Influence of nano nickel oxide (NNO) particles on hydrogen production in dark fermentation of palm oil mill effluent

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Abstract - Batch experiment with initial pH of 5.5, was conducted to produce hydrogen from palm oil mill effluent (POME). Nano nickel oxide (NNO) particles were supplemented (20-140 mg\l) to POME to enhance biohydrogen production. *Clostridium species* LS2 was used as inoculum. At initial hydrogen production rate was significantly increased from 2 to 15 ml H_2/h with the increasing NNO particle concentration from 20 to 80 mg/l.. While the NNO concentration continued to increase from 80 to 140 mg/l, the hydrogen production rate gradually decreased. The maximum COD removal potential of 59 % was obtained in the presence of 80 mg/l of NNO after 96 h of dark fermentation. Addition of nano micronutrient to the production medium enhanced the hydrogen production rate and COD removal efficiency thus improved concurrent hydrogen production and pollution reduction from POME with activated sludge.

Keywords: Biohydrogen; Nano nickel oxide particles; COD removal; Dark fermentation

1. INTRODUCTION

Shortfall of fossil fuel makes it necessity to find out the alternative way of fuel. Hydrogen production via waste fermentation has significant approach due to its zero carbon emission at the point of use and a high energy yield of 142.35 kJ/g, which is 2.75 times than that of any other hydrocarbon fuel[1, 2]. [Ni–Fe] hydrogenases and the [Fe–Fe] hydrogenases are the enzymes which are able to catalyze reduction reactions inside the bacterium during hydrogen production. They content metal ions in their active sites [3].The [Ni–Fe] hydrogenases are widely distributed among bacteria, whereas the [Fe–Fe] hydrogenases are restricted to a few bacteria. Moreover, the [Ni–Fe] hydrogenases have a higher substrate affinity than the [Fe–Fe] hydrogenases [4]. Since nickel is a fundamental component making up the [Ni–Fe] hydrogenases, it may influence the fermentative hydrogen production by influencing the activity of [Ni–Fe] hydrogenases and thus may play an important role in fermentative hydrogen production.

The potential of using palm oil mill effluent (POME) as a fermentation medium to produce hydrogen gas has been recently researched [5,6]. Study of nano particle functions is gaining impetus because nanoscience and nanotechnology encompass a wide range of fields, including chemistry, physics, materials engineering, biology, catalysis, medicine, and electronics. Small quantities of many metals, including copper, magnesium, sodium, potassium, calcium, and iron are essential for proper functioning of biological systems[7].Important parameters such as yield, efficiency, release rates, and cost have all seen benefits from the application of nano particles [8].

Considering the essentiality of nickel to fermentative hydrogen production, the objective of this study was to investigate the effect of Ni^{2+} oxide nano particles ranging from 20 to 140 mg/l on fermentative hydrogen production by using culture of Clostridium sp. LS2 from palm oil mill effluent in batch test with the purpose of obtaining the appropriate concentration of nano nickel oxides.

2. MATERIALS AND METHODOLOGY

2.1. Palm oil mill effluent and Microbial culture

The raw POME was collected from the final discharge point of Felda palm oil industry, Lepar Hilir, Gambang, Pahang, Malaysia. POME was preserved and refrigerated at 4°C prior to use for the study to decrease the biological degradation and acidification. The bacterial culture *Clostridium species LS2* used for fermentation was procured from our lab [9]. The medium containing per litre of distilled water: NH_4HCO_3, 2 g; K_2HPO_4, 100 mg; $MgSO_4$ .7H_2O, 100 mg: NaCl, 10 mg; Na_2MoO_4 .2H_2O, 10 mg; $MnSO_4$ .7H_2O, 15 mg; $FeCl_2O$, 10 mg; $CaCl_2$, 2.78 mg was supplemented for the growth of hydrogen producing bacteria.

2.2. Nickel oxide nano particles

The commercially available Nano nickel oxide (NNO) particles (density- 0.69 g/ml at 20^oC, 0.5 % - 0.7 % as nickel, particle size- 6.5nm± 3nm) in the clear liquid form were procured from Sigma Aldrich, Malaysia, used to investigate the effect of NNO particles on the hydrogen production. Oleic acid and Heptane and were used as a stabilizer.

