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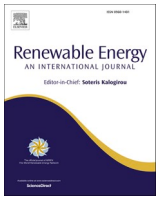

Evolution of microbial community structure during biohydrogen production process of palm oil anaerobic sludge

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ABSTRACT

Anaerobic microbial communities and their functions can provide information about waste management practices, as well as reveal novel degradation metabolisms that can be utilized in wastewater treatment. This study was aimed towards sustainable palm oil industry with assessing the microbial evolution during the dark fermentative processing of palm oil anaerobic sludge and palm oil mill effluent (POME) in batch-mode over two-months with a distinct incubation time of 24 h. The process was designed using response surface methodology (RSM) by acclimatization of inoculum using POME with a chemical oxygen demand (COD) of 36 g-COD/l. The effect of mesophilic and thermophilic conditions and pH on COD removal, hydrogen content, and hydrogen yield (HY) were investigated. A potential sample was selected for microbial analysis after the first and second months of operation based on the maximum outputs achieved throughout the experiment. The bacterial community at pH 5.6 and temperature 55 °C with maximum hydrogen content of 64.13 %, and HY of 0.93 ml H_2/g-COD was dominated by three phyla: Firmicutes, Actinobacteria, and Bacteroidota. The results obtained from high throughput pyrosequencing showed that how acclimatization period affects the microbial community structure towards Firmicutes phylum, which includes the majority of hydrogen-producing microorganisms.

1. Introduction

The production of bioenergy from organic waste is becoming a crucial part of sustainable energy development. A significant proportion of Malaysia's palm oil production is crude palm oil (CPO), which is produced annually at a rate of 13 million tons per year [1]. It is projected that about 1.5 tons of palm oil mill effluent (POME) are produced per ton of fresh fruit bunch (FFB) during palm oil extraction [2]. POME is a thick brownish wastewater with a high amount of chemical oxygen demand (COD) and biological oxygen demand (BOD) which are defined as the amount of oxygen needed to oxidize chemical compounds or biologically breakdown organic matter by microorganisms, which can cause severe environmental pollution, mainly contamination of the water resources [3]. POME contains considerable amounts of carbohydrates, nutrients, and fermentable organic materials, which makes it a potential substrate for biological processes [4]. Amongst the biological processes, dark fermentation suggests two profits of wastewater treatment along with sustainable biogas production, which plays a significant role in endorsing the circular economy perspective in the palm oil sector [5]. In addition to producing bio-hydrogen, dark fermentation is a promising method for treating agro-industrial wastes. Dark fermentation or anaerobic digestion occurred in mixed-culture or pure culture of microorganisms. In pure culture where only single species of bacteria is present that are known to produce H_2 can be used as microbial inoculum, facultative anaerobes such as *Klebsiella* sp., *Enterobacter* sp., or strict anaerobes such as *Clostridium* sp. from the *Clostridiaceae* family are potential strains of bacteria that can be selected for this process [6,7]. The inoculum can also be derived from engineered ecosystems, such as

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