Report by UWI doctoral researcher Marcella Nega (N1)

Project number: N1

First and last name of doctoral researcher: **Marcella Nega**

(Working) title of doctoral project: **Degradation of recalcitrant organic trace pollutants**

Name of supervisors: Prof. Dr. rer. nat. Ulrich Szewzyk (TUB), Dr. rer. nat. Burga Braun (TUB), Dr. rer. nat. Peter Casper (IGB), Prof. Dr. rer. nat. Mark Gessner (IGB & TUB)

2. Description of doctoral project and research results achieved to date:

Motivation

Emergent organic compounds, such as pharmaceuticals, and others, are only partially retained in wastewater treatment plants (WWTP). These substances are detected in a nanogram per litre range, as micropollutants, in surface water and pose a danger to communities in the waters (Heberer, 2002). In fact, micropollutants can enter the water system as biologically active substances, and concern exists about their presence and influence on living organisms. The fate of these compounds in the environment continues to be an essential object in research. Furthermore, there is still a lack of knowledge about the role of the microbial degradation in the fate of these compounds. In conclusion, this project aims the investigation of two research issues: (i) analysing the microbial communities and further parameters in the river Panke in Berlin, which is impacted by a WWTP, (ii) and proving the biodegradation potential of iron/manganese oxidising bacteria at the laboratory scale.

There are data on the occurrence of around 160 different active pharmaceutical ingredients in aquatic environments (Kümmerer, 2010). Accordingly, updated European framework legislation promotes the reduction of micropollutants. A few years back, diclofenac (DCF) has been included in the updated compounds watch list of the European Union (Directive 2013/39/EU) (Schröder et al., 2016). This nonsteroidal anti-inflammatory drug is widely used, and it belongs to the most frequently detected pharmaceutical in WWTP effluents (Schröder et al., 2016; Verlicchi et al., 2012). In previous studies, it was monitored that DCF was detected in concentrations up to 1200 ng L^{-1} in surface waters in Germany, and in Berlin too (Heberer et al., 2002; Ternes, 1998).

The target of this dissertation project is to show the biodegradation ability of iron/manganese oxidising bacteria for specific pharmaceutical compounds, as diclofenac, carbamazepine and gabapentin (ionizable, non-ionizable compounds). Besides this, the impact of effluents from a WWTP is identified by measuring micropollutant concentrations and structures of microbial communities in aquatic environments. Therefore, bacterial strains from environmental and technical systems were screened for the degradation potential of carbamazepine, diclofenac and gabapentin. The target compounds were quantified by the Liquid Chromatography – Tandem Mass Spectrometry analysis (LC-MS/MS) and the Mn(II) oxidation of the bacteria in parallel experiments. Moreover, the study in the river Panke was conducted by measuring the physicochemical conditions, micropollutant concentrations and MiSeq Illumina sequencing targeting the variable region (V3-V4) of bacterial 16S ribosomal (rRNA) gene.

Current State of Work

The current state of the work is described for the two part-projects.

(i) Analysing the microbial communities and further parameters in the river Panke in Berlin

The sampling sites in this study were located along the river Panke in the north-eastern suburban area Berlin-Karow. Due to capacity problems of the wastewater treatment plant Schönerlinde, the Panke received the discharge of clear water through the Buchholzer Graben (Fig. 1). Some of the physicochemical parameters are shown in Table 1. Significant differences were observed between seasons and sampling sites (oxygen (P2, P3), dissolved organic carbon (DOC) (P1-P3) ($p < 0.05$)). The lower O_2 concentrations just after the inflow of the Buchholzer Graben indicate a O_2 consumption mainly due to higher DOC concentrations. These higher DOC concentrations from the sampling site P1 indicate an influence of the inflow of the WWTP Schönerlinde. Whereas, due to heavy rain events before our campaign in June 2017 a fluctuation in precipitation and runoff has led to an increase of DOC levels (Table 1). Subsequently increased nitrate concentrations of up to 38.33 mg L^{-1} were observed in the water samples downstream of the WWTP effluent and can be due to agriculture uses. Especially, the highest micropollutant concentrations were found in pore water samples at the sampling site P1. Gabapentin (1.6 µg L^{-1}), diclofenac (1.3 µg L^{-1}), clofibric acid (0.25 µg L^{-1}) and bezafibrate (0.10 µg L^{-1}) were the pharmaceuticals with the highest detected concentrations and had shown increasing values from P3 up to the sampling site after the Buchholzer Graben P1 (Fig. 2). It has to be noted that these findings indicate an effect of the effluent of a wastewater treatment plant in the river Panke.

