Extended Summary

Pesticides constitute a significant group of environmental pollutants. The pollution of natural resources by pesticides is attributed to diffuse and point sources. The contribution of the latter in environmental contamination is of outmost importance and measures to diminish their impact have been proposed. On farm improper activities before, during or after spraying could be a significant point source of pollution of natural water resources by pesticides. Biobeds constitute an established, cost-effective and efficient on-farm biodepuration system for the treatment of wastewaters produced by on-farm activities. However post-farm activities involving pesticides application also contribute to the point pollution of natural resources. Such an example are fruit packaging plants where fungicides (thiabendazole (TBZ), imazalil (IMZ), ortho-phenylphenol (OPP)) and antioxidants (diphenylamine (DPA), ethoxyquin(EQ)) are used to protect fruits from fungal infestations and physiological disorders during storage. This results in the formation of large volumes of wastewaters which contain high loads of toxic and persistent pesticides and should be treated *on site.* The environmental risk associated with the postharvest use of pesticides is exemplified in the registration documents of all relevant pesticides which specify the need for treatment of the effluents produced before their environmental release. However no efficient, cheap and sustainable treatment methods are available at the moment in Europe.

In the absence of effective depuration methods, these effluents are discharged in municipal wastewater treatment plants or spread onto agricultural land compromising the quality of water and soil resources. Biobeds modified to cope with the characteristics of these particular effluents (large volumes, seasonality in production, high pesticide loads, low BOD/COD etc) could be an applicable solution for their treatment. Based on all the above, the main aim of this PhD thesis was to assess the potential use of biobeds as treatment methods for the depuration of wastewaters from fruit packaging plants. To achieve this main aim a gradually scalled up experimental approach was implemented which aimed (a) to identify, initially at lab scale level, biobeds packing materials based on Spent Mushroom Substrate (SMS), which exhibit high dissipation and sorption capacity against the pesticides

used in the fruit packaging plants (b) to further test, at leaching columns, the capacity of the best performing biobed packing material to retain pesticides under conditions of high hydraulic load simulating realistic conditions and (c) to finally test the depuration performance of biobed systems at pilot scale level. Additional points were explored like means to maximize the depuration performance of biobeds against recalcitrant pesticides via bioaugmentation with tailored-made inocula, the response and structure of the microbial community in biobed systems and practical aspects for their implementation like (i) the quality and post-treatment handling of the biobed-treated effluent and (ii) the decontamination of the spent biobed packing material upon the end of the life cycle of biobeds.

In Chapter 2 we studied the dissipation of TBZ, IMZ, OPP, DPA and EQ used by the fruit-packaging industry, in anaerobically digested sewage sludge and in liquid aerobic sewage sludge to test the dissipation capacity of sewage treatment plants. At a second stage we evaluated the dissipation (and the metabolism of EQ) and sorption of all these pesticides to various organic substrates composed of soil, straw and spend mushroom substrate (SMS) in various volumetric ratios to identify the best performing biobed packing material. TBZ and IMZ showed higher persistence especially in the anaerobically digested sewage sludge (DT_{50}=32.3-257.6 d), in contrast to OPP and DPA which were rapidly dissipated especially in liquid aerobic sewage sludge (DT_{50}=1.3-9.3d). EQ was rapidly oxidized mainly to quinone imine (QI), which did not persist, and dimethyl ethoxyquinoline (EQNL, minor metabolite) which persisted for longer. Sterilization of liquid aerobic sewage sludge inhibited pesticides decay verifying the microbial nature of pesticides dissipation in those substrates. Organic substrates rich in SMS showed the highest dissipation capacity with TBZ and IMZ DT_{50s} of *ca*. 28 days compared to DT_{50s} of > 50 days in the other substrates and the general recalcitrance of those compounds in soil (DT_{50} >100 d). TBZ and IMZ showed the highest sorption affinity, whereas OPP and DPA were weakly sorbed. These findings suggested that municipal wastewater treatment plants could not guarantee an efficient removal of the recalcitrant fungicides IMZ and TBZ, whereas SMS-rich biobed organic substrates show much higher dissipation capacity for those chemicals, and also for the less persistent OPP, DPA and EQ.

