I. Introduction

Natural gas (methane) is emerging as a viable power source for many industrial, commercial, and domestic applications. Bio-methane provides a promising replacement for mined natural gas. Methanogenic bacteria produce this bio-methane. These anaerobic bacteria pertain to the Domain Archaea, and are found in extreme environments where few other bacteria survive. They are employed by Up-Flow Anaerobic Sludge Blanket (UASB) reactors in the digestion of wastes to a marketable product (methane). The genome of methanogenic bacteria contains plates.

II. Objective

Design a new set of primers and develop a PCR protocol that will allow identification of archaeabacteria.

III. Methods

1. Obtain samples containing archaeabacteria from methane producing UASB reactor
2. Extract DNA with a commercially available kit (MO BIO PowerSoil® DNA Isolation Kit)
3. Identify archaeal primers through literature search and purchase primers
4. Adjust PCR protocols for optimal amplification
5. Verify amplification of DNA via gel electrophoresis
6. Clone amplified DNA into pCR®4-TOPO® vectors
7. Transform electrocompetent E. coli cells with vector
8. Culture transformed cells with vector on antibiotic-containing plates
9. Extract plasmids from selected colonies
10. Use the Basic Local Alignment Search Tool (BLAST) and National Center for Biotechnology Information (NCBI) database to identify DNA sequences

IV. Results

Before Optimization

After Optimization

Flow Chart of Methods

UASB Reactor

Biogas

Effluent

Three-phase separator

Baffles

Sludge bed

Sampling port #1

Sampling port #2

Biogas

Effluent

BIological ENGINEERING

Development of Archaeal and Algalytic Bacteria Detection Systems

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V. Conclusions

- Obtained clones with archaeabacterial 16S rRNA from environmental samples
- Achieved specific binding of archaea-targeting primers A571F and UA1204R
- Found optimal PCR reaction conditions for archaeabacterial primers

VI. Further Studies

Apply presented methods to:
- Identify algalytic bacteria
- Optimize bio-methane production

Bacteria Detection Systems

Development of Archaeal and Algalytic
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