2.3. Experimental setup for hydrogen production

The batch hydrogen production experiments were carried out in a 500 ml conical flasks with working volume of 300 ml. 10% active inoculum of *Clostridium species LS2* (OD_{660}~1) was carefully in the conical flasks and the experiments were done for seven different NNO concentration (20, 40, 60, 80, 100, 120, and 140mgl^{-1}). The medium was spurge with nitrogen gas to provide anaerobic condition, sealed with butyl rubber stopper and incubated under stirring condition at 200 rpm and 35^oC. The initial pH was set to 5.5 before the fermentation.

2.4 Analytical methods

Hydrogen percentage was measured by using Gas chromatograph (GC 8500 Perkin Elmer, Shimadzu, Japan) equipped with a thermal conductivity detector (TCD) and two Porapak columns. The hydrogen content was measured by using a 2.4 m×6 mm stainless column packed with Porapak Q (80/100 mesh). Nitrogen was used as gas carrier at a flow rate of 25 ml/min. Helium was applied as the carrier gas for GC with a flow rate of 25 ml/min. Measurements of chemical oxygen demand (COD) were carried out by using standard procedure according to Standard Methods for the Examination of Water and Wastes [10]. The hydrogen production rates were measured by using water displacement method. Hydrogen yield was calculated by dividing the amount of hydrogen produced over the amount of production medium (ml H_2/ml medium) (1).

$$\textbf{Hydrogen yield(y)} = \frac{\textbf{Total volume of hydrogen obtained(ml H2)}}{\textbf{Amount of production medium applied (ml)}} \quad (1)$$

3. RESULTS AND DISCUSSION

To analyze the effect of NNO on hydrogen production using POME as a substrate, dark fermentation was performed in the presence of *Clostridium species* LS2 as hydrogen producer. Figure 1 shows that when the production medium were supplemented with the NNO between the concentration of 20 to 80 mg/l, maximum hydrogen production rate of 15 ml H_2/h was observed at concentration of 80 mg/l.. Further increase of NNO concentration ranged from 80- 140 mg/l shows the gradual decrease in the hydrogen production rate as shown in Fig. 1. The result has an agreement with the study in which Li and Fang (2007a) showed that the hydrogen production potential can be influenced by using nickel oxides. In this study NNO showing the different pattern of hydrogen production rate in compare to study done by Li and Fang (2007a) [11]. The possible reasons behind this may be due to the substrates and pH variation. The hydrogen yield is calculated by dividing the total volume of hydrogen producing in batch test by volume of the production medium. Fig. 2 shows the effect of NNO particles concentration on the hydrogen yield. The result shows that the hydrogen yield in batch tests increases NNO concentration from 20 to 100 mg/l, but it decreases with further with increasing NNO particle concentration from 100to 140 mg/l. Hydrogen yield was higher than that of the control test with increasing NNO concentration from 20 to140 mg/l. Because [Ni–Fe] hydrogenases presents in generally all the hydrogen-producing bacteria, NNO may accelerate the fermentative hydrogen production by influencing the activity of [Ni–Fe] hydrogenases. Therefore, in an optimum range of NNO can enhance the ability of hydrogen-producing bacteria to produce hydrogen with increasing concentration, which was also made evident by [12], but Ni^{2+}at much higher concentration can decrease it [11].

In the mean while the enhanced hydrogen production rate was perceived due to the utilization of complex organic matters for nourishing Clostridium sp. LS2 and hydrogen production. During batch fermentation COD of the production medium was measured at every 12 hour interval of time (Fig. 3). The result showed that 59 % of COD was removed in POME at the concentration of 80 mg/l NNO particles. At the beginning the COD removal was observed very low e.g. After 12 the COD removal was only 4%. This is obviously because of the bacteria were low in number and had low catalytic activity as well. Later on it trended to increase gradually with further increasing in hydrogen production rate. This indicates that the bacteria couldn't utilize the substrate effectively because of the presence of complex organic compounds in the POME [13].COD removal was higher than that of control that 51% of COD removal was analysed without adding the NNO while, addition of this increase the COD removal percentage from 51 to59 %. The results showed in an appropriate range, NNO particles could increase COD removal percentage with increasing concentration.

