it in the system?*



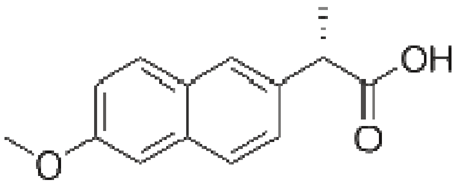
2nd objective:

➢ **Design of a fungal filter** for micropollutant removal in WWTP effluents, which allows **long-term survival** of the fungus

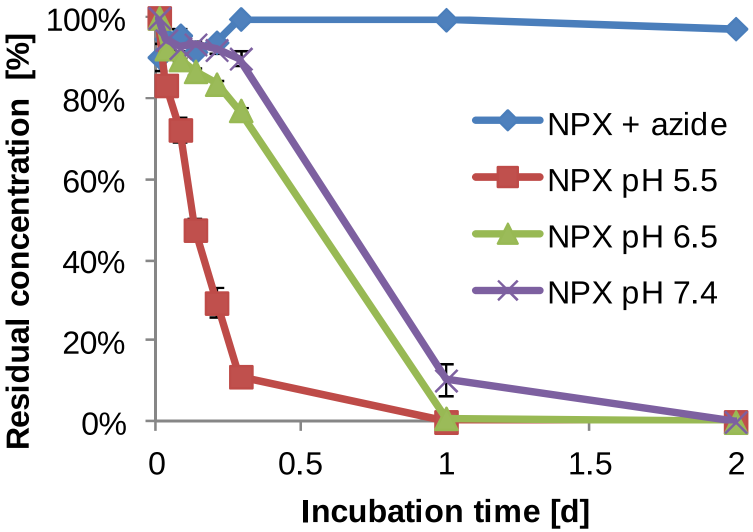
Micropollutant degradation with *T. versicolor* in defined culture medium

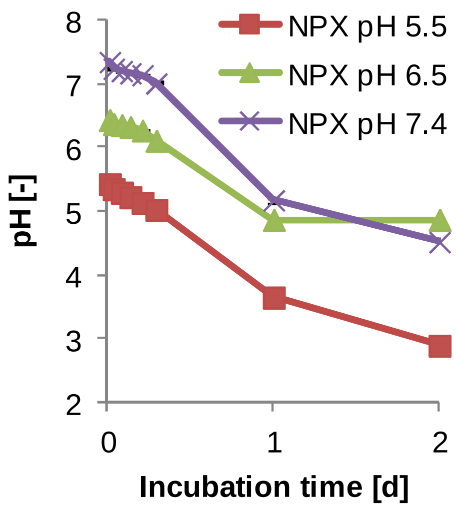
- Batch test with *T. versicolor* pellets (3 g TSS/l)
- In sterile defined culture medium (pH 5.5-7.4, glucose, NH_4)
- High pollutant concentration: 10 mg/l (HPLC-DAD)

Naproxen
(anti-inflammatory drug)



Known to be dregraded by *T. versicolor*
(Marco-Urrea et al. 2010)





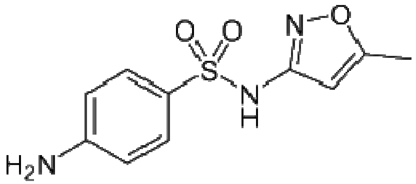
- 100% naproxen removal in less than 24 h
- Faster degradation at lower pH

Acidification due to glucose consumption

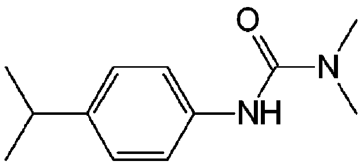
Micropollutant degradation with *T. versicolor* in defined culture medium

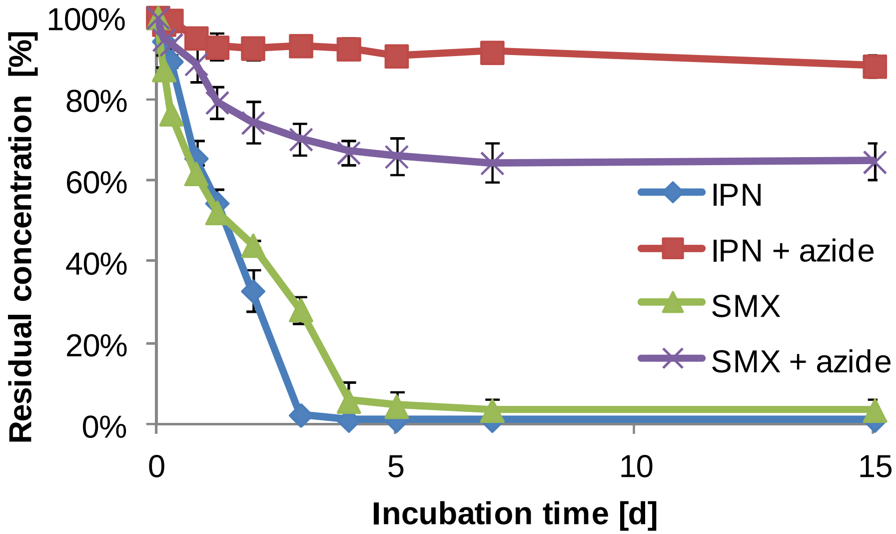
- Batch test with *T. versicolor* pellets (3 g TSS/l)
- In sterile defined culture medium (pH 5.5, glucose, NH_4)
- High pollutant concentration: 10 mg/l (HPLC-DAD)

Sulfamethoxazole (antibiotic)