Table 1: Some physicochemical parameters of water samples obtained from the river Panke at three sampling sites during two seasons (autumn 2016 and summer 2017) and analysed with the two-way ANOVA analysis.

Sampling sites	P1	P2	P3	P1	P2	P3
Date	12.10.16	12.10.16	13.10.16	26.06.17	27.06.17	26.06.17
Number of samples	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6
Dissolved oxygen (mg L^{-1})	4.68 ± 0.18	8.33 ± 0.34*	8.08 ± 0.07*	4.9 ± 1.03	7.49 ± 0.13*	6.45 ± 0.33*
DOC (mg L^{-1})	6.55 ± 1.62*	3.76 ± 0.61*	2.01 ± 1.49*	8.82 ± 1.11*	7.44 ± 0.22*	4.4 ± 0.53*
Nitrate (NO_3^-) (mg L^{-1})	38.33 ± 11.79*	3 ± 0	2.67 ± 0.47	30 ± 0*	3.33 ± 0.47	1.33 ± 0.47

* Values denoted with an asterisk (*) are significantly different (Tukey test, $p < 0.05$)

Figure 1: Sampling sites located in the river Panke (P1-P3) and the discharge from WWTP enters in the Panke through the Buchholzer Graben (orange label)

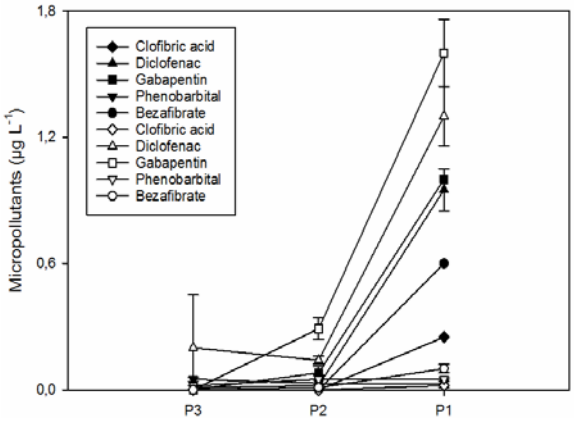

Figure 2: Some micropollutants concentrations in pore water samples along the three sampling sites in the river Panke. The campaign in 2016 is shown in black legend symbols, whereas the campaign in 2017 is shown in white legend symbols.

The investigation in the river Panke included as a central part the survey of the microbial community at the desoxy ribonucleic acid (DNA) and ribonucleic acid (RNA) level with next-generation sequencing (NGS), to interpret the impact of the river condition on the microbial patterns.

In the first place, differences in the phylogenetic structure were detected at the phylum level (Fig. 3). Obtained results showed that sediment samples were dominated by Proteobacteria at every sampling site (Fig. 3, just P1 and P2 are shown). Proteobacteria are a ubiquitous and metabolically diverse group of Gram-negative bacteria that are frequently detected in freshwater sediments. More particularly, it was obvious that Nitrospirae species were more abundant at the site P1 as before the entrance of the WWTP discharge through the Buchholzer Graben. These results and further upcoming results from the collected sequence data will result in the first journal paper which is currently under preparation.