4. CONCLUSION

The effect of NNO particles concentration ranging from 20 to 140 mg/l on fermentative hydrogen production from POME by Clostridium species LS2 was investigated in batch test. The following conclusion could be drawn. At 35°C and initial pH 5.5, NNO particles were able to increase hydrogen production rate along with hydrogen yield. The maximum hydrogen production rate of 15 ml H_2/h and the maximum yield of 2.60 ml H_2/ml POME were obtained at 80 and 100 mg/l of NNO. NNO particles had effect on COD removal efficiency with concentration of 80 mg/l, 59% of COD removed from the POME while in control 51 % of COD removed. This study indicates the application of nano nickel oxides can achieve higher hydrogen yield and simultaneous treatment of waste water.

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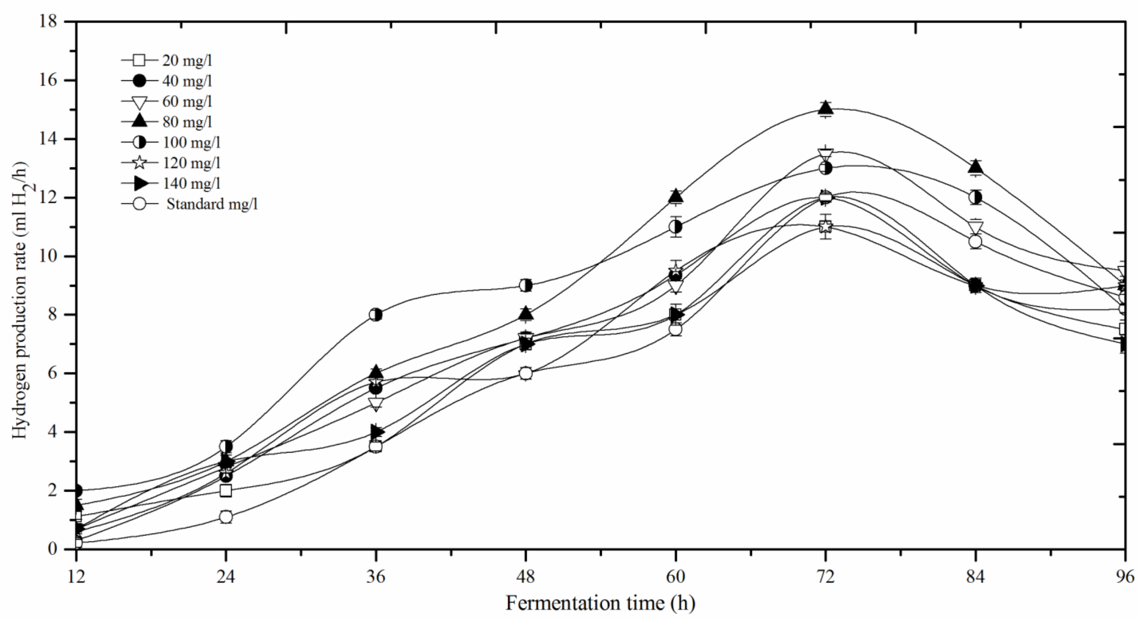

Figure 1: Time course of hydrogen production in the presence of NNO in various concentration (Standard = without nickel oxide)

