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# Influence of thermal and chemical pretreatment on structural stability of granular sludge for high-rate hydrogen production in an UASB bioreactor

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## ABSTRACT

Fermentation of organic waste materials presents an alternate route instead of photo-synthetic and chemical routes for hydrogen production. Low yield of biohydrogen production is the major challenge in the fermentative hydrogen-producing technology. Improvement of fermentation process by various sludge pretreatment methods is one of the ways that have been applied to boost hydrogen productivity. This study sheds new light on the impact of thermal and chemical pretreatments on the hydrogen-producing granular sludge morphology and strength as well as up-flow anaerobic sludge blanket (UASB) reactor performance treating palm oil mill effluent (POME). Thermal pretreatment showed devastating effects on the morphological and structural characteristics of the granules. However, the chemically pretreated granules remained structurally stable and relatively undamaged. The thermal pretreatment increased the cumulative hydrogen production by 40% and 76% over chemical pretreatment and control test (untreated), respectively.

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## Introduction

The rising global demand for energy along with growing concerns over adverse environmental conditions has made it of paramount importance to substitute carbon-containing fossil fuels with renewable and clean energy sources [1]. Among the alternative sources of energy, hydrogen-based systems feature various advantages, such as nominal or zero

consumption of hydrocarbons, high energy density per unit mass (122 kJ/g), low environmental impact (H<sub>2</sub>O is the sole by-product of H<sub>2</sub> combustion), versatile resources, and good storability [2]. As a synthetic energy carrier, hydrogen has many potential applications, including as a starting material in chemical and petrochemical industry (ammonia and methanol production), petroleum processing, metal refining, food processing (oil and fat hydrogenation), as a fuel in automobiles, rockets and fuel cells [3,4].

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Because hydrogen is not easily accessible in nature like convenient fossil fuels, hydrogen fuel must be produced from other feedstock and resources, such as fossil, nuclear and renewable resources. A wide range of processes and technologies may be employed for hydrogen production which can be organized into two general groups based upon the raw material used, conventional and renewable. In the former technology, fossil fuels, especially natural gas, serve as a resource for hydrogen production through different methods, mainly including hydrocarbon reforming processes (steam reforming, partial oxidation (POX), and autothermal reforming (ATR)) [5]. Presently, conventional techniques account for most of industrially produced hydrogen due to the ease of use and relatively low costs. High energy intensity, the emission of potent greenhouse gases (CO<sub>2</sub> and CH<sub>4</sub>), and fossil fuel exhaustion are the driving forces behind replacing fossil fuel based methods for hydrogen production [6,7]. The green hydrogen generation from renewable resources has been of a great importance over the past three decades and fall into three general groups: electrolysis (water splitting), biomass conversion (thermochemical, biochemical and fermentation techniques), and solar conversion (thermolysis and photolysis) [8–10]. The fermentative hydrogen production presents a promising route because of its low energy intensity, economic and technical feasibility, simple operation, applicability to different waste materials as substrate and rapid hydrogen production rate [11–13]. Recently, many studies have focused on biohydrogen production through dark fermentation as a sustainable approach to generate clean hydrogen energy. Among factors affecting the dark fermentative hydrogen production (DFHP), reactor design considerations play a pivotal role. Various bioreactor configurations have been used for a wide range of wastewaters as feedstock to produce biohydrogen [14]. More energy-efficient reactor types include continuous stirred tank reactor (CSTR) [15], sequencing batch reactor (SBR) [16], packed bed reactor (PBR) [17], fluidized bed reactor (FBR) [18], immobilized cell reactor (ICR) [19], up-flow anaerobic sludge blanket (UASB) reactor [20], membrane bioreactor (MBR) [21]. Hydraulic retention time (HRT) is a key operational parameter which affects the efficiency of the reactors for biohydrogen production [22]. The high-rate up-flow anaerobic sludge blanket (UASB) technology has gained popularity for biological hydrogen generation over the last decade due to its operation at very short HRT, high concentration of self-granulated biomass, and more stable operation under extreme conditions [23]. In order to overcome the detrimental impact of hydrogen-consuming bacteria (HCB) in the processes using mixed microflora and subsequent low hydrogen yield, inoculum must be pre-treated to dominate hydrogen-producing bacteria (HPB) [24]. In literature, numerous pretreatment techniques have been proposed to select HPB and to suppress HCB including mechanical treatment [25], thermal treatment [26], pH stress (acid or alkaline treatment) [27,28], wave and radiation stress (microwave [29], ultrasound [30], ultraviolet [31], infrared [32], and gamma [33] irradiation), chemical treatment [34], and aerobic stress [35]. Successful results have been reported with the use of chemical (chloroform) and thermal pretreatment methods to enrich HPB [43,44]. One strategy for shortening the long start-up period (2–4 months), a substantial problem linked to the

stable operation of UASB systems, is to use the granulated hydrogen producing sludge as seed. Pretreatment methods may cause damage to the structural and morphological properties of granular sludge. So far, there is little information on the impact of these methods on the structural stability of granular sludge. Thus, this study was conducted to compare the morphology, structural stability, and hydrogen production yield of intact granular sludge with that of thermal and chemical pre-treated in a UASB reactor treating POME.

## Materials and methods

### Feed substrate

The POME was collected from raw and pretreated (after first anaerobic pond) POME (sampling location is shown in Fig. 1S (Supplementary materials)). Raw POME samples were stored in a cold room at 4 °C before use. The feed did not undergo any noticeable change (in composition) during the storage. The characteristics of POME used in this study is shown in Table 1.

### Inoculum source and treatment condition

The granular seed inoculum was taken from an up-flow anaerobic sludge blanket (UASB) reactor of Carlsberg Brewery Company (Kuala Lumpur Malaysia Berhad). At the outset, the sludge was traversed through a screen (size 2.0 mm) to remove fragments and rinsed with tap water. Then, the sludge was acclimated to ensure that the bacteria would adjust to their new environment by feeding gradually of POME as organic source. 10 mL of the sample sludge was evaporated to dryness at 100 °C for 30 min and then well mixed and ignited at 450 °C for 15 min. The chemical pre-treatment was conducted by adding 0.1% (v/v) of chloroform into the sludge followed by 24 h incubation. The thermal pre-treatment was conducted by heating the sludge to 90 °C for 1 h in the water bath.

### Batch tests

In this study three 500 mL Schott bottles were used, which two bottles were filled with 50 mL acclimated and pretreated sludge and one bottle with untreated sludge as a control and 150 mL of POME. The initial pH of the mixture was adjusted to 5.5 and sparged with nitrogen to obtain an anaerobic condition. All experiments were incubated at 35 ± 2 °C, 120 rpm for 72 h in an incubator shaker and were carried out in three replicates.

**Table 1 – Characteristics of wastewater samples used.**

Parameter	Value		Unit
	Sample 1	Sample 2	
TCOD	61600	56700	mg/l
SCOD	21500	24000	mg/l
PCOD	40100	32700	mg/l
TSS	18800	20100	mg/l
pH	5.1	5.0	–

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