



## Article Preliminary Investigation of Different Types of Inoculums and Substrate Preparation for Biohydrogen Production

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Abstract: A pre-culture stage is required to obtain robustly-dividing cells with high hydrogen (H<sub>2</sub>) production capabilities. However, a step-by-step process for biohydrogen production is scarcely reported, mainly from palm oil wastewater. Therefore, this study developed a guideline to find the best inoculum heat treatment conditions and implement the selected conditions for biohydrogen production using palm oil wastewater. This study used raw palm oil mill effluent (POME) and POME sludge as substrate and inoculum, respectively. Our findings reveal that 80 °C and 30 min were the best conditions for inoculum heat treatment. When testing the conditions on POME sludge and inoculating with raw POME (28 g COD/L) at 37 °C (reaction temperature), 24 h (reaction time), and pH 5.5, 34 mL H<sub>2</sub>/d was recorded. A slight increase (1.1-fold) was observed compared to 5 g COD/L POME co-digested with 5 g/L glucose (31 mL H<sub>2</sub>/d). This discovery indicates that raw POME is a potential source for biohydrogen production under anaerobic fermentation and can be directly used as substrate up to 30 g COD/L. The proposed guideline could also be implemented for different organic wastes for biohydrogen production study.

Keywords: anaerobic digestion; biohydrogen; heat treatment; palm oil; renewable sources; energy

## 1. Introduction

Hydrogen ( $H_2$ ) is considered a clean and long-lasting energy carrier. For the past 15 years, the focus on hydrogen production from organic waste has been increasing, fuelling research in the field. Organic wastes, such as agricultural waste, contain specific microbes that may contribute to hydrogen production in the future and prove to be a viable renewable energy source. Increasing the performance of biohydrogen production from lignocellulosic biomass is thus an important research direction since biohydrogen is a green and environmentally friendly energy carrier that has the potential to reduce our reliance on fossil fuels.

Palm oil mill effluent (POME) is one such source of agricultural waste, and palm oil milling is one of Malaysia's most important industries. POME is produced by milling either dry or wet palm oil fruit bunches. Several studies reported that 5 to 7.5 tonnes of water are used for every metric tonne of crude palm oil (CPO) produced, with more than half of this typically ending up as POME [1,2]. Generally, POME contains a high amount of organic



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