Report by UWI doctoral researcher Niranjan Mukherjee (H4)

Project number: H4

First and last name of doctoral researcher: **Niranjan Mukherjee**

(Working) title of doctoral project: **Redox gradients in natural and technical systems: Population structure and physiological properties**

Name of supervisors: Prof. Ulrich Szewzyk (TUB), Dr. Burga Braun (TUB), Prof. Lorenz Adrian (UFZ and TUB), Dr. Peter Casper (IGB)

2. Description of doctoral project:

State of research

A redox gradient in natural and technical urban water systems refers to the difference in reduction/oxidation potential between different depths of the urban water system in question. The hyporheic zone is one such interface which derives steep redox gradients from active mixing of groundwater and surface water. The hyporheic zone is the saturated transition zone between surface water and groundwater bodies that derives specific physical and biogeochemical characteristics to provide a habitat for obligate and facultative anaerobic and aerobic species (Krause et al. 2009).

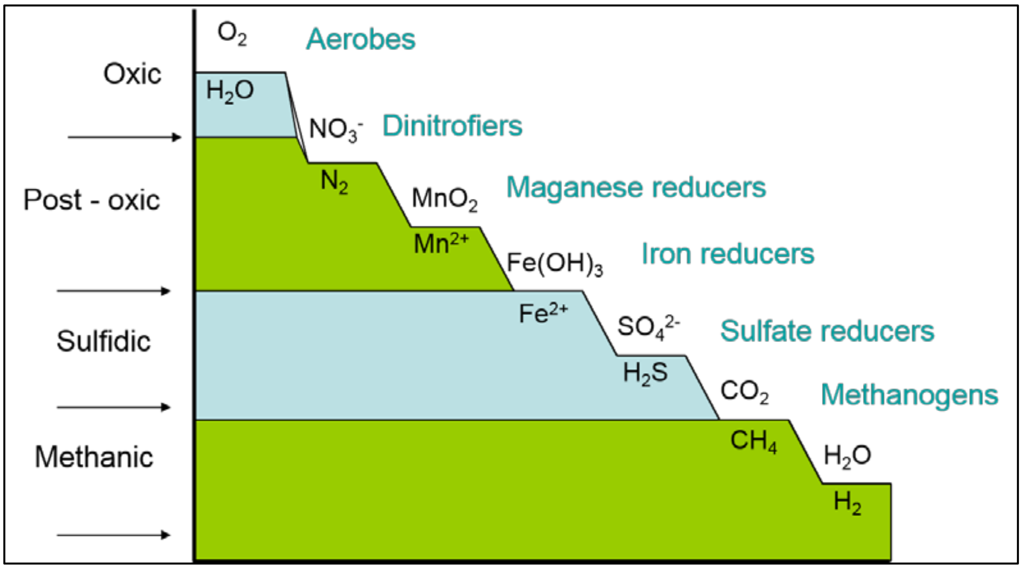

*Figure 1: The Redox Ladder. The redox-couples are shown on each stair-step, where the most energy is gained at the top step and the least at the bottom step (*https://www.uvm.edu *2018)*

Redox gradients drive life processes. The transfer of electrons between oxidants and reactants is harnessed as the battery, the source of metabolic energy for organisms. Natural waters can be classified into three based on redox properties. Oxic waters are those that contain measurable dissolved oxygen. Suboxic waters lack measurable oxygen or sulphide but do contain significant dissolved iron (> ~0.1 mg/L). Reducing or anoxic waters are those that contain both dissolved iron and sulphide. (*https://www.uvm.edu* 2018)
There has been progress in the past decades in the interdisciplinary exchange of information between hydrological, hydrogeological, biogeochemical, hydroecological and microbiological research on the hyporheic zone (Krause et al. 2009), but there are still gaps regarding the role of biodiversity in hyporheic processes. The role of microbial communities has been identified as one of five major issues regarding the role biodiversity plays in the processes at groundwater–surface water interfaces. (Marmonier et al. 2012)

The major part of the microbial populations in these zones is considered to live attached to inert surfaces as a biofilm. The hyporheic zone has been identified to be of importance in maintaining the ecological function of running water and a natural reactor taking the main responsibility for the impressive self-purification capacity of rivers (Lewandowski et al. 2011; Sterba et al. 1992). Microbial diversity within a hyporheic zone has been shown to influence the degradation rates of certain pollutants in a river (European Hyporheic Forum 2018). Moreover, redox gradients have been shown to shape microbial community structure and relative abundance of specific taxa (Lipson et al. 2015).

