

# ICFOST 2000

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Focal Theme:  
MODERN TRENDS & PERSPECTIVES IN  
FOOD PACKAGING FOR 21ST CENTURY



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conditions. On acclimatization the degradation rates improved. Degradation of 5, 10 and 25 mg ml<sup>-1</sup> of 2,3-xyleneol by the unacclimatized strain took 48, 72 and 144 h, respectively, whereas the acclimatized culture degraded completely 50, 100 and 200 mg ml<sup>-1</sup> within 72, 72 and 96 h, respectively. An inoculum of 6.22 x 10<sup>8</sup> colony forming units ml<sup>-1</sup> was found to be optimal, when the substrate concentration was 100 mg ml<sup>-1</sup>. The degradation occurred at a wide range of pH between 4.0 and 10.0, the optimum being at pH 6.5. Similarly, effective degradation was obtained within a temperature range of 4 to 50°C, the optimum being at 35°C. Increased aeration improved the degradation rate. Strain CP4 also degraded low concentrations of 3,5-xyleneol.

D-08

#### Preparation of Inoculum of Hexachlorocyclohexane – Degrading Microbial Consortium – Selection of Carbon Sources

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Technical grade hexachlorocyclohexane (HCH) has been used extensively as an insecticide in our country for the last 4-5 decades. This has resulted in widespread contamination of air, water and soil with its residues and persist in these environments for long periods. However, HCH-degrading microbial consortium developed in our laboratory was found to degrade all the major isomers of HCH. For bioremediation of soil in a big way, large amounts of inoculum will be required. As only limited quantities of HCH can be used as substrate, the biomass build up is rather low. Hence, it is imperative to use other easily utilizable carbon sources as co-substrates for the preparation of bulk inoculum, without losing the degrading ability. Several simple or complex carbon sources were screened along with 25 ppm tech-HCH, for their ability to build up biomass. Molasses supported highest biomass production, followed by glucose, sucrose, rice straw extract supplemented

with glucose, rice straw hydrolysate, nutrient broth and wheat bran hydrolysate (WBH). However, the inoculum grown on WBH and 25 ppm tech-HCH showed the best ability to degrade HCH. disappeared with 72 h of incubation. Next best was molasses - HCH. Nearly 80 to 90% of all four isomers of 25 ppm tech-HCH grown inoculum which showed 65 to 70% of degradation. Growth of the inoculum for 72 h containing WBH equivalent to 0.75% reducing sugar and 25 ppm gave an inoculum which was efficient in degrading tech - HCH residues, both in liquid and soil media.

D-9

#### Effect of Induction and Acclimatization of a Microbial Consortium on its Ability To Degrade Isomeric Hexachlorocyclohexane (HCH)

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Biodegradation processes are characterized by an acclimatization or pre-adaptation period, during which time, the enzyme systems of the biodegradation pathway(s) get induced, facilitating effective removal of the pollutant. The effect of different modes of induction of the inoculum of a HCH-degrading microbial consortium on the degradation of technical grade HCH, inoculum, grown on wheat bran hydrolysate (containing 1.5% reducing sugars) was studied. Tech - HCH (25 ppm) was induced for 24, 48 and 72 h, with incremental feeding of 25 ppm tech-HCH every 24 h. Induction for a short period was found necessary to obtain effective degradation of the HCH. The degradation of all the four major isomers of the tech-HCH added (25 ppm) was complete within 72 h, when 24 h induced inoculum was used, whereas degradation of  $\beta$  and  $\delta$ -isomers were not very effective when the inocula induced for 48 and 72 h were used. Induction with  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ -HCH separately also helped in complete degradation of tech-HCH. Inoculum biomass prepared by growth on molasses (with 1.5% reducing sugars) was not very

effective in the degradation of 25 ppm tech-HCH, irrespective of the induction periods.

D-10

#### Effect of Different Isomers of Hexachlorocyclohexane (HCH) on the Activity of a Microbial Consortium and the Survival of its Members

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The composition of the microflora, natural or added, of a consortium is governed by many factors such as the biological equilibrium among the individual members, their dependence on one another for growth substrates, detrimental influences through their activities towards the pollutant- chemical, competition, ability to utilize and tolerance towards the starting pollutant material and its metabolites etc. In the present study, a microbial consortium capable of degrading  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ -HCH was used to understand the interactions among the 9 bacterial members during the degradation of tech-HCH. Members of the consortium were grown individually on nutrient broth/acetate/wheat bran hydrolysate, reconstituted at equal quantities and induced with  $\alpha$ -,  $\beta$ -,  $\gamma$ -,  $\delta$ -HCH or tech-HCH. The induced inoculum was used for degradation of 25 ppm of tech-HCH in mineral medium. Degradation of the substrate was complete by 24-72 h in shake flasks and it was partial in soil. A passage for 24 h through  $\beta$ -,  $\delta$ -HCH and tech-HCH eliminated 3, 2 and one bacterial types, respectively, whereas, all the types were retained in the consortium induced with  $\alpha$ - and  $\gamma$ -HCH. After the growth in tech-HCH (25 ppm) for 72 h, all the consortia showed the survival of only 5 to 6 members. The survival in soil was better with 7-8 members. It is evident that  $\alpha$ -,  $\beta$ - and  $\delta$ -HCH or probably their degradation intermediates are becoming toxic to some of the members of the consortium.