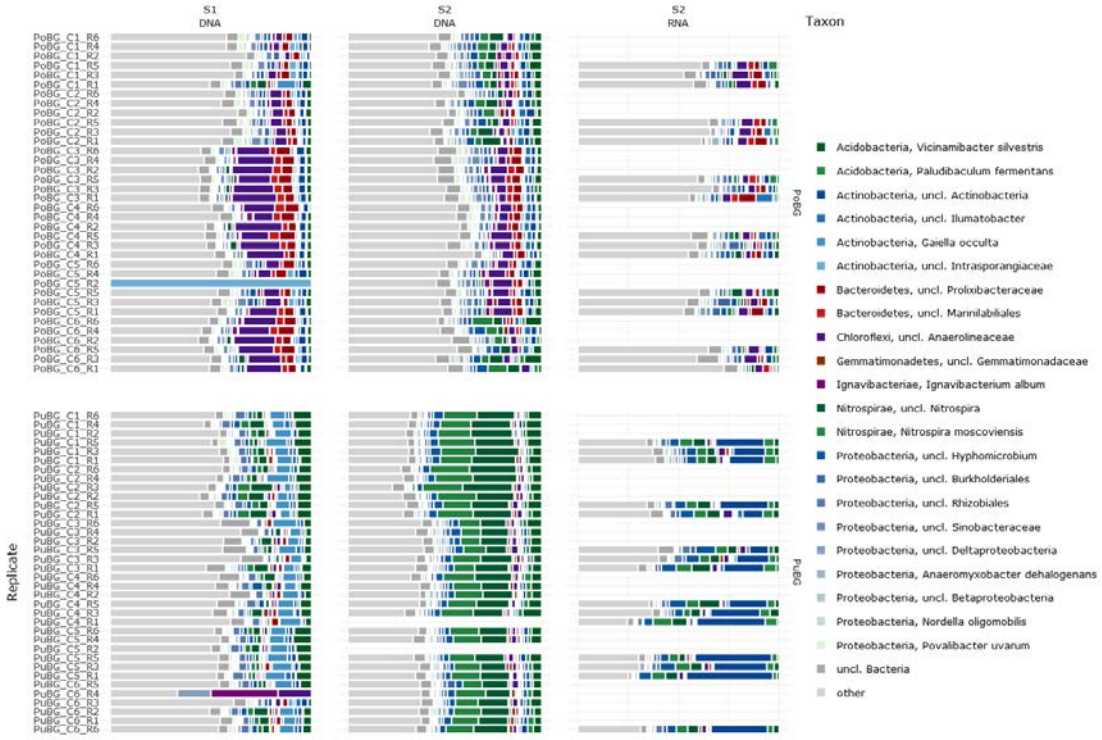

Figure 3: Taxon frequencies from DNA and RNA samples from the sampling sites P1 (PuBG) and P2 (PoBG) monitored during S1 (season 1, autumn) and S2 (season 2, summer) summarised by phylum and species

(ii) Determination of the biodegradation potential of iron/manganese oxidising bacteria

In the beginning, the biodegradation of certain iron/manganese oxidising bacteria (IMOB) was analysed with diclofenac, carbamazepine and gabapentin. The IMOBs included in this study are representatives of Proteobacteria and could play an essential role as biodegrading microorganisms in natural systems, and bioreactor systems. In experimental batch setups with *Ideonella, Leptothrix, Pedomicrobium* and *Pseudomonas* spec.only a biodegradation potential was observed with diclofenac (Table 2).

Table 2: Overview of the biodegradation potential of IMOBs to degrade diclofenac (DCF) obtained by LC-MS/MS method; A210 and A288 were isolated from the Lower Oder Valley National Park, Germany, Pedomicrobium DSM 1540 was obtained from the German collection of Microorganisms and Cell Cultures (DSMZ), and OF001 was isolated from the outflow of a bioreactor.

