## Experimental Assessment of Mesophilic and Thermophilic Batch Fermentative Biohydrogen Production from Palm Oil Mill Effluent Using Response Surface Methodology

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Abstract – The present work evaluated the production of biohydrogen under mesophilic and thermophilic conditions through dark fermentation of palm oil mill effluent (POME) in batch mode using the design of experiment methodology. Response surface methodology (RSM) was applied to investigate the influence of the two significant parameters, POME concentration as substrate (5, 12.5, and 20 g/l), and volumetric substrate to inoculum ratio (1:1, 1:1.5, and 1:2, v/v.%), with inoculum concentration of 14.3 g VSS/l. All the experiments were analyzed at 37 °C and 55 °C at an incubation time of 24 h. The highest chemical oxygen demand (COD) removal, hydrogen content (H<sub>2</sub>%), and hydrogen yield (HY) at a substrate concentration of 12.5 g COD/l and S:I ratio of 1:1.5 in mesophilic and thermophilic conditions were obtained (27.3, 24.2%), (57.92, 66.24%), and (6.43, 12.27 ml H<sub>2</sub>/g COD<sub>rem</sub>), respectively. The results show that thermophilic temperature in terms of COD removal was more effective for higher COD concentration of 14.3 g COD/l showed higher results in both temperatures. It is recognized how RSM and optimization processes can predict and affect the process performance under different operational conditions.

Key words: Biohydrogen production, Mesophilic, Thermophilic, Dark fermentation, Palm oil mill effluent

## 1. Introduction

Palm oil mill effluent (POME) as a renewable resource for biogas production has received attention in Malaysia. It is projected for each ton of crude palm oil, around 3.5 tons of POME are produced [1,2]. POME is a complex thick brownish effluent discharged from the palm oil mill industry. It is not toxic, but due to its high organic content, is considered particularly contaminating [3]. Its characterization might vary according to the operational process and raw material used. This industrial effluent has been projected as a potential substrate for biohydrogen production due to its large quantity, low cost, and reliability [4]. Among different biological methods, dark fermentation is the most studied and promising method for biohydrogen production [4]. In this process, microorganisms in pure or mixed culture are responsible for treating wastewater and producing biohydrogen simultaneously [1]. Biohydrogen has been considered as the most capable energy carrier among all the current fuels. To produce hydrogen, it is required to remove H<sub>2</sub>-consuming bacteria (HCB)

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This is an Open-Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/bync/3.0) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited. from the anaerobic sludge as a mixed culture medium, avoiding methanogenesis bacteria [1]. Several types of pre-treatments can be preferred considering the microflora in the inoculum [5]. To develop  $H_2$  production efficiency, some key parameters need to be studied. The factors that mostly affect the hydrogenase enzymes activity are pH, temperature, types of substrate and substrate concentration [6].

It is essential to control pH value in an optimum range to preserve hydrogen production. During the building up the hydrogen through dark fermentation process, volatile fatty acids such as butyric and acetic acids with high molecular weight are accumulated, resulting in a pH drop in the system. Hence, it is necessary to control the pH in the desired range, mostly between 5 to 6; otherwise it will confine microbes from growing and stop hydrogen production [1,3]. Biohydrogen production process through dark fermentation could be produced in a different range of temperatures, including mesophilic, thermophilic, and extreme thermophilic. From economical and technological points of view, the mesophilic condition is desirable to the thermophilic condition; however, thermophilic dark fermentation, due to a higher amount of hydrogen yield and production rate, shows more potential than the mesophilic conditions [7]. Moreover, the rate of biochemical processes is influenced by temperature due to the effects of the enzymatic activity. Whereas substrate concentration affects the metabolic pathways of microbial community structures.