**Simultaneous removal and detoxification of neonicotinoid insecticides by a bacterial degrading consortium at reactor scale**

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**Highlights**

* Microbial consortia capable to degrade imidacloprid were isolated
* Cross degradation of other neonicotinoid insecticides was achieved
* Reactor scale treatment (STBR) removed binary and ternary mixtures of neonicotinoids
* The treatment partially detoxified the matrix towards honeybees and other biomarkers

**1. Introduction**

Neonicotinoid insecticides are widely applied for crop protection, hence their presence in the environment is common, where they exhibit high persistence, particularly in soil. Of particular current concern is the negative effect exerted by neonicotinoid residues on honey bees, causing the decline in their populations [1,2,3], which has led to an European Union decree that banned since the end of 2018 the use of three compounds (clothianidin, thiamethoxam and imidacloprid) on crops pollinated by bees. Biological approaches such as biopurification systems used in the removal of pesticide-containing wastewaters have shown mostly inefficient removal of such compounds, reason why the liquid-phase systems with specialized degrading consortia are explored. This work aimed to isolate and employ degrading-consortia to remove several neonicotinoid insecticides simultaneously at reactor scale, and to monitor the ecotoxicological changes during the treatment.

**2. Methods**

A selective enrichment approach was employed to isolate microbial degrading consortia with the ability to transform imidacloprid; members of the relevant consortia were identified by sequencing of 16S rRNA or ITS genes. The elimination of imidacloprid and the cross-degrading ability to remove the neonicotinoid insecticides acetamiprid and thiamethoxam was determined at flask scale and monitored by LC-MS/MS. The removal process was scaled-up in a stirred tank bioreactor (STBR) using the consortium exhibiting the best performance to treat mixtures of neonicotinoids. Detoxification in the reactors was monitored by ecotoxicological tests including: seed germination (*Lactuca sativa*), bioluminescence inhibition (Microtox ®) and acute oral test on honeybees.

**3. Results and discussion**

The selective enrichment using soil pre-exposed to imidacloprid permitted to obtain cometabolic imidacloprid-degrading consortia. The consortia were composed by eight bacterial and one yeast strains, capable of degrading not only this compound, but also thiamethoxam and acetamiprid, as demonstrated in cross-degradation assays. The scaling-up of the process in batch STBR was able to simultaneously remove mixtures of imidacloprid+thiamethoxam or imidacloprid+thiamethoxam+acetamiprid, reaching elimination of 95.8% and 94.4% of total neonicotinoids respectively after 30 d. Removal rates in the bioreactors followed the pattern imidacloprid>acetamiprid>thiamethoxam, including >99% elimination of imidacloprid in 6 d and 17 d (binary and ternary mixtures, respectively). Ecotoxicological evaluation in the STBR revealed partial detoxification of the matrix, with clearer detoxification patterns in the binary mixture compared to the ternary mixture.

**4. Conclusions**

Considering that the residual ecotoxicity of the synthetic wastewater was partially decreased by the reactor treatment, the liquid-phase approach here described seems a promising strategy to remove the highly toxic and persistent neonicotinoids from agricultural wastewaters. Further research should be focused on optimizing the process to achieve higher detoxification levels, and to adapt it to feasible conditions for farms of different sizes and devoted to the production of diverse crops.

**References [Calibri 10]**

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