SUMMARY

Agro-food processing industries use large amounts of fungicides to ensure availability of fresh plant products during storage and transport. These include seed-producing industries (SPI), which treat seeds with systemic fungicides like carboxin (CBX), metalaxyl-M (MET-M) and fluxapyroxad (FLX), bulb handling industries (BHI) which immerse bulbs into dense solutions of fungicides such as chlorothalonil (CHT), thiabendazole (TBZ) and fludioxonil (FLD), and fruit-packaging industries (FPI) that make use of fungicides like imazalil (IMZ) and fludioxonil (FLD) for the control of fungal infections of fruits during storage. As a result, they generate large amounts of pesticide-contaminated effluents which constitute serious environmental threats due to the high environmental persistence and toxicity of the pesticides contained in them. Despite relevant regulation, the lack of efficient and cost-effective treatment methods has pushed agro-food industries into improper and environmentally harmful disposal practices.

Through the years, many depuration methodologies have been studied, but their full implementation has not been achieved due to poor results concerning mineralization, high operational costs and formation of toxic by-products. Biological treatment systems like biobeds could provide an efficient and sustainable solution to the depuration of pesticide-contaminated effluents. Some recent studies have demonstrated the efficient decontamination of FPI effluents in biobeds, while the use of biobed systems for the treatment of SPI and BHI effluents is still not explored. The high depuration capacity of biobeds is attributed to the microbiome of the packing material, but the composition and succession of the microbial communities and the dynamics of mobile genetic elements (MGE) involved in the dispersion of pesticide-catabolic genes in the bacterial community of the packing material during biobed operation, are not yet adequately explored. The performance of biobed systems against persistent and particularly mobile pesticides can be greatly enhanced by bioaugmentation of the packing material with tailored-made microbial inocula specialized in the degradation of target compounds. With these in mind, we aimed (i) to provide evidence for the capacity of biobed systems to depurate pesticide-contaminated effluents from SPI, BHI and FPI, (ii) to shed some light on the composition of biobed microbiome and identify factors that drive microbial succession during biobed operation, (iii) to provide insight on the occurrence and distribution of MGEs in biobed systems during operation and (iv) to isolate microorganisms able to degrade IMZ, a highly persistent fungicide that is widely used by agro-food processing industries, especially FPI, with the potential to be used in future biological treatment systems such as biobeds and bioreactor units.

In Chapter 2 we studied the degradation and adsorption, two major processes controlling the environmental fate of pesticides in biobed packing material composed of 25% soil, 25% straw and 50% spent mushroom substrate and comparatively in soil with no previous pesticide exposure. The degradation of CBX, MET-M, FLX, FLD, TBZ and CHT, was studied under individual and in-mixture application relevant to their industrial use, to simulate realistic exposure conditions, while FLD was also tested at different concentrations (10, 20, and 150 mg/kg) representing the dose rates used by the different industries. The majority of fungicides, regardless of the mode of application, resulted in higher dissipation in the biobed packing material (DT_{50} = 2.34 – 142.9 days) than in soil (DT_{50} = 6.67 – 784.1 days). In most cases

application in mixtures retarded fungicides' degradation, with CHT having the most pronounced inhibitory effect in the degradation of TBZ and FLD. FLD degradation showed a dose-dependent pattern with its DT_{50} increasing from 42.4 days at 10 mg/kg to 107.6 days (at 150 mg/kg). In addition, all pesticides showed higher adsorption affinity in the biomixture (K_f = 3.23 - 123.3 g mL^{-1}) compared to soil (K_f = 1.15-31.2 g mL^{-1}). The findings of Chapter 2 provided initial evidence of the depuration potential of biobeds against fungicides contained in effluents generated by SPI, BHI and FPI which allowed us to proceed with our research of biobed systems treating agro-industrial effluents.

Consequently in Chapter 3, we employed a biobed column experiment using the same packing material as in Chapter 2, in order to assess the efficiency of biobed systems to depurate agro-industrial effluents containing mixtures of fungicides in a realistic loading scenario. We demonstrated that the biobed columns could effectively retain and dissipate the fungicides contained in agro-industrial effluents with 93.1 - 99.98 % removal efficiency in all cases. Lipophilic substances like FLX were mostly retained in the biobed while more polar substances were mostly dissipated like CBX or showed higher leaching potential like MET-M. The effect of continuous effluent application in the packing material's microbiome was also explored in the same experimental setup through amplicon sequencing analysis. Contrary to our expectation, biobed column supported resilient bacterial and fungal communities, which were not affected by fungicide application but showed temporal patterns along the different horizons. Facultative or strict anaerobic bacteria like *Chloroflexi*/*Anaerolinae*, *Acidibacter* and *Myxococcota* showed significant increase in the abundance supporting the hypothesis that the temporal patterns were driven by microaerophilic conditions upon water saturation of the packing material. Lastly, we investigated the dynamics of MGE, namely *Intl1*, *IS*1071 and IncP-1 and IncP-1ε, expecting the continuous exposure to high pesticide loads will promote their dissemination. However, our hypothesis was not confirmed, as continuous wastewater application did not affect the dynamics of MGE in biobeds. Instead, we observed temporal increase in the abundance of most MGE tested, suggesting the influence of biotic or abiotic factors, beyond pesticide-related pressure. All in all, the findings of Chapter 3 reinforce the high potential of biobed systems for the depuration of agro-industrial effluents and showed that the packing material contains a resilient microbiome that is not affected by pesticide exposure, but responds to abiotic and biotic conditions that gradually develop in the biobed system.

Chapter 4 was dedicated in the isolation and characterization of a microorganism able to degrade the widely used and persistent fungicide IMZ, and in the investigation of its potential use as inoculum in biotic wastewater treatment systems. A *Cladosporium herbarum* strain capable of degrading IMZ was isolated via enrichment cultures from a soil that was receiving regular discharges of FPI's IMZ-contaminated effluents. The *C. herbarum* strain did not show any pathogenicity on fruits commonly processed by FPIs, a trait essential for its biotechnological exploitation in the treatment of FPI effluents. The isolate was able to degrade up to 100 mg/L of IMZ but its degrading capacity and growth was reduced at increasing IMZ concentrations in a dose-dependent manner, indicating that the degradation of IMZ is a detoxification mechanism instead of a growth-linked process. The isolated strain was able to tolerate the presence other fungicides, which are commonly used by FPIs and are, thus, expected to co-occur with IMZ in their effluents, and showed partial dissipation of FLD, TBZ and 3,5 dichloroaniline (3,5DCA), a toxic transformation product of iprodione (IPR) degradation. On the contrary *ortho*-phenylphenol (OPP) inhibited the growth and, therefore, degradation capacity of the isolate. The ability of *C. herbarum* to depurate IMZ-contaminated effluents was assessed in a benchtop bioreactor fed with artificial IMZ-contaminated wastewater (200 mg L-1). Amplicon sequencing analysis showed that *C. herbarum* was able to successfully establish and dominate the fungal community of the bioreactor throughout the study and successfully removed >96% of IMZ. Overall, the findings of Chapter 4 demonstrate the high potential of *C. herbarum* to remove IMZ under lab and bioengineering conditions.

As a whole our study demonstrated the high potential of the use biobed systems for the depuration of fungicide-contaminated effluents from seed-producing, bulb-dipping and fruit packing industries. We also showed that the biobeds support a resilient microbiome, whose composition was most probably affected by microaerophilic conditions that gradually developed in the packing material. Lastly we reported the isolation of a *C. herbarum* strain with the capacity to degrade IMZ and examined its potential for the depuration of FPI effluents that contain IMZ.