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Integrated biodepuration of pesticide-contaminated wastewaters from the fruit-packaging industry using biobeds: Bioaugmentation, risk assessment and optimized management

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HIGHLIGHTS

- Fruit-packaging plants wastewaters constitute a serious point source contamination.
- Biodepuration with pilot biobeds achieved >99.5% removal of the pesticides tested.
- Bioaugmentation with tailored-made inocula maximized depuration for thiabendazole.
- Risk assessment suggested no unacceptable risk by the release of biobed effluents.
- Bioaugmentation or composting could decontaminate the spent biobed packing material.

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ABSTRACT

Wastewaters from fruit-packaging plants contain high loads of toxic and persistent pesticides and should be treated *on site*. We evaluated the depuration performance of five pilot biobeds against those effluents. In addition we tested bioaugmentation with bacterial inocula as a strategy for optimization of their depuration capacity. Finally we determined the composition and functional dynamics of the microbial community via q-PCR. Practical issues were also addressed including the risk associated with the direct environmental disposal of biobed-treated effluents and decontamination methods for the spent packing material. Biobeds showed high depuration capacity (>99.5%) against all pesticides with bioaugmentation maximizing their depuration performance against the persistent fungicide thiabendazole (TBZ). This was followed by a significant increase in the abundance of bacteria, fungi and of catabolic genes of aromatic compounds *catA* and *pcaH*. Bioaugmentation was the most potent decontamination method for spent packing material with composting being an effective alternative. Risk assessment based on practical scenarios (pome and citrus fruit-packaging plants) and the depuration performance of the pilot biobeds showed that discharge of the treated effluents into an 0.1-ha disposal site did not entail an environmental risk, except for TBZ-containing effluents where a larger disposal area (0.2 ha) or bioaugmentation alleviated the risk.

1. Introduction

Postharvest treatment of fruits with pesticides guarantees their protection from fungal infestations and physiological disorders during storage. However, it leads to the production of large volumes of pesticide-contaminated effluents whose discharge without prior treatment entails serious environmental risks [1]. This is exempli-

Abbreviations: TBZ, thiabendazole; IMZ, imazalil; OPP, ortho-phenylphenol; DPA, diphenylamine; SMS, spent mushroom substrate; RACs, regulatory acceptable concentrations; TER, toxicity exposure ratio; PECs, predicted environmental concentrations; HQ, hazard quotient; EFSA, European food safety authority; EC, European Commission.

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fied by the high aquatic toxicity of the pesticides used in this sector like thiabendazole (TBZ) [2] imazalil (IMZ) [3], *ortho*-phenylphenol (OPP) [4] and diphenylamine (DPA) [5].

The need for treatment of those effluents is stressed in the relevant pesticide regulatory documents which state that member-states should ensure *that appropriate waste management practices to handle the waste solution remaining after application are put in place* [6]. Several methods have been tested for the treatment of those effluents but integrated full scale implementation has not been achieved yet. Garcia-Portilo et al. [7] patented a treatment system based on activated carbon which showed high removal efficiency for TBZ. However its high cost have prevented its wide implementation in fruit-packaging plants. Recent studies by Sanchez Perez et al. [8] proposed a combined membrane biological reactor/Fenton-Photo Fenton process for the dissipation of TBZ. However this study was performed at pesticide levels (0.1 mg L^{-1}) which are multi-fold lower than the levels found in the effluents. In addition, those methods lead to the formation of oxidation products of unknown toxicity [9]. In the absence of treatment systems industries dispose their effluents in municipal sewage treatment plants which are not effective in the removal of those pesticides transferring the contamination to receiving water systems [10].

Biological treatment systems like biobeds could be a possible solution for the treatment of those effluents. They are simple and efficient systems used up to now for the depuration of pesticide-contaminated effluents at on farm level [11]. In their simplest form they are composed of a pit packed with a mixture of bioorganic material [12]. Omirou et al. [13] first tested biobeds for the depuration of wastewater produced by the citrus fruit production chain, thus pesticides like DPA used in pome fruit-packaging plants were not considered. In their study, TBZ and IMZ were retained by the biobed packing material leading to a potential build-up of high pesticide residues stressing the need for decontamination of the spent packing material. This constitutes a key regulatory issue withholding the wider adoption of biobeds [14]. However only a few studies have addressed it [15,16].

Little attention has been given also to the post-treatment handling of biobeds-treated effluents. Despite the high depuration performance of biobeds [12], pesticide residues are still present in their effluents and their environmental release should be allowed pending risk assessment. This is feasible for biobeds receiving wastewaters from the fruit-packaging industry where a limited number of pesticides is used, in contrast to on-farm systems which receive a much wider pesticide range and thus complex risk assessment approaches are required.