Motivation and research idea

Organic trace pollutants and recalcitrant pharmaceuticals are found in urban fresh water systems seen to originate from effluent of wastewater treatment plants (WWTP). This has then been shown to affect the ecology of the fresh water system and can also have potential impacts on human health (Snyder et al. 2009). The hyporheic zone has been assessed for its self-purification capacity. The microbial populations attached to inert sediment surfaces as microbial mats (Storey et al. 1999) have been considered crucial in the degradation or the transformation of trace pollutants and recalcitrant pharmaceuticals.

The microbial communities and their diversity are yet to be assessed in these zones. Since the microbial diversity is likely to vary based on the redox conditions of the different gradients, the research idea is to firstly establish the different redox gradients in a lab-based sediment column reactor. Subsequently, the microbial communities in the different gradient zones should be analyzed.

The potential of these biofilm communities to degrade recalcitrant pharmaceuticals in urban water systems is to be evaluated. The knowledge of mechanistic pathways for reduction/transformation of contaminants is limited. The aim is to determine if the pollutant-degrading organisms can live on trace pollutants alone or if they require co-substrates or partner organisms to maintain their energy metabolism. Significant interactions like syntrophy and co-metabolism between organisms for determining degradation efficiency can then be identified.

Scientific project relevance
It is of special interest to what extent the pharmaceuticals are transformed to compounds which can be integrated in the pool of natural humic substances. Responsible organisms are then planned to be detected and identified in both laboratory reactors and natural biofilms.

Identifying the microorganisms, substrates and metabolic pathways of degradation/transformation can then also be useful in enriching the microbial community used in the treatment of wastewater such that treated WWTP-effluent being discharged into urban freshwater streams no longer contain these trace pollutants. Of course, time required for degradation would be important to consider in this case, among other parameters.

Research demands
Hyporheic zones providing a wide range of redox gradients in the river Erpe would first have to be identified. It would then be crucial to identify and characterize the gradient zones before the diversity of the microbial communities is evaluated. This would then make it possible to determine the substrate/co-substrate available and being potentially used by the pollutant-degrading organisms.

Selection of the iron-oxidising bacteria, identified for degradation in UWI project N1 (1st cohort), would be important during the setup of lab-scale reactors to study the degradation/transformation of selected contaminants. Transformation products would have to be identified to be able to determine possible metabolic pathways and significant interactions between microorganisms.

Objectives
Some of the main objectives of the project are:

1. Identification of different redox gradients present in the hyporheic zone of the river Erpe in Berlin
2. Assessment of microbial diversity present in the different redox gradients
3. Evaluation of the degradation potential of contaminants by iron-oxidizing bacteria in a hyporheic zone
4. Identification of substrate/co-substrates for the pollutant-degrading organisms
5. Determination of metabolic pathways for the degradation/transformation of selected contaminants
6. Assessing impact of WWTP-effluent on microbial diversity of hyporheic zone

Work programme incl. proposed research methods

WP 1 ***'Setup of sediment column from river Erpe'***

This work package involves the setting up of a suitable sediment column reactor from known sites in the river Erpe which can then be used to establish subsequently formed redox gradients. The sediments collected from the river are also going to be used for the characterization of microbial communities using Illumina Next Generation Sequencing (NGS) followed by predictive functional profiling.

The first step of this work package is going to involve the identification of appropriate sites in the river Erpe by collaborating with projects in the Leibniz Institute of Freshwater Ecology and Inland Fisheries. Sites with thick sediments containing diverse redox gradients are likely to be selected. Sediment samples will be used for total genomic DNA extraction for MiSeq Illumina Sequencing. The sequencing would allow for predictive functional profiling of microbial communities using 16S rRNA marker gene sequences (Langille et al. 2013). This would help us in identifying not just the microbial communities that are present in the hyporheic sediments but also associate the likely function with the organisms and point out the potential pollutant-degrading organisms.

Furthermore, Real-Time Polymerase Chain Reaction (qPCR) and Fluorescence In-Situ Hybridisation (FISH) would be used for (i) quantification of rRNA gene copies and (ii) spatial localization of the specific bacteria inhabiting the microbial mats with respect to the hyporheic sediments by using a confocal laser scanning microscope (CLSM).

