Extracellular enzymatic activity of intact heterotrophic biofilms is decreased upon exposure to TiO_2 nanoparticle and environmentally realistic UV radiation

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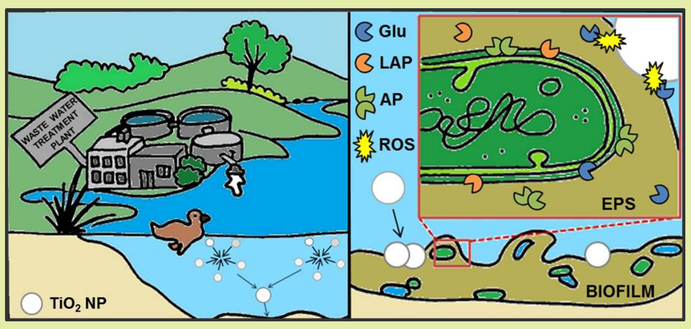
Introduction

Background

The growing use of TiO_2 nanoparticles (TiO_2 NP) will inevitably result in an increased environmental release of these materials. Depending on the water chemistry, TiO_2 NP entering aquatic environments are prone to agglomeration and sedimentation[1], which results in increased exposure of biofilms to TiO_2 NP.[2] Since TiO_2 NP are highly photoactive, one possible mechanism by which TiO_2 NP may affect biofilm function is through oxidation by reactive oxygen species (ROS). In the biofilm matrix, extracellular enzymes represent a likely target of primary TiO_2 NP interaction. These extracellular enzymes are of great importance for biofilm fitness because they mediate the uptake of nutrients from water.



Aims

In this study the influence of different TiO_2 NP with and without UV radiation on the activity of extracellular enzymes of freshwater biofilms was investigated

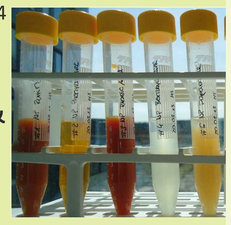
Three enzymes of essential macronutrient cycling were examined:
β – Glucosidase: hydrolyses β-linked polysaccharides (*Carbon cycling)*
L-Leucine-Aminopeptidase: cleaves peptides/amino acids *(Nitrogen cycling)*
Alkaline Phosphatase: breaks organophosphoric esters (*Phosphor cycling*)

- To test the effect of TiO_2 NP on enzyme activity in absence of a biofilm matrix, pure alkaline phosphatase isolated from *Escherichia coli* was investigated
- TiO_2 NP were coated with different substances, which mimic the variety of engineered and naturally occurring surface modifications
- TiO_2 NP were characterized for their behavior in freshwater including size, zeta potential, absorbance, sedimentation and photocatalytic activity

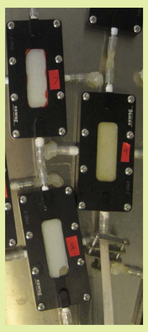
Materials & Methods

Types of TiO_2 NP

- Flame synthesized TiO_2 NP with 1 % Nb from Empa (Dübendorf, Switzerland)[3]
- Degussa P-25 from Evonik (Essen, Germany)
- Solution synth. TiO_2 NP surface coated with molecules having an enediol moiety[4]
- Coatings included acids/bases, nonpolar organic, environmentally & biologically relevant and molecules with different chromophores



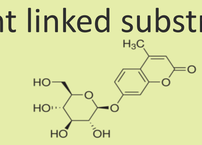
Colonization of biofilms



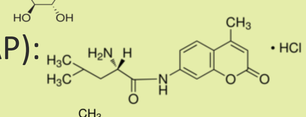
- Heterotrophic biofilms were colonized in a flow-through system (flow rate = 2 cm s^{-1}) at 15°C over a membrane support (cellulose acetate)
- For cultivation, water from the Chriesbach river (small stream in Dübendorf, Switzerland) was filtered through a 1.5 µm filter
- Biofilm was sampled after 3 weeks of colonization

Extracellular Enzyme Assay

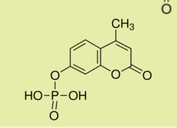
- Effects of TiO_2 NP on extracellular enzyme activities were assayed by determining the utilization rate of fluorescent linked substrate.
- β-Glucosidase (Glu):
  (MUF - β-D-glucopyranoside)