Isoproturon (herbicide)



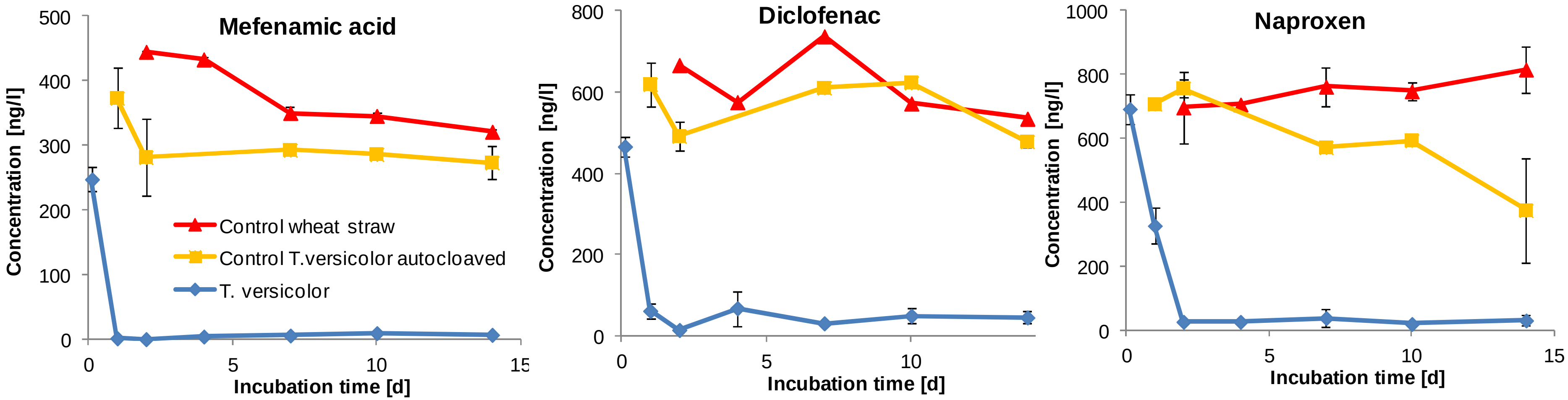


- >90% isoproturon and sulfamethoxazole removal in less than 4 days

Promising but **far from real conditions** (glucose, pure culture, etc)

Micropollutant degradation with *T. versicolor* in treated wastewater

- Batch test with *T. versicolor*
- In biologically treated municipal wastewater (filtrated at 0.45 µm, not sterile) (not spiked)
- Wheat straw as sole substrate
- 20 pollutants analysed by SPE UPLC-MS/MS

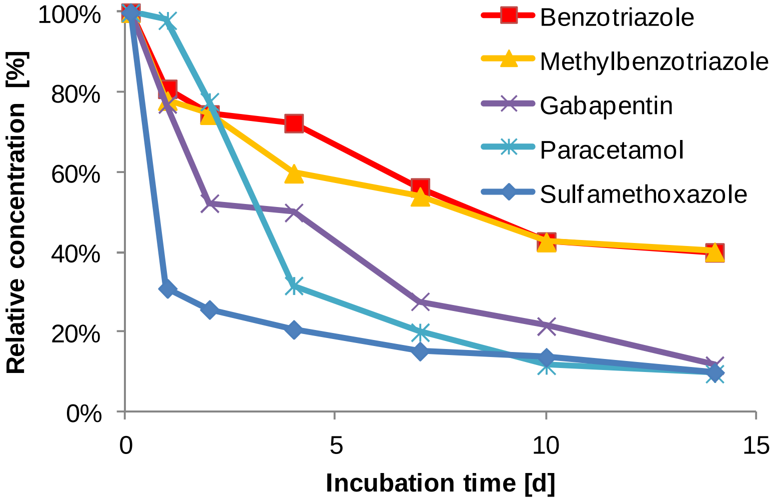


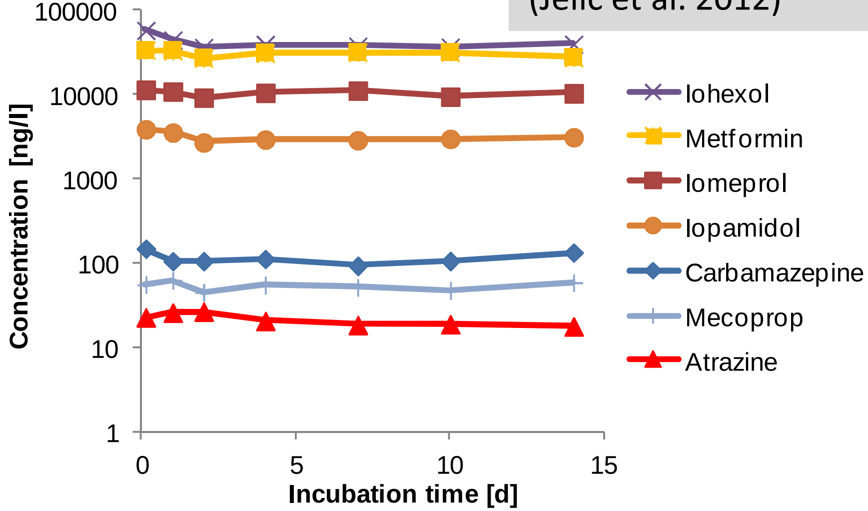
- >90% DFC and MFA removal in less than 24 h
- >90% Naproxen removal in less than 2 days

In **real wastewater**, at **real pH** (7.9) and **real concentration**!

