A new test method to determine the bioaccumulation of manufactured nanomaterials in filtering organisms (Bivalvia) using the freshwater mussel *Corbicula fluminea*

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Introduction

The identification and scientific assessment of compounds that bioaccumulate in organisms and biomagnify in food webs play a key role within the PBT -assessment. The bioaccumulation potential of compounds is commonly expressed as bioconcentration factors (BCF) determined in flow-through studies with fish according to OECD 305. Comparable studies with manufactured nanomaterials (MNMs) are difficult to carry out due to the lack of suitable test systems that allow a permanent and constant exposition of the compounds. MNMs tend to sediment in water and are supposed to be primarily taken up by benthic species in aquatic ecosystems. Different studies have shown that mussels are able to ingest and to incorporate MNMs suspended in water. However, existing standardized test methods to investigate the bioaccumulation of substances in mussels have only been developed and optimized for soluble, non-particulate substances.[1&2] Therefore, an alternative test concept was developed allowing to investigate the bioaccumulation of MNMs in mussels under flow-through conditions. First studies were carried out with the freshwater mussel *Corbicula fluminea* using titanium dioxide and silver MNMs (NM 105 & NM 300K). In addition, silver nitrate was testes to compare the accumulation and elimination of ionic and nanoparticulate silver.

Testsystem & Methods

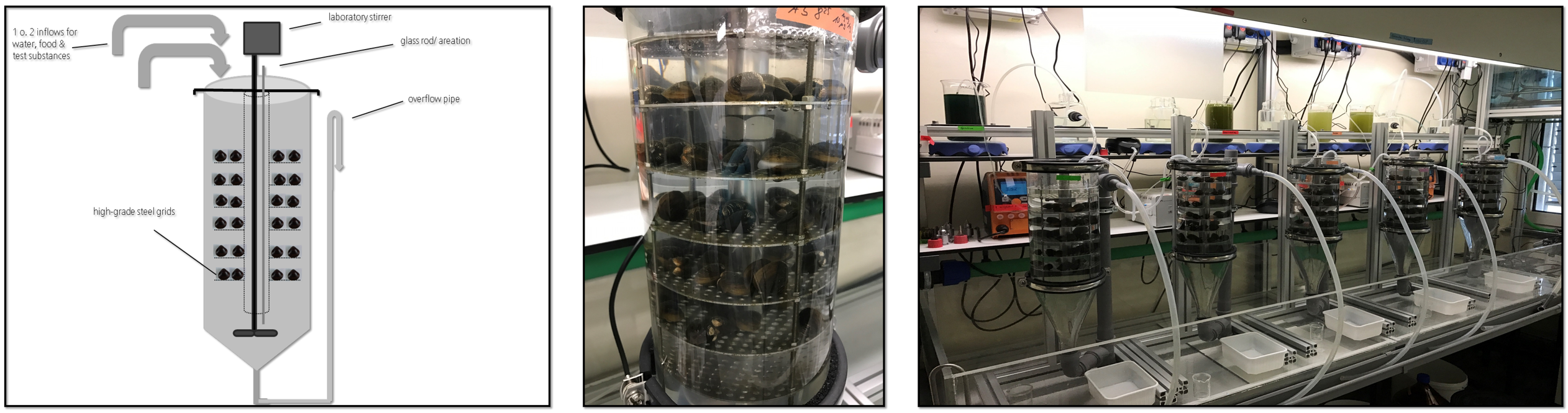

Fig. 1: Test system to determine the bioaccumulation of manufactured nanomaterials in filtering organisms (Bivalvia)

- Flow-trough system with a test volume of 8 L and a flow rate of 4 L per hour
- 170 animals per unit
- Continuous addition of $AgNO_3$ / AgNP - suspension and aeration
- Continuous, minimalized addition of food suspension (milled stinging nettle)
- Uptake phase 96h (NM 300K) /144h ($AgNO_3$)
- Sample collection: triplicate samples consisting of 2-3 animals each
- Samples (water & tissue) for total Ag content analysis are digested by microwave following addition of aqua regia and measured by ICP-MS and ICP-OES
- Further tissue samples digested by proteinase K and analysed using (AF4-coupled-) SP-ICP-MS

