



Identification of Methane-producing Bacteria from Palm Oil Mill Sludge (POMS) with Solid Cud from Ruminant Stomach

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Abstract

Biological generation in anaerobic environments such as enteric fermentation and anaerobic waste treatment from agriculture sector are the major contributor of methane gas which has the potential as biogas. The aimed of this study was to identify methane-producing bacteria in anaerobic vessel which contained a mixture of Palm Oil Mill Sludge (POMS) and solid cud taken from the first compartment of cow's stomach (1:2 and 2:1 ratio) as co-mixture. The co-mixture was incubated at 50 °C in a 2 L vessel with initial starter of 400 ml and sampling was conducted every 4 weeks interval during 12 weeks of incubation. For specific detection of methanogens, 16S rRNA-cloning analysis was carried out. *Methanobrevibacter* sp. and *Methanosaeta* sp. were confirmed to be presence within the 2:1 ratio of co-mixture while only *Methanobrevibacter* sp. was found in 1:2 ratio of co-mixture on both Week 0 and Week 4. No methanogens were detected for both co-mixtures on Week 8 and Week 12.

Introduction

Crude palm oil (CPO) production in Malaysia has been increasing continuously over the years, from 4.1 million tonnes in 1985 to 6.1 million tonnes in 1990. The production is further increased by 11.29% to 18.9 million tonnes in 2011 [1]. However, increase of production leads to generation of huge quantities of wastes. During oil extraction process, about 50% of water used results in palm oil mill effluent (POME) while others are lost as steam, mainly through sterilizer exhaust, piping leakages as well as wash water [2]. POME contains suspended solids and total dissolved solids in the range between 18,000 mg L⁻¹ and 40,000 mg L⁻¹ respectively [3]. Both solids are known as palm oil mill sludge (POMS). POMS consists of 3.6, 0.9 and 2.1 mg L⁻¹ of total nitrogen, phosphorus and potassium, respectively, which results in bad odors and is consider as a source of ground pollution [4].

POMS can be applied as fertilizer as it has high nutrient value [5]. However, during rainy season, the drying process of POMS becomes difficult as the rate of drying become slower. Due to this limitation, anaerobic treatment of POMS such as anaerobic digester offer more attractive solutions for biogas production and clean development mechanism (CDM).

Anaerobic digestion process involves a wide variety of microbial community. In order to produce higher biogas yield, inoculum source is crucial for optimization of inoculum ratio. In this study,

the aim of the present work was to determine the methane-producing bacteria community in POMS with solid cud from ruminant stomach using 16S rRNA clone library techniques.

Material and Methods

Samples Collection

Palm Oil Mill Sludge (POMS) was collected from the anaerobic pond from Bau Palm Oil Mill (BAPOM), Kuching, Sarawak. The solid cud from the first compartment of cow's stomach was collected from a slaughter house located at Ladang Lapan, Kuching. Both samples were stored in sealed container immediately after collection and preserved at 4 °C in order to avoid biodegradation due to microbial activities.

Anaerobic vessel set up

Co-mixture with different ratio (Table 1.0) were incubated at 50 °C in a 2 L vessel with initial starter of 400 ml. Sampling for both ratio were conducted every 4 weeks interval during 12 weeks of incubation.

DNA Extraction and PCR Amplification

Bacterial DNA of both ratio of co-mixture were extracted using Power Soil™ DNA Isolation Kit (Mo Bio Laboratories, USA) and amplification of 16S rRNA region was amplified using