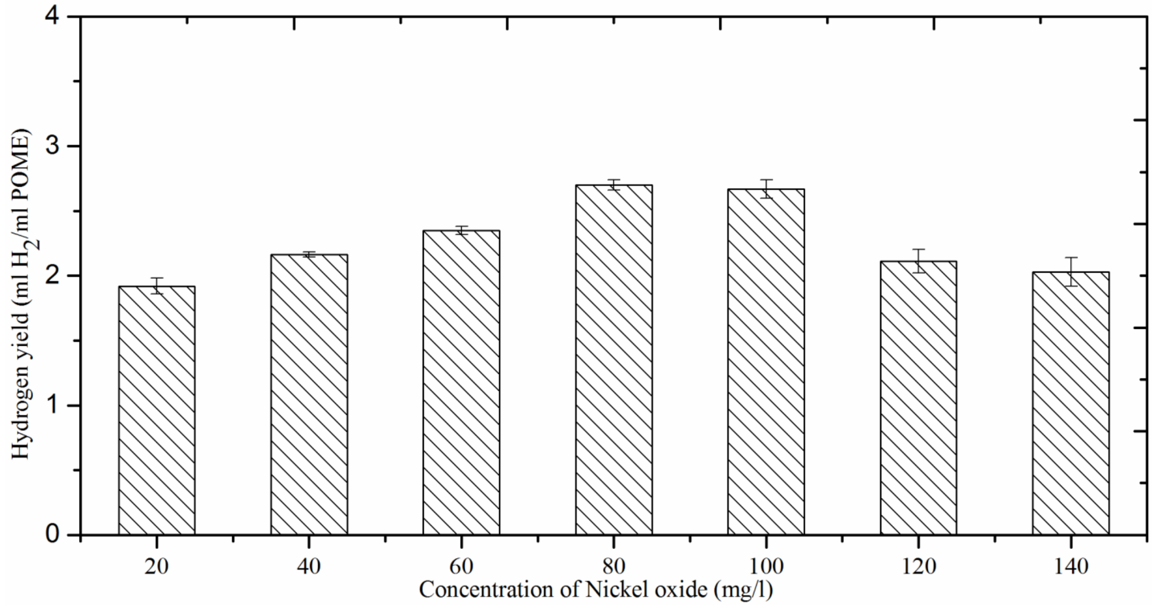

Figure 2: Hydrogen yield, in the presence of nickel oxides in various concentrations

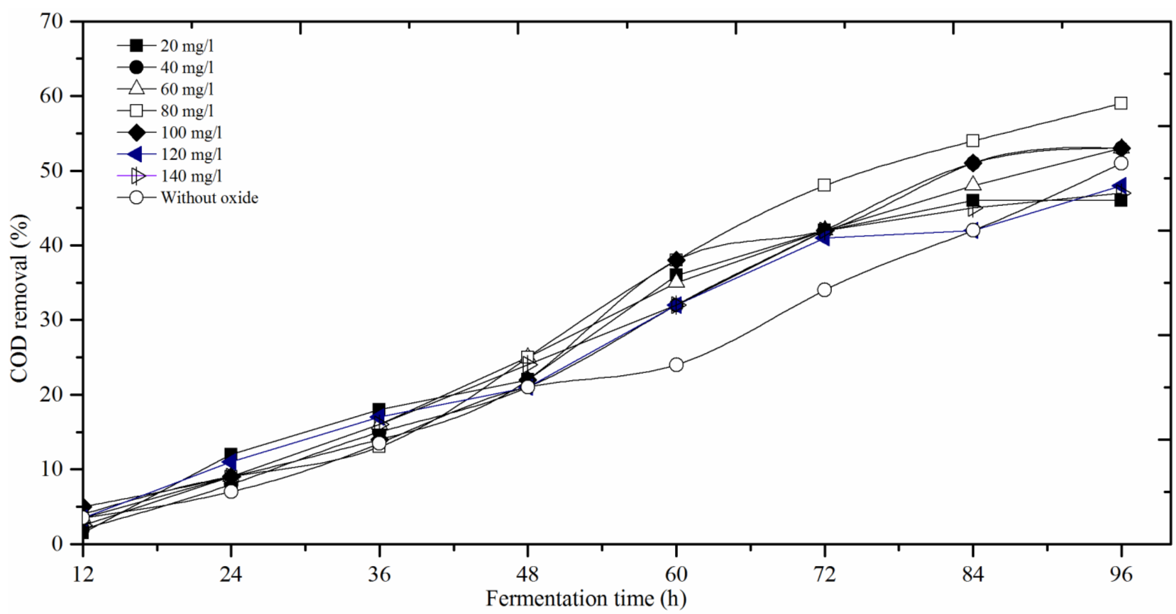

Figure 3: COD removal from POME in the presence of NNO in various concentrations

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