This is likely to add further clues to the physicochemical characterisation of the sediment layers and possibly the steps in the metabolic pathway of degradation/transformation.

Enough time in the order of a year (or longer based on requirements) would be given to the stabilization of redox gradients in the hyporheic sediments of the sediment column setup in the lab. This work programme would thus take up to a minimum of a year. Lab experiments mentioned above would be conducted simultaneously. Characterization of redox gradients and identification of microbial communities are then likely to be published.

WP 2 ***'Bioreactor setup for the determination of metabolic pathway'***

The second stage of the project would start after the stabilization and determination of the different redox gradients in the hyporheic sediment layers established in the sediment column. Suitable iron-oxidizing bacteria identified for degradation in the N1 project of the UWI would then be introduced into the hyporheic sediments. It would be important to identify bacteria which can survive different oxic/anoxic conditions of the sediments. The sediment column would be embedded into an agar block containing Fe (II) as a nutrient source for the iron-oxidizing bacteria.

The bioreactors would be spiked with a suitable pollutant such as diclofenac (a recalcitrant pharmaceutical) or iopromide (an X-ray contrast medium). Here, it can be useful to note that both compounds and their transformation products have been well-studied in general, excluding their degradation by iron-oxidizing bacteria. This would make it easier to identify transformation productions. This then combined with predictive functional profiling would allow for the determination of metabolic pathway of degradation/transformation of the microorganisms. Significant interactions between organisms for determining degradation efficiency are going to be identified here. The transformation products are also going to be assessed for the extent to which they could be integrated in the natural pool of humic substances.

As previously, for the detection and quantification of responsible microorganisms, FISH and qPCR methods are going to be employed. CLSM would be used to determine spatial organization of growing microorganisms in relation to the sediments. Approximately the second year of the PhD has been planned for this package. The following results of microbial pathway determination, substrates/co-substrates and responsible microorganisms is then likely to be published.

WP 3 ***'Comparison of hyporheic zone sediments upstream and downstream of WWTP effluent'***

The final stage of the doctoral thesis includes the analysis of microbial community diversity in two specially identified sites in the river Erpe, one upstream of the WWTP effluent and one downstream. Aim of this work package will be to find the impact of the effluent on the microbial community diversity.

This would be interesting because the WWTP effluent contains trace amounts of pollutants such as pharmaceuticals. The aim is to see if these organic trace compounds influence the type and quantity of microbial populations in the sediments of the hyporheic zone.

This analysis would be done with the data that would be collected in the first work package, where we are already performing NGS, qPCR, FISH and CLSM for microbial biofilms living in hyporheic sediments of the river Erpe.

Changes in the microbial populations will indicate the sensitivity of the hyporheic zone to receiving WWTP effluent and its potential ability to reduce incoming contaminants. This would further strengthen the claim of the hyporheic zone's self-purification capacity. This work package is planned for the third year of the doctoral thesis and the comparison of the microbial communities in the two locations and its subsequent implications will be submitted for publication.

Collaborations

Collaborations are being discussed with a couple of UWI doctoral candidates and a collegiate. Microbial communities of sewer biofilms will be analysed in collaboration with UWI collegiate Adrian Augustyniak and Micaela Pacheco Fernandez (S1, 2nd cohort). Birgit Müller (H1, 2nd cohort) and I could possibly work on characterizing the redox gradients of the Erpe river which is common to both of our projects. Yuki Sorgler (H3, 2nd cohort) who investigates the behaviour of iodinated X-ray contrast media during bank filtration and drinking water treatment could help in the identification of transformation products of iopromide which can then help me establish microbial metabolic pathways. Microbial communities involved in bank filtration processes in urban lakes will be analysed for Anna-Lena Kronsbein (F1, 2nd cohort).

References

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3. Comments on the qualification programme and supervision strategy:

Participation in the following Research Training Group events:

1. Core courses
    - Urban interface processes – fluxes, transport, interactions (3 ECTS)
    - Urban freshwater ecology (3 ECTS)
    - Modelling and measuring concepts of interface processes (3 ECTS)
2. Elective courses
    - Mikrobiologie (3 ECTS)
    - Schadstoffabbau (3 ECTS)
    - Introduction to experimental design and basic statistics (3 ECTS)

Research stays or internships at other research institutions both at home and abroad:

Omics2view, Kiel, Germany: 3-day internship planned to learn the analysis of results of MiSeq Illumina Sequencing for predictive functional profiling