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## **Biodegradation of Hexachlorocyclohexane in Solid and Slurry Phases**

Hexachlorocyclohexane (HCH) is an organochlorine insecticide widely used world-wide until the 1970s. Most countries have restricted its use because of the risk of health effects and environmental problems. The pesticide is a mixture of isomers ( $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ ) produced by the chlorination of benzene in the presence of UV light. Many countries, including Brazil, are still suffering with the pollution of its soil and water, so, obtaining bioremediation of this compound is important to reduce the exposure of people to contaminated areas. The most well-known HCH-degrading bacteria are members of the genus *Sphingomonas* (formally *Pseudomonas*), which have aerobic metabolism and are found in upland contaminated fields. This work focused on the biodegradation of HCH using microorganisms endogenous to a contaminated soil from São Paulo - Brazil. Firstly, this soil was spread-plated in TSA (Tryptic Soy Agar - Oxoid CM 129) and after 48 hours, it presented  $2,4 \times 10^6$  CFU/g of soil. Then, the experiments were carried in solid phase and slurry, both using aerobic condition, discontinuous reactors, temperature (25°C) and humidity controlled. The difference between them was the amount of water, the solid phase had 18% of water and the slurry had 70%. The solid phase test had the duration of 12 months and was carried in amber bottles with O<sub>2</sub> flow. The slurry lasted 6 months and was carried inside the shaker with agitation (120 rpm) at 30°C (O<sub>2</sub> could reach the interior of the flask through the cotton lid). Three conditions were tested in both phases: 1 - contaminated soil plus water; 2 - contaminated soil plus mineral solution (biostimulation); 3 - contaminated soil plus mineral solution plus microorganisms (biostimulation and bioaugmentation). Each flask from condition (1) had an amount of 225 g (solid phase) and 30 g (slurry) of soil; plus water (solid phase: 16 mL; slurry: 70 mL). The

other conditions had the same amount of soil and water of condition (1) plus mineral solution (condition (2), solid phase and slurry: 5 mL) or, plus microorganisms (condition (3), solid phase and slurry: 8 mL with 104 CFU/mL of water solution).

All the analysis were determined by chromatography, using: US EPA method 3541 (automated Soxhlet extraction), US EPA analytical method 8081 for organochlorine pesticides, and a Hewlett-Packard 5890 Series 2 gas chromatograph with an electron-capture detector (GC-ECD).

The results showed that the solid phase had percentages of degradation around 70% (solid phase conditions: (1) 71.10%, (2) 76.10%, (3) 74.20%). (conditions: (1) 98.40%, (2) 98.90%, (3) 99.30%). The slurry phase presented 90% of degradation,

The improvement was probably due to the dilution of the HCH crystals in water (bigger amount of solvent plus agitation), since this compound has low solubility (5-10 g/L at 20°C), turning it more suitable for the microorganisms to make contact and consume the pesticide. Adding mineral solution and more microorganisms in the soil did not show expressive differences in the final results. These parameters could be more valuable for stimulating degradation in shorter period tests.