- L-Leucine-Aminopeptidase (LAP):
  (L-Leucine-AMC)

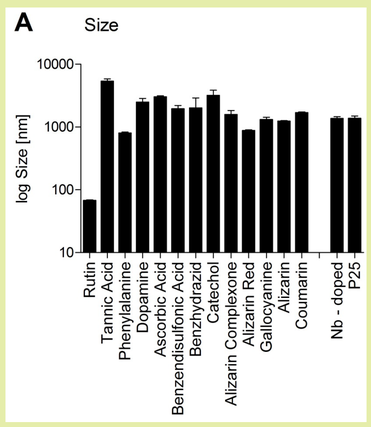


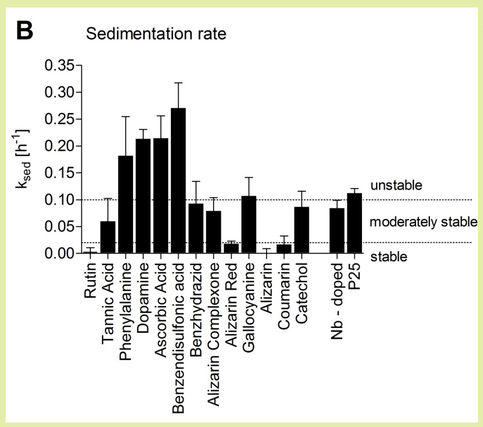
- Alkaline Phosphatase (AP):
  (MUF phosphate)



Characterization of TiO_2 NP

Size & Sedimentation

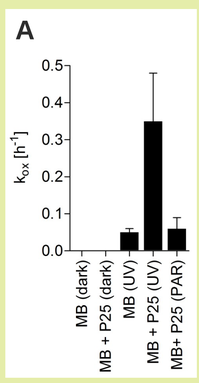


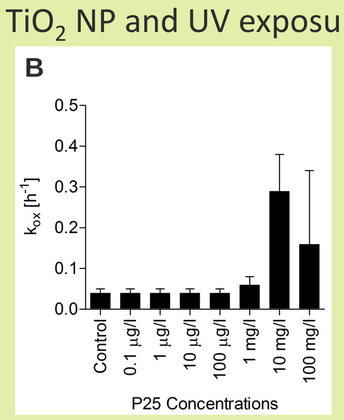


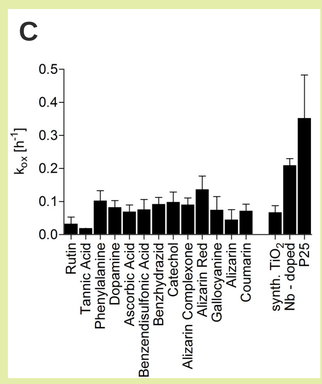
- TiO_2 NP suspended in Chriesbach river water agglomerated to 890 – 5400 nm, with the exception of Rutin coated TiO_2 NP (70 nm)
- Dry particle size for P25 was 28 nm, for solution synth. TiO_2 NP 5 – 10 nm (determined by XRD and TEM)
- Sedimentation, affecting the fate of NP in the water body, was altered by the surface coating leading to unstable, moderately stable and stable suspensions

Photocatalytic activity

- Photocatalytic activity was assayed by methylene blue (MB) degradation, which was increased under TiO_2 NP and UV exposure. In the dark no effect was found.



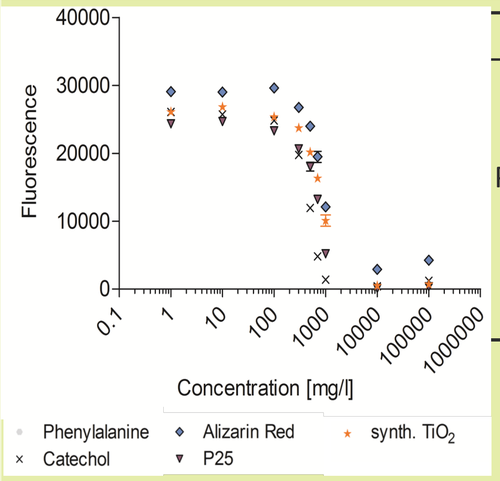




- MB oxidation is dependent on the TiO_2 NP concentration, with highest effect found at 10 mg l^{-1}
- Photocatalytic activity was affected by the surface coating of the TiO_2 NP