Micropollutant degradation with *T. versicolor* in treated wastewater

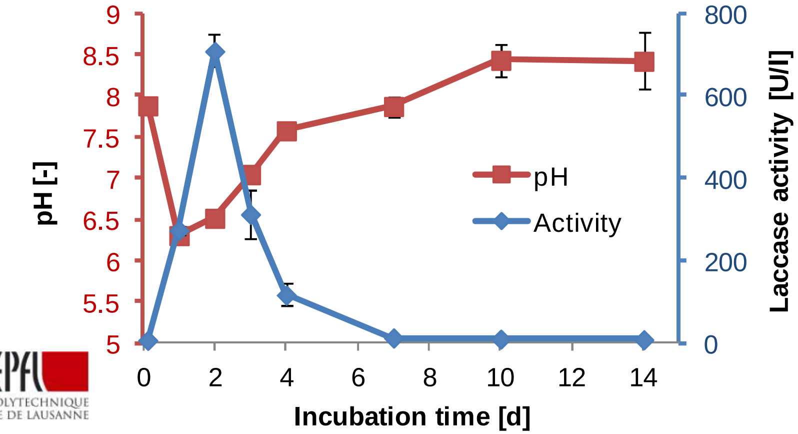
Carbamazepine can be degraded by *T. versicolor* (Jelic et al. 2012)





Removed also in the controls!
Adsorption, bacterial of fungal degradation???

Not removed!
- Recalcitrant to *T. versicolor*?
- Lack of easy carbon source?
- Too low fungal activity? Too high pH?



Feasible in real wastewater! But:
- ➢Very low fungal activity after a few days of submerged culture
- ➢ Short survival time in submerged culture in non sterile conditions

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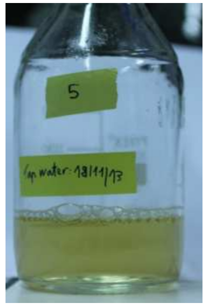
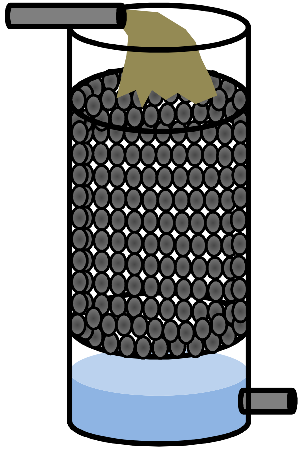
Development of a fungal filter

Woodchips as sole **substrate and support** in the filter
- ➢ Conditions closer to their **natural habitat**
- ➢ No other substrate addition
- ➢ Limitation of bacterial competition (not able to degrade wood)

Drawback: leaking of soluble wood components in the water at the beginning (yellowish color)





