5. Summary and Conclusion

Carbon dioxide is one of the potent greenhouse gases which become major concern over environment leading to environmental pollution to cause of global warming. Fermentation of carbon dioxide into methane was achieved by using methanogens. Direct sequestration of carbon dioxides directly implies on fourth generation biofuels. Biofuels are produced by biological process with high futuristic needs. Therefore, direct impact of carbon dioxide on environment stimulated our interest to conduct extensive research on sequestration of carbon dioxide into methane and bioconversion methane to methanol. This work is aimed to isolate potent carbon dioxide reducing methanogens for production of methane and to isolate effective methanotrophs for better conversion of methane to methanol.

About 12 Sediments samples were collected from various ecological niches of Tamilnadu, Pondicherry, Karanataka and Andaman Nicobar Islands. Sediment samples were enriched and processed. Upon initial screening, it appeared that total of 82 methanogenic isolates were isolated are purified and used for our studies. Out of 82 methanogenic isolates 66 isolates were able to utilize acetate as carbon source, sixteen isolates able to utilize both acetate and CO\textsubscript{2} as substrate and remaining 10 isolates were able to utilize H\textsubscript{2}:CO\textsubscript{2} (80:20). Isolates utilized CO\textsubscript{2} were M2, M3, M5, M7, M11, M12, M17, M19, M23 and M25. The microscopic examination of selected isolates revealed that all were gram positive short to filamentous long rods and all the isolates were non-motile under phase contrast microscope except M5 and M7 which were motile. All the isolates showed negative growth in aerobic heterotrophic medium. The methanogenic isolates are fast growers, able to grow in bicarbonate
medium in 6 days at H₂:CO₂ in gas space. Maximum gas production was achieved on 6th day of incubation. The isolate M11 produced highest gas production followed by M25, M3, M17, M12, M23, M2, M19, M7 and M5 and the presence of methane gas was confirmed by flame test and Gas Chromatography (GC) using Thermal Conductivity Detector (TCD).

The DNA base composition analysis of the isolates was analyzed.

The environmental parameter and effective CO₂ feeding rates were extensively studied to monitor the growth and methane production. The optimum methane production was found at 6th day of incubation. The methanogenic isolates showed good growth and methane by production at a broad temperature range 30-65°C with maximum methane production was by isolate M11 at 35°C. All the methanogenic isolates was able to grow at wide range of initial pH 5-9 and maximum was achieved by isolate M11 at pH – 7.0. Different ration H₂:CO₂ stimulated the methane production and the best production was exhibited by H₂:CO₂ (80:20). Isolate M11 produced higher amount of methane was selected reactor studies at optimized conditions, the isolate M11 able to convert 8.34 mole of carbon dioxide into 7.90 moles of Methane and remaining utilized for cell growth.

For phase two study, about 48 methanotrophic isolates were isolated from the collected samples. Further the isolates was differentiates into obligate methanotrophs and methylotrophs. Out of 48 isolates two isolates M29 and M31 found to be obligate methanotrophs. The specific activity of sMMO was expressed as nanomoles of naphthol formed per milligram of cell protein per minut. Therefore isolate M29 and M31 produced 275 and 450 nanomoles sMMO/ml culture. The isolate M31 which produced higher amount of sMMO used for reactor studies.
The Isolate M11 at optimized studies produced, the isolate M31 was able to convert 7.90 moles of methane into 4.65 moles of Methanol per hour.

In the conclusion the result of the study demonstrated a significant level of carbon dioxide sequestration rates. About 19.4% CO₂ was converted into methane and around 11% methane was effectively converted into methanol successfully. So, far limited studies have been done for cultivation of methanogens and methanotrophs for mitigation of carbon dioxide and methane respectively. In order to achieve more and enhanced production a detailed studies are needed in field of methanogenic archaea and methanotrophs.