

Summary

- Soil samples were collected from two locations, agricultural soil (sugarcane field) from Junagadh district and industrial site soil (engaged in γ-HCH manufacturing for more than 20 years) Vadodara district of Gujarat state, India.
- \checkmark Total 82 bacteria were isolated from the above mentioned soil samples.
- 22 bacteria were isolated from agricultural soil; however, none of these bacterial isolates showed γ-HCH biodegradation activity.

49 bacteria were isolated from industrial site soil by direct method without enrichment. One of the isolate showed zone of clearance on γ-HCH clearance assay. This isolate was identified by 16S rDNA sequence identity as *Shewanella* sp. CGR-L1.

- ✓ Shewanella sp. CGR-L1 was able to degrade about 68 % of 10 mg $l^{-1} \gamma$ -HCH within four days of incubation in MSM-L2 in presence of glucose.
- ✓ *Shewanella* sp. CGR-L1 was able to tolerate γ -HCH concentration of 50 mg Γ^1 in the MSM-L2 medium.
- \checkmark *lin*A gene involved in the biodegradation of γ -HCH was amplified and sequenced.
- ✓ Four copies of *lin*A were detected in southern blot analysis.

- One plasmid of size ~2.5 kb was observed in *Shewanella* sp. CGR-L1. Plasmid curing and PCR confirmed that it is not involved in the biodegradation of γ -HCH.
- Metabolite produced during the biodegradation of γ -HCH were identified as γ -PCCH and 1, 2, 4-TCB. We were isolated from industrial site soil after enrichment. One of the Eleven bacteria were isolated from industrial site soil after enrichment. One of the isolate showed zone of clearance in γ -HCH clearance assay. This isolate was identified by 16S rDNA sequence identity as *Sphingobium* sp. Strain CGR-L2.
 - Sphingobium sp. CGR-L2 showed around 97% biodegradation of γ -HCH after 3 days of incubation in MSM-L2 in absence of glucose.
- Increase in inoculum concentration augmented the degradation of γ -HCH residue in the medium.
- Maximum metabolic activity of *Sphingobium* sp. CGR-L2 was observed in the presence of glucose.
- ✓ Optimum pH and temperature for γ-HCH biodegradation by Sphingobium sp.
 CGR-L2 were 8 and 30^oC, respectively.
- ✓ Sphingobium sp. CGR-L2 degrades around ~87 % of the γ -HCH (10mg Γ^1) within the 9 hours of time.

Metabolite produced during the biodegradation of γ-HCH was identified as γ-PCCH.



- ✓ Reduction of other isomers, γ-HCH, β-HCH as well as α-HCH was observed during the biodegradation analysis with technical HCH.
- Genes involved in the biodegradation of γ-HCH *linA*, *linB*, *linC*, *linD*, *linE* and *linR* were present in the isolate *Sphingobium* sp. CGR-L2.
- ✓ Southern blot analysis indicated that isolate CGR-L2 carries a single copy of *lin*A, *lin*B and *lin* C gene.
- ✓ Sphingobium sp. CGR-L2 was able to degrade γ -HCH and β -HCH in microcosm study.
- ✓ Other bacterial isolates from enrichment culture showed random occurence of *linA*, *linB* and *linC*.
- ✓ To conclude, two γ -HCH degrading bacteria were obtained with different isolation approaches. Our results show that *Sphingobium* sp. CGR-L2 has the potential for use in bioremediation of soils contaminated with HCH.