Water trickles through the filter

Dry beech woodchips colonized by fungal mycelium

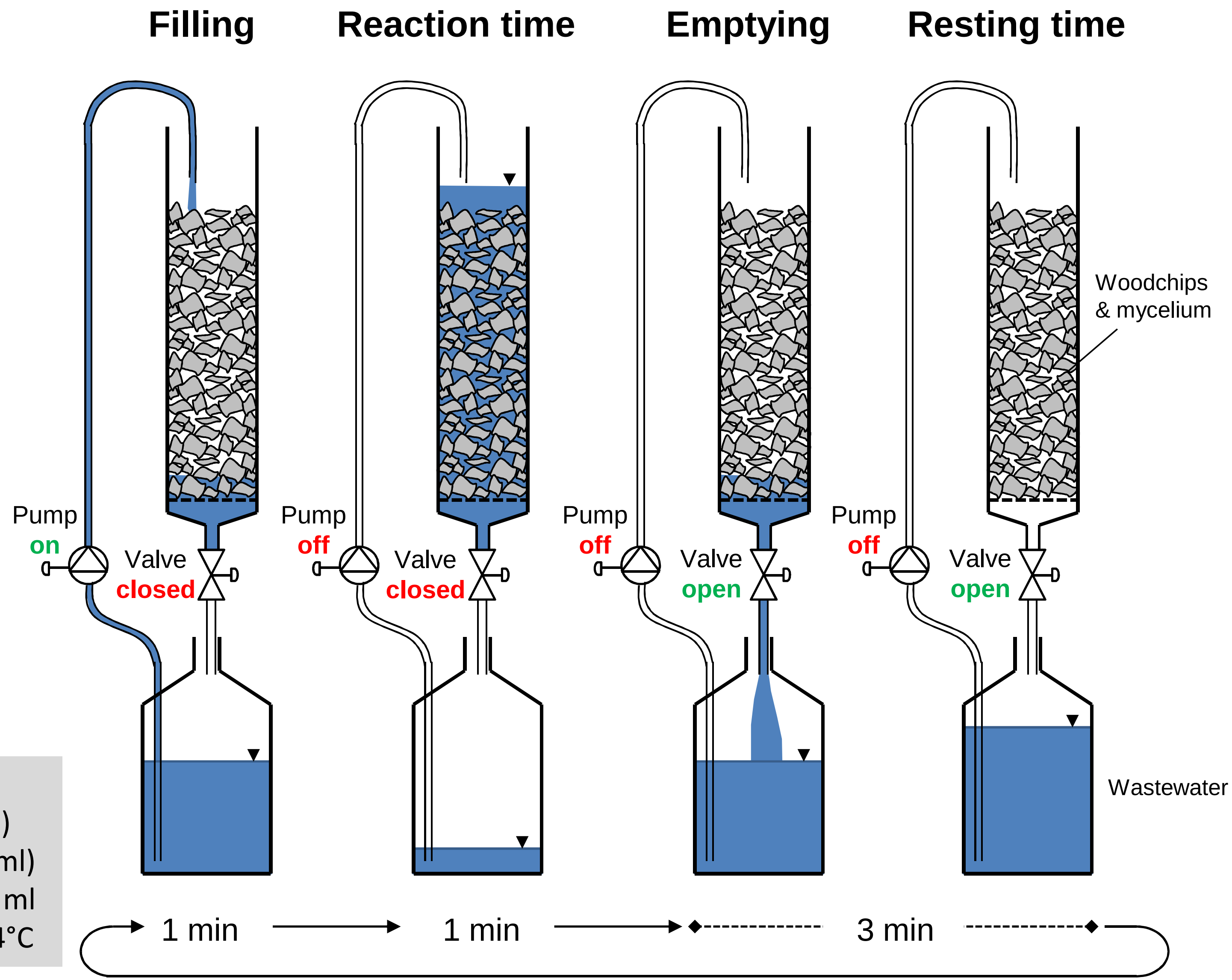
Operation of the fungal filter

Not in sterile condition!

- **Sequential batch** operation with recirculation (5 min cycle)
- Water discharged and **renewed every 2-3 d** (150 ml)

Monitoring:
- Laccase activity
- pH
- Pollutant degradation

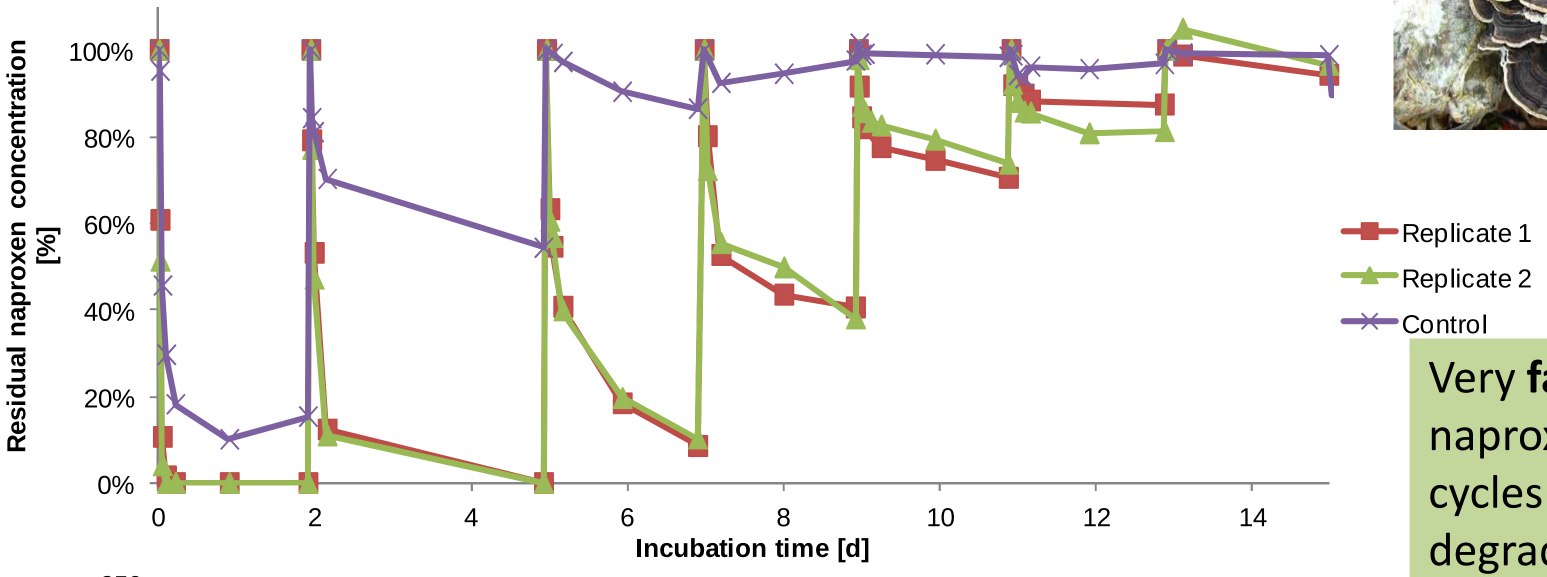
Characterisitcs:
Column: 25 x 3.6 cm (253 ml)
Woodchips: 21 g (d.w) (157 ml)
Volume pumped/cycle: 66.7 ml
Ambient temperature: 20-24°C



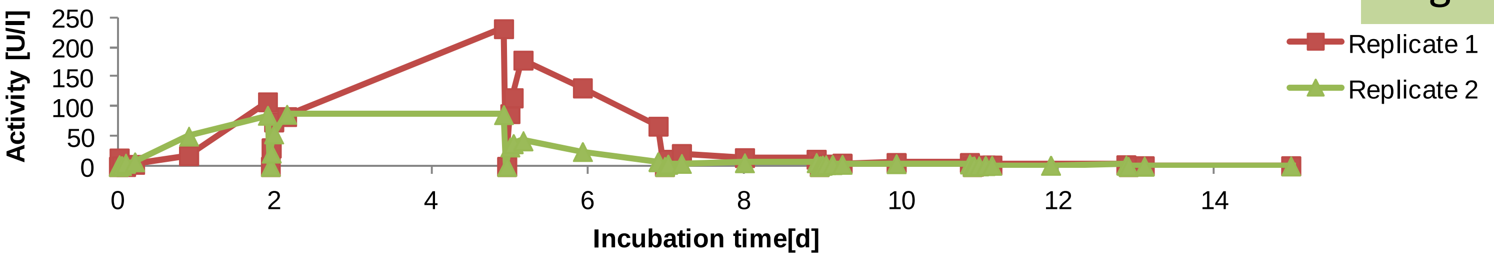
Naproxen in **tap water** (10 mg/l)