Biodegradation has been identified as the key process controlling biobeds performance [11]. Despite that little is known about the composition of the microbial community in biobeds and the microbial dynamics driving the biodegradation process. Good knowledge of the microbiology of biobeds will facilitate their optimization. Bioaugmentation has been explored as a strategy for optimization of biobeds performance. Karanasios et al. [17] showed that the use of spent mushroom substrate (SMS) from the edible fungus *Pleurotus ostreatus* in biobeds accelerated pesticide dissipation. Sniegowski and Springael [18] showed that the use of soil carrying a microbial community adapted to the rapid degradation of specific pesticides as a component of the packing material could ameliorate the depuration capacity of biobeds. This strategy or bioaugmentation with tailored-made microbial inocula could be ideal in cases where biobeds receive effluents containing a few known pesticides like in fruit-packaging plants.

The main aims of this study were to a) evaluate the depuration performance of pilot biobeds against pesticides used in fruit-packaging plants, and assess bioaugmentation as an optimization strategy, b) identify the key microbial groups, phylogenetically and functionally relevant to biobed systems, c) estimate the risk associated with the environmental disposal of the biobed-treated effluents and d) assess methods for the decontamination of the spent biobed packing material.

2. Materials and methods

2.1. Pesticides

Analytical standards of IMZ (99.8%), TBZ (99%) OPP (99.9%) and DPA (99.9%) (Pestanal®, Sigma-Aldrich) were used for residue analysis. Commercial formulations of TBZ (TECTO® 50% SC), IMZ (FUNGAZIL® 50% EC), OPP (FRUITGARD® 20%SL) and DPA (NO SCALD® 31.8%EC) were utilized for the preparation of the aqueous solutions applied on biobeds.

2.2. Biobed packing material

Following earlier optimization studies [19,20], a mixture of SMS, soil and straw (50:25:25 by volume) was used for the packing of the pilot biobeds. The soil used was collected from a field site in Larissa, Greece. It was sieved to homogenize (4 mm) prior to mixing with organic materials. Wheat straw was chopped into small pieces (1–3 cm) and passed through a 4.75 mm sieve. SMS was obtained from a *P. ostreatus* mushroom production unit (Mpoulogeorgos, Trikala, Greece) and it was chopped into small pieces. Soil, straw and SMS were mixed thoroughly and were left to mature for a month. Properties of the materials used are listed in Supplementary Table 1. Total organic C and N content were determined by the wet digestion [21] and the Kjeldahl digestion method [22] respectively. pH was determined in a mixture of 1:2.5-5 air dried solid substrate:water (w:v). Soil texture was determined with the Bouyoucos hydrometer method [23].

2.3. Set up of pilot biobeds

Five pilot biobeds composed of plastic containers of 1.1 m^3 (3 biobeds) or 0.24 m^3 (2 biobeds) volume were set up. The bottom of the biobeds was covered with a metal wire mesh and on top of this a 5-cm layer of well-washed gravel (2–3 cm diameter) was placed. The remaining volume was filled with the packing material described above. A 10-cm diameter hole was made at the bottom of the biobeds to allow collection of the draining effluent. A plastic funnel was positioned under the outer side of the hole and it was connected to a plastic tube (15 mm i.d.) leading to a 2.5-L amber glass bottle where effluents were collected.

The pesticide solutions applied on the biobeds were prepared in three 100-L tanks each containing an aqueous solution of two pesticides: IMZ + DPA (Tank 1), OPP + IMZ (Tank 2) and TBZ + OPP (Tank 3). The concentration of all pesticides in the aqueous solutions was 100 mg L^{-1} assuming a 10-fold dilution of their concentration in the water during the treatment process and considering the pesticides recommended dose rates (0.6 g L^{-1} for OPP, 1.2 g L^{-1} for TBZ, 1 g L^{-1} for IMZ and 2 g L^{-1} for DPA) [5,24–26]. Pesticides combinations were established according to their use patterns: IMZ + DPA and OPP + IMZ or TBZ are used in pome and citrus fruit-packaging plants respectively. In total 1080 and 252 L of pesticide solutions were discharged into the large and the small pilot biobeds respectively within a period of 160-d corresponding to the average operation period of a fruit-packaging plant [27]. Pesticide solutions were pumped (max capacity 10 L h^{-1}) into the biobeds daily (Three 10-min application periods per day). This resulted in a daily discharge of 7.5 and 2.0 L in the large and the small pilot biobeds respectively. Pesticide solutions were pumped at the top of the pilot biobeds via a drip irrigation system ensuring their uniform dispersion onto the biobeds surface. A schematic diagram of the experimental setup is

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