Effect on enzymatic activity

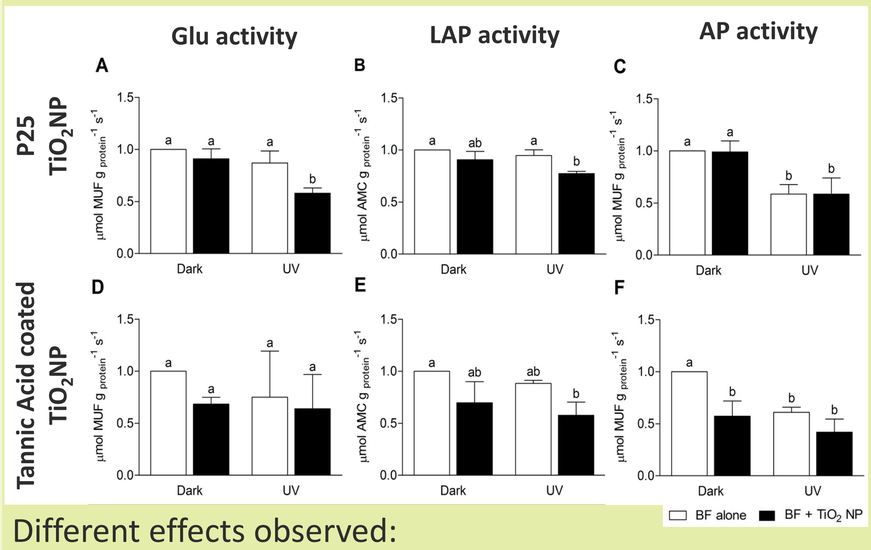
Isolated Alkaline Phosphatase from *E. Coli*



Particles	IC_{50} [µgl^{-1}] (95% CI)
Rutin – coated TiO_2 NP	847 (833 – 861)
Tannic Acid – coated TiO_2 NP	ND
Phenylalanine – coated TiO_2 NP	806 (755 – 839)
Alizarin Red – coated TiO_2 NP	1245 (630 – 2457)
Catechol – coated TiO_2 NP	495 (477 – 513)
P25 TiO_2 NP	686 (664 – 708)

- Similar IC_{50} values for different TiO_2 NP
- Oxidation through ROS unlikely to be the dominant mechanism of inactivation
- However UV radiation was a significant factor: in the dark, only minor effects

Enzymes from intact heterotrophic biofilms



- The three enzymes responded differently to the exposure (Glu > LAP > AP) possibly due to different localization patterns
- P25 TiO_2 NP was most effective in reducing enzyme activity

Different effects observed:

1. TiO_2 NP + UV affects enzyme activity (P25 TiO_2 NP on Glu & LAP, alizarin red coated TiO_2 NP on Glu, tannic acid coated TiO_2 NP on LAP)

2. TiO_2 NP in the dark affect enzyme activity (tannic acid coated TiO_2 NP on LAP and AP)

3. UV alone affects enzyme activity (on Glu and AP)

Summary & Conclusion

- Activity of β-Glucosidase and L-Leucin Aminopeptidase of intact heterotrophic biofilms is decreased by exposure to TiO_2 NP and UV radiation
- Exposure reflects environmental scenarios in shallow freshwater streams
- Loss of enzymatic function likely as a consequence of oxidation through ROS
- Tannic acid coated TiO_2 NP seem to have specific mode of inhibition
- Effect on diluted phosphatase was not correlated to ROS but UV-dependent
- The intact extracellular matrix of the biofilm reduces negative effects

Overall, the decrease in enzymatic activity may adversely affect nutrient acquisition in the biofilm and might have implications for nutrient cycling and degradation of pollutants in the aquatic environment

References

[1] Keller *et al.*, *Environmental Science and Technology*, **2010**, 44, 1962-7
[2] Ferry *et al. , Nature Nanotechnology,* **2009**, 4, 441-4
[3] Michalow *et al., Environmental science and pollution research international,* **2012**, 19, 3696-708
[4] Kotsokechagia *et al., Langmuir,* **2008**, 24, 6988-6997

Acknowledgment

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