Efficiency of the fungal filter

Trametes versicolor



Very **fast removal** of naproxen in the first cycles: adsorption + degradation

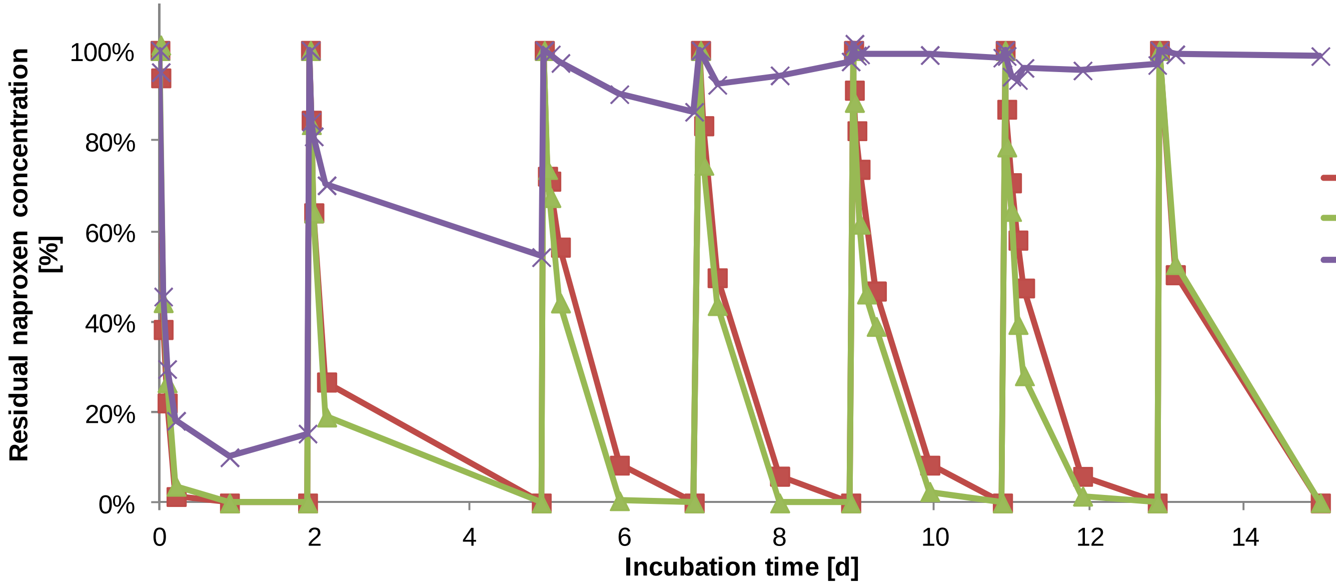


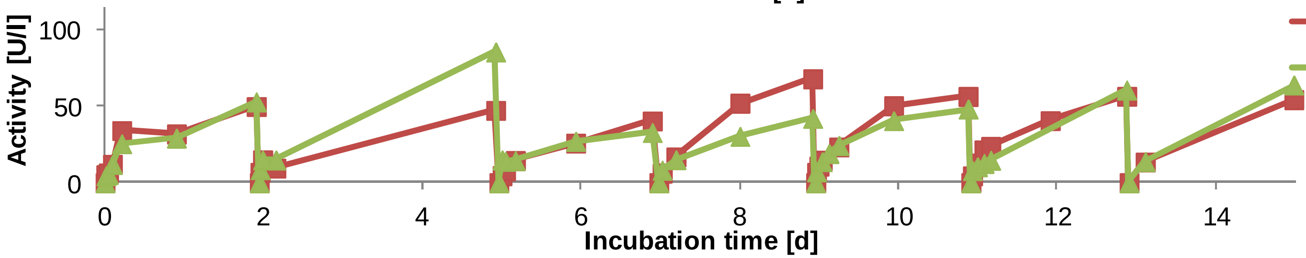
Very **low fungal activity** after **one week** → strong decrease in naproxen removal efficiency!

Trametes versicolor **not surviving long** in this system!
→ Need for **more competitive** fungus!

Efficiency of the fungal filter

Pleurotus ostreatus





Two removal processes:
- **Fast adsorption** (reversible), followed by
- **Biodegradation**