Intern strain number	Organism	Manganese source	Tested compound	Initial compound concentration [mg L^{-1}]	Degradation potential	Removal [%]
A210	*Leptothrix* spec.	$MnCO_3$	DCF	1	✓	100
A288	*Ideonella* spec.	$MnCO_3$	DCF	1	✓	57
OF001	*Pseudomonas* spec.	$MnCO_3$	DCF	1	✓	100
A210	*Leptothrix* spec.	$MnSO_4$	DCF	1	✓	75
A288	*Ideonella* spec.	$MnSO_4$	DCF	1	✓	69
Pedomicrobium sp. (DSM 1540)	*Pedomicrobium* spec.	$MnSO_4$	DCF	1	✓	66
OF001	*Pseudomonas* spec.	$MnSO_4$	DCF	1	✓	80

Our findings have shown that the ability to degrade DCF in higher concentration as 1 mg L^{-1} depends on the strain, the manganese sources ($MnCO_3$ and $MnSO_4$), and the external carbon content, like yeast extract concentration (data not shown). These results will be published in the second journal paper which is currently under preparation.

Future Work planned

A detailed analysis with the collected sequence data will follow, and an additional investigation with a specific bioinformatic tool Tax4Fun is planned for predicting functional profiles from metagenomic 16S rRNA data. These results gained from the sequence data will help to provide a deeper insight into the phylogenetic and functional diversity of the microbial communities. The results will then be published in the first journal paper which is currently under preparation.

Since it is likely, that the biodegradation of DCF follows different metabolic pathways, further experiments should detail if 2,6-dichloroaniline is produced during the biodegradation of DCF as transformation product. Because the understanding is still incomplete, and it is supposed that different batch conditions could differ in their produced transformation products. This subject will be part of the second planned paper.

Collaboration

The thematic work related to the microbial degradation of pharmaceutical compound included collaboration with project T6 (Fatima El-Athman). The quantification of organic compounds by LC-MS/MS was realised within the cooperation with project T6 (Fatima El-Athman) in the laboratories of the Chair of Water Quality Control (TU Berlin). Further laboratory equipment was shared with the projects T6 and N1.
In addition to that, micropollutant concentrations in the river Panke were measured by the Berlin Water Works (BWB). The profiling of microbial communities from the river Panke was conducted in collaboration with Dr. Sven Künzel at the Max-Planck-Institute (MPI) for Evolutionary Biology in Plön by using high-throughput 16S rRNA gene amplicon sequencing. During the period of residence at the MPI, the knowledge about the library preparation for sequencing was provided by Dr. Sven Künzel. Subsequently, the library preparation was implemented in the laboratories at the Max-Planck-Institute.
Likewise, the research project is part of the common topic groups in UWI “Micropollutants” and “Biogeochemical processes“.

References

1. Heberer, T. (2002): Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data. *Toxicol. Lett.* 131, 5–17. https://doi.org/10.1016/S0378-4274(02)00041-3
2. Heberer, T., Reddersen, K., Mechlinski, A. (2002): From municipal sewage to drinking water: Fate and removal of pharmaceutical residues in the aquatic environment in urban areas. *Water Sci. Technol.* 46, 81–88.
3. Kümmerer, K. (2010): Pharmaceuticals in the Environment. *Pharm. Environ. Sources, Fate, Eff. Risks* 75–94. https://doi.org/10.2146/ajhp050123
4. Schröder, P., Helmreich, B., Škrbić, B., Carballa, M., Papa, M., Pastore, C., Emre, Z., Oehmen, A., Langenhoff, A., Molinos, M., Dvarioniene, J., Huber, C., Tsagarakis, K.P., Martinez-Lopez, E., Pagano, S.M., Vogelsang, C., Mascolo, G. (2016): Status of hormones and painkillers in wastewater effluents across several European states—considerations for the EU watch list concerning estradiols and diclofenac. *Environ. Sci. Pollut. Res*. 23, 12835–12866. https://doi.org/10.1007/s11356-016-6503-x
5. Ternes, T. (1998): Occurrence of drugs in German sewage treatment plants and rivers. Water Res. 32, 3245–3260. https://doi.org/10.1016/S0043-1354(98)00099-2
6. Verlicchi, P., Al Aukidy, M., Zambello, E. (2012): Occurrence of pharmaceutical compounds in urban wastewater: Removal, mass load and environmental risk after a secondary treatment-A review. *Sci. Total Environ*. 429, 123 155. https://doi.org/10.1016/j.scitotenv.2012.04.028

