PCR-based DGGE and FISH analysis of methanogens in an anaerobic closed digester tank for treating palm oil mill effluent

ABSTRACT

16S ribosomal RNA (rRNA)-targeted fluorescent in situ hybridization combined with polymerase chain reaction (PCR)-cloning, light microscopy using Gram stains, scanning electron microscopy and denatured gradient gel electrophoresis were used to reveal the distribution of methanogens within an anaerobic closed digester tank fed with palm oil mill effluent. For specific detection of methanogens, 16S rRNA-cloning analysis was conducted followed by restriction fragment length polymorphism (RFLP) for presumptive identification of methanogens. To cover the drawbacks of the PCR-cloning study, the organization of the microorganisms was visualized in the activated sludge sample by using fluorescent oligonucleotide probes specific to several different methanogens, and a probe for bacteria. In situ hybridization with methanogens and bacterial probes and denatured gradient gel electrophoresis within activated sludge clearly confirmed the presence of Methanosaeta sp. and Methanosarcina sp. cells. Methanosaeta concilii was found to be the dominant species in the bioreactor. These results revealed the presence of possibly new strain of Methanosaeta in the bioreactor for treating palm oil mill effluent called Methanosaeta concilii SamaliEB (Gene bank accession number: EU580025). In addition, fluorescent hybridization pictured the close association between the methanogens and bacteria and that the number of methanogens was greater than the number of bacteria.

Keyword: Anaerobic digestion; DGGE; FISH; Methanogens; POME