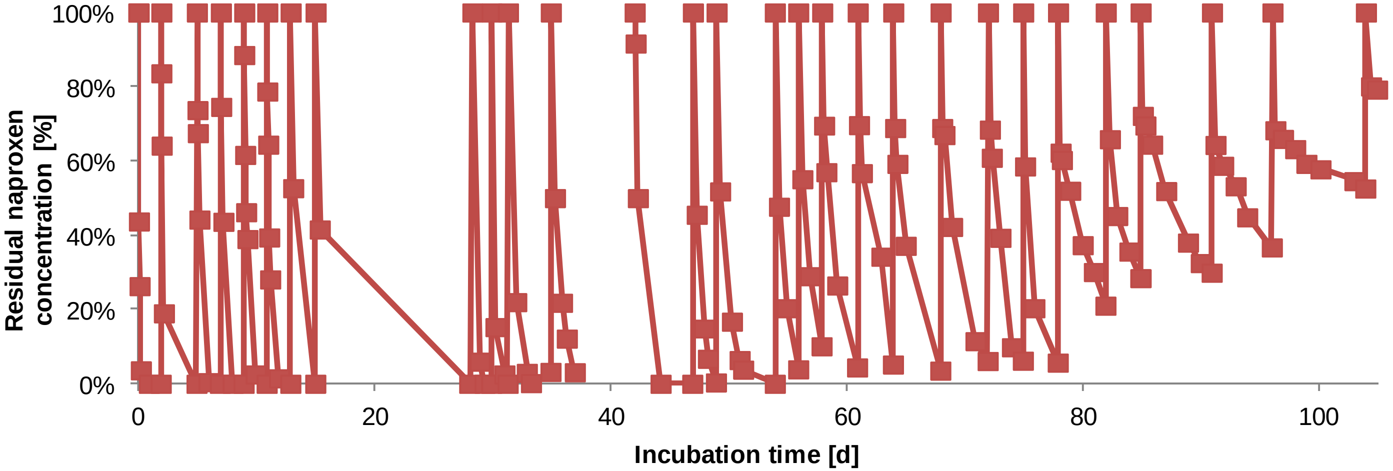
Good efficiency during 2 weeks!
Pleurotus ostratus **able to survive** in the filter

How long does it work?

Efficiency of the fungal filter

Naproxen in **tap water** (10 mg/l)

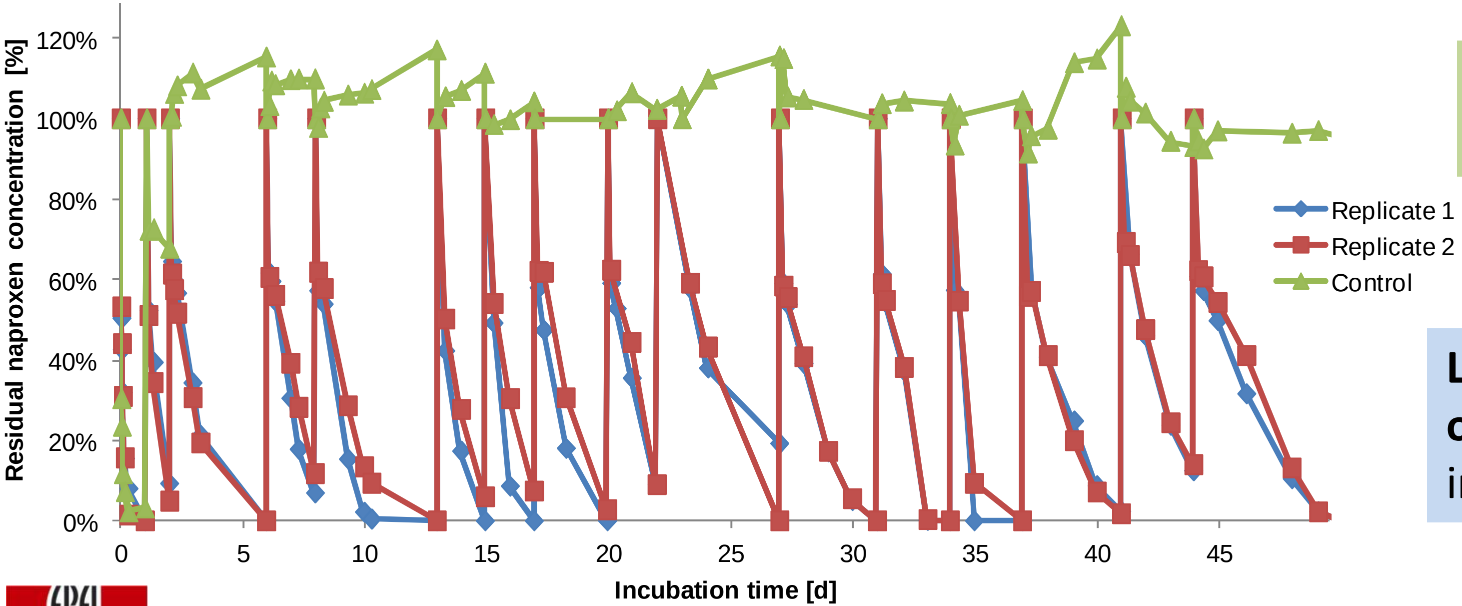
Pleurotus ostreatus



Good efficiency during 80 d! (>90% NPX removal in 48h)

Long term survival (> 3 months) without any other substrate addition!

Naproxen (10 mg/l) in **treated wastewater** (DOC: 11 mg/l, TN: 15 mg/l, 10^5 CFU/ml)



