

## CHAPTER VI: SUMMARY

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Culture independent molecular analysis was performed for analysis of microbial/methanogenic diversity present in the landfill leachate and marshland. 16S rDNA gene was used a biomarker for identification of bacterial and archaeal species present in both landfill site and marshland. Methyl coenzyme reductase A (mcrA) was used as a biomarker for identification of methanogens specifically. Bacteria belonging to family *Bacillus* and *Clostridia* were found to be abundant in the landfill site. In landfill site these microbes play an important role during anaerobic digestion of municipal solid waste and stabilisation. These microbes produce volatile fatty acids and H<sub>2</sub> by degrading cellulose and other biopolymers which are subsequently utilized by methanogens for production of methane. Bacteria like *Bacillus cereus*, *Bacillus licheniformis* and *Thermoanaerobacter tengcongensis* were detected which has industrial applications. 16S rDNA sequences affiliated to methylotrophic bacteria were also detected in the landfill site Delhi. Sequences obtained in this study were distantly related to the cultured species of *Methylophilaceae*. These microbes play an important role in biological mechanism for mitigation of methane and can oxidize upto 10-100% methane emitted from landfill cover soil. Methanotrophic communities enriched from the environment can also be used for bioremediation of the toxic chemicals and compounds present in contaminated environments more economically and in eco-friendly manner.

Denaturing gradient gel electrophoresis based *Lactobacillus* identification ladder was created for detection of lactic acid bacteria (LABs) in the landfill site. It indicated presence of *Lactobacillus acidophilus*, *Lactobacillus casei* and *Lactobacillus fermentum* species in the MSW leachate. Accumulation of lactic acid was also found during anaerobic digestion of MSW leachate in lab scale bioreactor. Accumulation of lactic acid was found to be higher in the reactor containing cow dung pus leachate in comparison to reactor containing only cow dung. The possible reason behind this may be the presence of heavy metals like molybdate and nitrite which suppress Sulphate reducing bacteria (SRB) which consumes lactic acid produced and compete with methanogens for H<sub>2</sub> during anaerobic digestion of MSW leachate.

Both 16S rDNA and mcrA gene sequencing revealed similar type of methanogens presence in the landfill site. But denaturing gradient gel electrophoresis (DGGE) based community profiling of methanogenic 16S rDNA and mcrA gene indicated contradictory results. DGGE profiling methanogens using 16S rDNA as a marker revealed lower diversity of methanogens in comparison to mcrA gene. Band pattern analysis of both gel in Gel 2K software indicates more number of bands in respective samples for mcrA gene in comparison to 16S rDNA gene. Therefore, mcrA gene can be used for identification and diversity analysis of active methanogens in a sample and for understanding methane dynamics.

Real time experiment indicated that landfill site has higher methanogenic richness than marshlands. This may be due to the presence of higher volatile fatty acids and other substrates in the anthropogenic system like landfill. Methanogens belonging to order *Methanomicrobiales* and *Methanobacteriales* were detected by both mcrA and 16S rDNA gene in the landfill. Real time experiment indicated presence of acetoclastic methanogens i.e. *Methanosarcina mazei* in both landfill site and Silicoorie Lake of Southern Assam. However, hydrogenotrophic methanogens *Methanobacterium mobile* has higher richness in both landfill and marshland samples.

In short, global warming and water pollution is among one of the major causes of concern to humanity in this modern era. Water vapour, carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>) and nitrous oxide (N<sub>2</sub>O) are the most important greenhouse gases, and human activities such as industry, livestock and agriculture, contribute to the production of these gases. Landfill cover soils play an important role in the CH<sub>4</sub> cycle as methanotrophy (oxidation of CH<sub>4</sub>) and methanogenesis (production of CH<sub>4</sub>) take place in them. The information about methanogenesis and methanotrophy and microbes associated will enrich our knowledge about how landfill, wetlands and human activity contribute to green house gas emission and global warming. It will be also useful in establishing new agriculture techniques and industrial processes that will be ecofriendly and provide a better balance of greenhouse gas.