## On-Farm Biopurification Systems: Fungal Bioaugmentation of Biomixtures For Carbofuran Removal

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Abstract : One of the main causes of contamination linked to agricultural activities is the spillage and disposal of pesticides, especially during the loading, mixing or cleaning of agricultural spraying equipment. One improvement in the handling of pesticides is the use of biopurification systems (BPS), simple and cheap degradation devices where the pesticides are biologically degraded at accelerated rates. The biologically active core of BPS is the biomixture, which is constituted by soil pre-exposed to the target pesticide, a lignocellulosic substrate to promote the activity of ligninolitic fungi and a humic component (peat or compost), mixed at a volumetric proportion of 50:25:25. Considering the known ability of lignocellulosic fungi to degrade a wide range of organic pollutants, and the high amount of lignocellulosic waste used in biomixture preparation, the bioaugmentation of biomixtures with these fungi represents an interesting approach for improving biomixtures. The present work aimed at evaluating the effect of the bioaugmentation of rice husk based biomixtures with the fungus Trametes versicolor in the removal of the insectice/nematicide carbofuran (CFN) and to optimize the composition of the biomixture to obtain the best performance in terms of CFN removal and mineralization, reduction in formation of transformation products and decrease in residual toxicity of the matrix. The evaluation of several lignocellulosic residues (rice husk, wood chips, coconut fiber, sugarcane bagasse or newspaper print) revealed the best colonization by T. versicolor in rice husk. Pre-colonized rice husk was then used in the bioaugmentation of biomixtures also containing soil pre-exposed to CFN and either peat (GTS biomixture) or compost (GCS biomixture). After spiking with 10 mg/kg CBF, the efficiency of the biomixture was evaluated through a multi-component approach that included: monitoring of CBF removal and production of CBF transformation products, mineralization of radioisotopically labeled carbofuran (14C-CBF) and changes in the toxicity of the matrix after the treatment (Daphnia magna acute immobilization test). Estimated half-lives of CBF in the biomixtures were 3.4 d and 8.1 d in GTS and GCS, respectively. The transformation products 3-hydroxycarbofuran and 3-ketocarbofuran were detected at the moment of CFN application, however their concentration continuously disappeared. Mineralization of 14C-CFN was also faster in GTS than GCS. The toxicological evaluation showed a complete toxicity removal in the biomixtures after 48 d of treatment. The composition of the GCS biomixture was optimized using a central composite design and response surface methodology. The design variables were the volumetric content of fungally pre-colonized rice husk and the volumetric ratio compost/soil. According to the response models, maximization of CFN removal and mineralization rate, and minimization in the accumulation of transformation products were obtained with an optimized biomixture of composition 30:43:27 (pre-colonized rice husk:compost:soil), which differs from the 50:25:25 composition commonly employed in BPS. Results suggest that fungal bioaugmentation may enhance the performance of biomixtures in CFN removal. Optimization reveals the importance of assessing new biomixture formulations in order to maximize their performance.

Keywords : bioaugmentation, biopurification systems, degradation, fungi, pesticides, toxicity

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