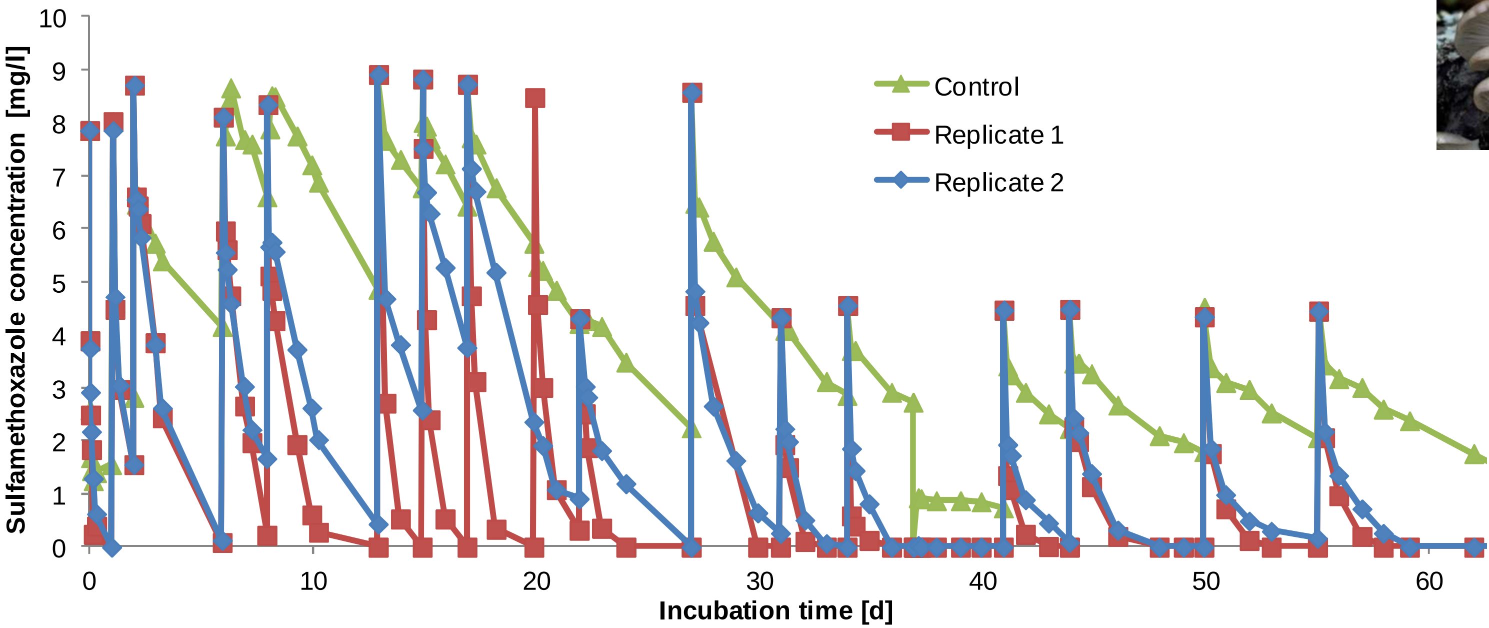
Good efficiency during > 50 d!

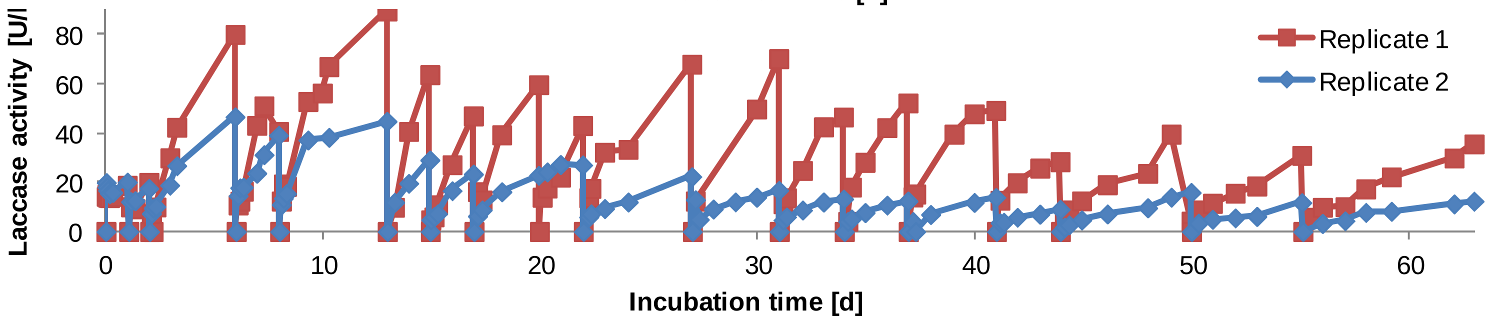
Long term operation feasible in real conditions!

Sulfamethoxazole
in **tap water**
(5-10 mg/l)

Efficiency of the fungal filter

Pleurotus ostreatus





Good efficiency during >60 d!
(>90% SMX removal in 24-48h)

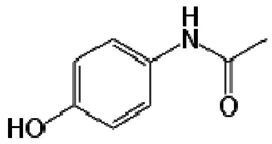
Slow **degradation in the control**

Long term activity
(> 2 months)

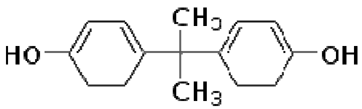
Removal efficiency of other micropollutants? → still need to be tested!