3. Comments on the qualification programme and supervision strategy:

Being part of the UWI group gave me many possibilities to gain a more profound knowledge in my research field by being promoted to participate in elective courses that I chose for my specific research topic. Primarily, the participation in the core courses, e.g. related to modelling of hydrosystems broadened my horizon and deepened my understanding in another research field. This excellent support to participate in different classes leads to a better and further education. It promotes exchange with other scientists and students and encourages interdisciplinary exchange in research. Also, the scientific and personal dialogue between UWI students took place during annual UWI Summer Schools and the interim workshop which produced new ideas and approaches for my work.

Besides this excellent support of UWI in the education programme, there was also the opportunity to appoint a UWI doctoral student speaker, who attended the meetings with the speakers to speak about our concerns if it was needed.

When I needed further information about opportunities for doctoral scholarships to complete my dissertation, the UWI provided great communication and support with that. I’ve got all the support from my supervisor and UWI, so, therefore, it is possible for me that I will be funded with a doctoral scholarship for the last three months.

Participation in the following Research Training Group events:

1. Core courses
   - I – Urban interface processes – fluxes, transport, interactions (3 ECTS)
   - II – Modelling and measuring concepts of interface processes (3 ECTS)
   - III – Urban freshwater ecology (3 ECTS)
2. Elective courses
   - Summer School – Aquatic Microbial and Molecular Ecology (SDU Denmark) (10 ECTS)
   - NGS Introduction and Library Preparation Workshop (TU Berlin) (1 ECTS)
   - R Grundlagenkurs (FU Berlin) (1 ECTS)
   - Stay Abroad workshop (TU Berlin)
   - English Academic Writing (TU Berlin)
3. Gender courses
   - Self-management
   - Positioning and negotiation
   - Effective collaboration
   - Project coaching and agile leadership
4. UWI lectures: Participated in all UWI lectures
5. Other UWI events
   - Orientation Seminar and UWI Opening Ceremony (08. – 09.09.2015)
   - Exposé Talks (08.12.2015)
   - Summer School (13.–14.09.2016)
   - Kollegiate Seminar (22.09.2016)
   - Interim Meeting (19.05.2017)
   - Student Research Council (17. – 18.03.2017)
   - Summer School (05. – 06.09.2017)
   - Summer school (19. – 20.09.2018)

Research stays or internships at other research institutions both at home and abroad.
Internship: 12.11-17.11.2017 at the Max-Planck-Institute for Evolutionary Biology in Plön (Germany)

Participation in conferences, congresses, etc., at home and abroad:

2015: - ASKURIS - Closing event of joint projects ASKURIS and IST4R (14.09.2015, Technische Universität Berlin)
- 17th IWA International Conference on Diffuse Pollution and Eutrophication (15.09.2015, Berlin)

2016: - Spring School – Water in Urban Areas (09.03.2016, Department of Fluid System Dynamics, Technical University Berlin)
- Biofilms 7 Conference (26.06-28.06.2016, Porto, Portugal)

2017: - ATHENE Workshop – Integrative approaches to remove compounds of emerging concern (CECs) in wastewater treatment (08.03.2017, BfG Koblenz)
- Wasser Berlin International, Berlin (28. – 31.03.2017)
- Symposium – Biofilms in Nature, Technology and Medicine (04.05.2017, Philipps University Marburg (Symikro))

4. Individual publications:

II. Conferences, poster presentations etc.:

- Nega, M., Braun, B., Szewzyk, U. (2016): Effect of pharmaceuticals on growth behaviour of iron/manganese depositing bacteria, Biofilms 7 Conference 2016 (26. – 28.06.2016, Porto, Portugal), poster presentation