Results

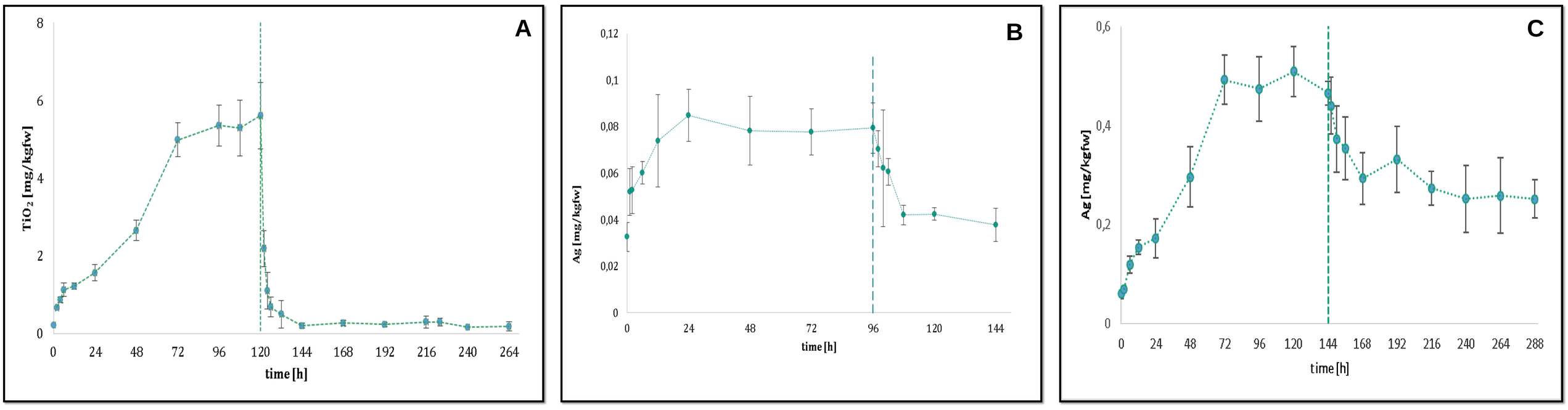

Fig. 2: Ag content in mussel tissue: **A)** NM 105 exposure (0,589 µgTiO_2/L) for 120h, BAF_{ss}= 9022; **B)** NM 300K exposure (0,624µg Ag/L) for 96h, BAF_{ss}= 128; **C)** $AgNO_3$ exposure for 144h (0,682 µg Ag/L), BAF_{ss}= 711

Conclusions

- The BAF study design with *Corbicula fluminea* allowed to elucidate the uptake and elimination of TiO_2 from TiO_2NPs (NM 105) as well as Ag from AgNPs (NM 300K) and $AgNO_3$
- The bioaccumulation of TiO_2NPs could result from physical sorption/accumulation e.g. in the gastrointestinal tract, instead of real bioaccumulation; further investigations are in progress
- Bioavailability and bioaccumulation of Ag at comparable total Ag concenrations is depending on the exposure form (nanoparticluar or dissolved ions)
- The bioaccumulation of Ag from NM 300K may mainly result from Ag^+-ions; further investigations are in progress
- Results of $AgNO_3$ exposure indicates the presence of a silver sink for ionic silver in the tissue of *Corbicula fluminea*

References:
[1] American Society for Testing and Materials, “ASTM E1022 - 94(2013) Standard Guide for Conducting Bioconcentration Tests with Fishes and Saltwater Bivalve Mollusks,” 2003.
[2] United States Environmental Protection Agency, Ecological Effects Test Guidelines OPPTS 850.1710 Oyster BCF, 1996

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Different studies have shown that mussels are able to ingest and to incorporate MNMs suspended in water. However, existing standardised test methods to investigate the bioaccumulation of substances in mussels have been developed and optimized for soluble, non-particulate substances. Therefore, an alternative test concept was developed allowing to investigate the bioaccumulation of MNMs in mussels under flow-through conditions.

First studies were carried out with the freshwater mussel *Corbicula fluminea*. By using silver MNMs (NM300K) and silver nitrate we were able to compare the accumulation and elimination of ionic and nanoparticulate silver. Mussels were exposed for a period of 4 - 6 days. In both cases steady state concentrations of total silver in the mussel tissue were reached within 24 hours. The quantification of the total content of silver in water and tissue samples was carried out by ICP-MS or ICP-OES. The determined tissue and water concentrations were used to determine bioaccumulation factors for both test items. In a further study the bioaccumulation of a titanium dioxide nanomaterial (NM 105) was tested. The studies have shown that the new test system is suitable to investigate the bioaccumulation of MNMs.