Potential of extracellular laccase produced in the filter

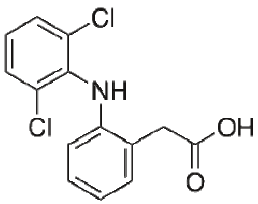
T. versicolor filter



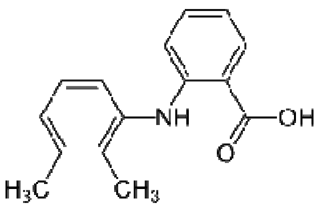
Paracetamol



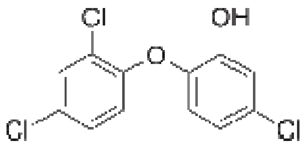
Bisphenol A



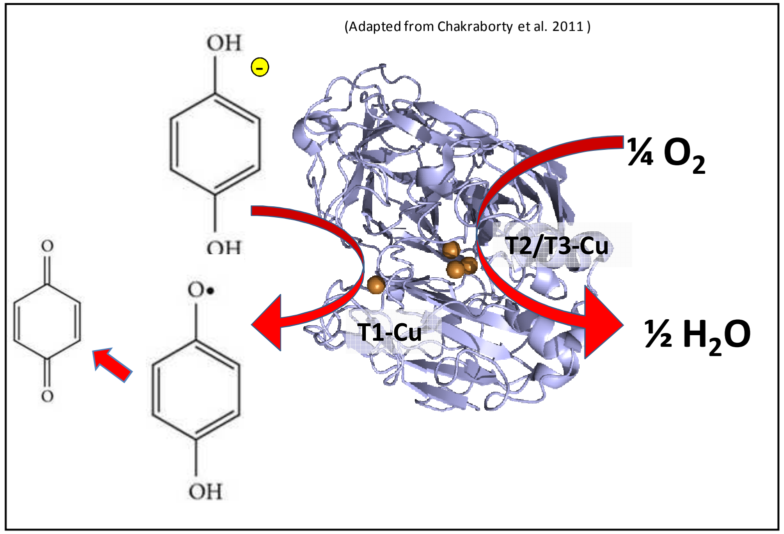
Diclofenac



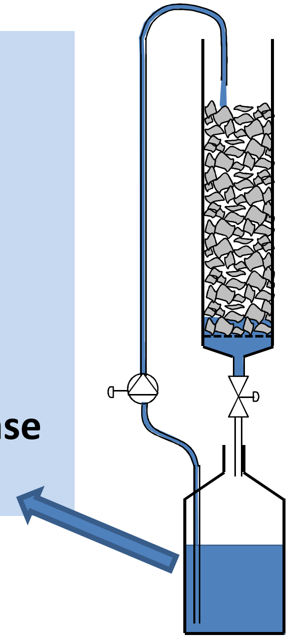
Mefenamic acid

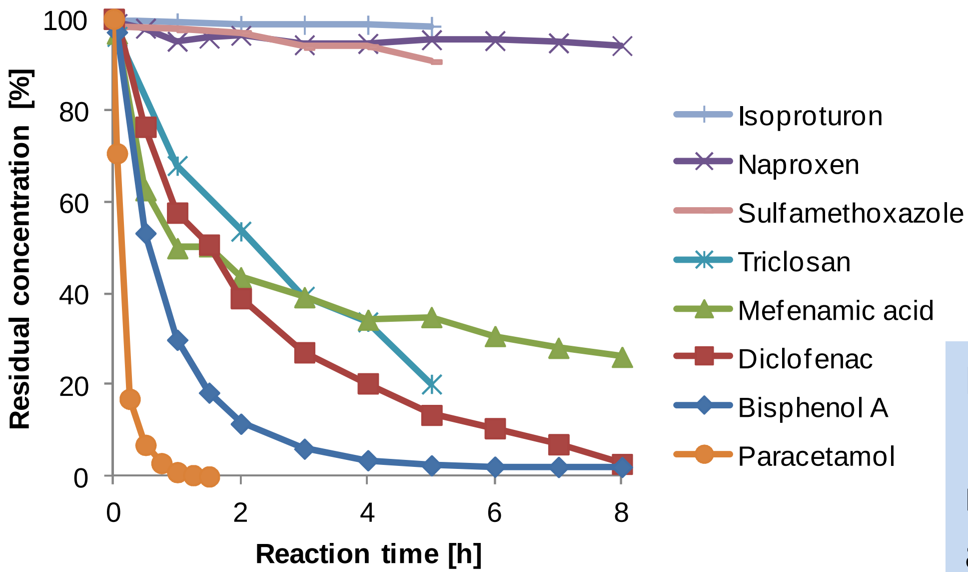


Triclosan



Water collected from the filter:
- **filtrated** at 0.2 µm
- **spiked with pollutants** at 10 mg/l
-pH 5, **high laccase activity**: 934 U/l



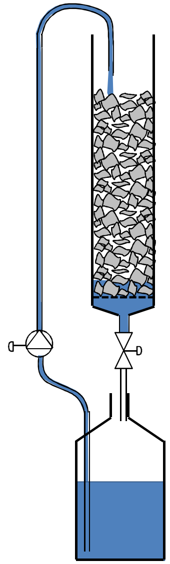


✓ **>80%** removal of most pollutants in **5h**

❖ **No degradation** of IPN, NPX and SMX by laccase alone!

Extracellular laccase
→may increase the **removal of phenolic - aniline pollutants**

Conclusions



- **Fungal treatment** with *P. ostreatus* grown on wood substrates: **promising solution** to improve micropollutants removal in wastewater
  - Many pollutants potentially degraded
  - Cheap and widely available substrate (woodchips)
  - No need to add other external carbon source, simple system, low maintenance (only pumps and valves)
  - Long term operation (>3 months) without renewing the substrate
- Still **research to perform** before the development a fungal trickling filter suitable for small WWTPs
  - Efficiency on a **wider range of pollutants**? Toxicity removal?
  - **Design optimization** to reduce (i) **treatment time** (now >24-48h), (ii) **electricity consumption** for recirculation (now up to 0.35 kWh/m^3), (iii) **space needed** for the filter (now up to 0.5-1 m^2/capita)
  - Strategy for wood inoculation and replacement (long term operation)
  - Reduction of input of organic compounds leaking from the wood


Thank you for your attention!