In Chapter 3 we focused on the citrus fruit-packaging plants which produce large wastewater volumes with high loads of fungicides like OPP and IMZ, two chemicals showing contrasting persistence (OPP is non persistent while IMZ is persistent). In accordance with a gradual scaling up approach we employed a column study to assess the capacity of SMS of *Pleurotus ostreatus*, either alone or in mixture with straw and soil plus a mixture of straw /soil to retain and dissipate IMZ and OPP. The role of *P. ostreatus* on fungicides dissipation was also investigated by parallel studying the performance of fresh mushroom substrate of *P. ostreatus* (FMS) and measuring lignolytic enzymatic activity in the leachates. We employed a sequentil treatment scheme which simulated a realistic worst case operation of a citrus fruit packaging plant. All substrates effectively reduced the leaching of OPP and IMZ which corresponded to 0.014-1.1% and 0.120-0.420% of their initial amounts respectively. Mass balance analysis revealed that FMS and SMS/Straw/Soil (50/25/25 by vol) offered the most efficient removal of OPP and IMZ from wastewaters respectively. Regardless of the substrate, OPP was restricted in the top 0-20 cm of the columns and it was bioavailable (extractable with water), compared to IMZ which was less bioavailable (extractable with acetonitrile) but diffused deeper in the columns (20-50 & 50-80 cm) in the SMS- and Straw/Soil-columns. The distribution of the living microbial community was measured via phospholipid fatty acids analysis (PLFAs). Fungal abundance was significantly lower at the top layer of all substrates from where the highest pesticide amounts were recovered suggesting an inhibitory effect of fungicides on total fungi. These results suggested that biobeds packed with SMS-rich substrates could ensure the efficient removal of IMZ and OPP from wastewaters of citrus FPP even under particularly high hydraulic and pesticide loads.

Based on the results of Chapters 2 and 3 and in accordance with the gradual scaling up of the experimentation, we constructed and tested pilot biobed systems under practical conditions of citrus and pome fruit packaging plants. Further aspects tested were (a) the optimization of the depuration capacity of the pilot biobeds through bioaugmentation with tailored-made bacterial inocula, and (b) the composition and functional dynamics of the microbial community in pilot biobeds using molecular approaches (q-PCR). Practical issues were also addressed including the risk associated with the direct environmental disposal of biobed-treated effluents and methods for the decontamination of the spent packing material. Three pilot biobeds of 1 m^3 (non bioaugmented) and 2 pilot biobeds of 0.24 m^3 (bioaugmented) were constructed and treated for a period of 160 days with different combinations of pesticides simulating practical scenarios from citrus and pome fruit packaging plants. Pilot biobeds showed high depuration capacity for the less persistent OPP, DPA (>99.9%) but also for the more recalcitrant chemicals IMZ and TBZ (>99.5%). Bioaugmentation maximized the depuration capacity of pilot biobeds for the persistent fungicide TBZ which was fully dissipated by the end of the study. This was followed by a significant increase in the abundance of bacteria, fungi and of catabolic genes *catA* and *pcaH*. Bioaugmentation was the most potent method for the decontamination of the spent packing material, although composting with fresh organic matter and even storage at ambient temperature offer effective alternatives when inocula are not available. Risk assessment based on practical scenarios (pome and citrus fruit-packaging plants), the depuration performance of the pilot biobeds and the currently implemented regulatory framework for pesticides showed that the discharge of the biobed-treated effluents into an 0.1-ha disposal site did not entail an unacceptable risk for aquatic and terrestrial ecosystems, except for TBZ-containing effluents produced by pome fruit packaging plants where a larger disposal area (0.2 ha) or bioaugmentation of biobeds alleviated the risk.

Overall our study provided a comprehensive evaluation of biobeds as a method for the treatment of pesticide contaminated wastewaters produced by the fruit packaging industry. We showed that these systems could be a viable solution for the treatment of these agro-industrial effluents. Further studies will aim explore the application of biobeds for the treatment of the wastewaters produced by other agro-industries (i.e. bulb disinfection, seed-coating).