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Implications of Microbial Adaptation for the Persistency Assessment of Organic Chemicals

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Summary

The research presented in this thesis focuses on microbial adaptation to organic chemicals and the resultant effects on ready biodegradation tests. Biodegradability is a key parameter for the environmental risk assessment of organic chemicals. Readily biodegradability is mostly determined using screening tests such as the ready biodegradability tests (RBTs) developed by the Organization for Economic Cooperation and Development (OECD).

Chapter 1 is a general introduction about the environmental risk assessment of organic chemicals. It also describes the sources and compositions of microbial communities that are used in the RBTs. Chapter 2 gives a more fundamental insight and reviews the current literature. We emphasize that the RBTs suffer from several problems that lead to a high variability of the results and, hence, to difficulties in their interpretation. The origin and exposure history of the inocula used for biodegradability testing can lead to highly variable outcomes. Microbial adaptation to chemicals and its impact on biodegradation requires further investigation in order to have a better understanding of their effects on persistency assessments of chemicals. Microbial adaptation appears to enhance biodegradation of organic chemicals as judged by different studies. This review described the several mechanisms responsible for these phenomena, amongst which are i) shifts in community composition or abundances, ii) mutations within populations, iii) horizontal gene transfer or iv) recombination events. It also shows that these adaptation processes may well be mimicked under laboratory conditions, but that the outcome remains difficult to predict as we lack a fundamental understanding of the adaptive responses. This chapter aimed to bring together our current knowledge regarding microbial adaptation and its implication for the testing of biodegradation of chemicals, and to provide theoretical and practical background information to this thesis.

As described in chapter 2, exposure history and adaptation of the inoculum to chemicals have been shown to influence the outcome of ready biodegradability tests. In chapters 3 and 4, we used chemostats to investigate the impact of a long-term exposure to N-methylpiperazine (NMP) and 4-chloroaniline (4CA) (in chapter 3) and metformin (MET) (chapter 4) of an activated sludge microbial community that was obtained from a wastewater treatment plant in Amsterdam. The objective was to characterize the influence of adaptation to the chemicals on an enhanced biodegradation test, following the OECD 310 guideline. The inoculum was exposed to each chemical independently in the chemostat for a total period of nine months. Changes in community diversity and composition were followed by 16s rRNA gene amplicon sequencing. The original activated sludge inoculum was unable to degrade NMP within the 28 d frame of the test while 4CA was completely eliminated by the same initial community. However, after one month of exposure, the community cultivated in the chemostat that contained NMP was adapted and could completely degrade it within 10 d in the biodegradability test. On the other hand, the culture exposed for 3 months to 4CA and the one

exposed to MET lost part of their ability to degrade these compounds. Long term incubation in the chemostat system led to a progressive loss of the initial biodegradation capacity of the community, apparently as a consequence of the loss of key degrading microorganisms. This chapter highlights the potential of chemostat systems to induce adaptation to a specific chemical which is persistent according to the ECHA database (ECHA, 2013b), ultimately resulting in its biodegradation. However, it also shows the limits of chemostat systems and the difficulty to maintain a stable microbial community activity. In addition, a MET-degrading strain belonging to the genus *Aminobacter* was isolated from the community, as described in chapter 4. Results of this chapter show that environmental microbial communities are most likely already adapted to MET and that further exposure in a chemostat does not improve the biodegradation of such compounds.

However, biodegradation of metformin is influenced by the nitrogen concentration in the medium, as demonstrated in chapter 5. In this chapter, activated sludge from the municipal wastewater treatment plant of the city of Eindhoven was used to inoculate four different chemostat systems with two different media. Both of these media were performed in duplicate, and contained the same concentration of MET (60 mg/l) but at different nitrogen concentrations. Two of the chemostat contained a media that followed the OECD 310 guideline with 1 mg/l of nitrogen, while the two others contained a higher concentration of nitrogen at 100 mg/l. MET and its transformation product, guanylurea (GUA) were completely removed from the chemostat systems fed with an OECD 310 medium, while high nitrogen concentration in the other medium resulted in an inability to transform MET to GUA and and to metabolize GUA any further. However, biodegradability tests in batches with OECD 310 medium using the community cultivated with an excess of nitrogen resulted in a full transformation of MET within 14 d. Furthermore, CO_2 production data indicated mineralization of MET without intermediate transformation to GUA. This chapter confirmed that more than one taxon is involved in the biodegradation of MET. The results show that MET and GUA degraders are present and active in activated sludge, as already demonstrated in chapter 4. However, consumption of MET and GUA is linked with the presence of easily accessible carbon and nitrogen sources in the environment.

In chapter 6, chemostat systems were used to expose an activated sludge microbial community to the persistent pharmaceutical carbamazepine (CBZ) at an environmentally relevant concentration for a period of 12 months. The objective was to measure the capacity of a microbial community to adapt and biodegrade CBZ. The results showed that, despite the change in community diversity and composition, no biodegradation was achieved by the community. Adaptation to CBZ did therefore not take place in the chemostats, and CBZ can therefore be considered to be non-biodegradable by activated sludge microbial community, even after a pre-exposure time of the culture of 12 months. This chapter highlights that enhanced RBTs with exposed inocula are still not lenient to all chemicals.

In chapter 7, five different activated sludge sources were used to perform biodegradability tests on 4-chloroaniline (4CA), N-methylpiperazine (NMP), metformin (MET) and

carbamazepine (CBZ), before and after short- or long-term exposure. Activated sludge from the wastewater treatment plant of the Dutch cities of Amsterdam, Amstelveen, Utrecht, Bennekom and Eindhoven were sampled in autumn and winter. A pre-exposure of 7 days to one of the test compounds was conducted in batch while long term exposure of maximum 4 months, to a mixture of the four chemicals, was performed in independent chemostats for each location. RBTs outcome showed that none of the test compound can be considered as readily biodegradable in the standard tests. However, after pre-exposure in batch culture or long term exposure in the chemostat, 4CA was degraded in some cases, and less variability between inocula was observed for the biodegradation of MET. Microbial communities were found to be significantly different from each other according to their location and season of sampling. Furthermore, significant differences were observed after each treatment performed on the communities. The communities from winter sampling were found to be less diverse in terms of taxa or abundance than the autumn communities. Moreover, this chapter revealed that RBT outcomes, for these specific chemicals, are apparently not affected by the sample or the seasonal time of sampling, even if the inocula composition was significantly different, but likely more by the test volume and the inoculum quantity.

Finally, the synthesis of this thesis presented in chapter 8 discusses the contribution of this thesis to the current biodegradability tests guidelines. Future research and the recommendation to implement microbial adaptation to the existing test are also discussed in this chapter.