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A N I N T E R N A T I O N A L J O U R N A L

Immobilized Biofilm in Thermophilic Biohydrogen Production using Synthetic versus Biological Materials

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ABSTRACT

Biohydrogen production was studied from the vermicelli processing wastewater using synthetic and biological materials as immobilizing substrate employing a mixed culture in a batch reactor operated at the initial pH 6.0 and thermophilic condition (55 ± 1ºC). Maximum cumulative hydrogen production (1,210 mL H_2/L wastewater) was observed at 5% (v/v) addition of ring-shaped synthetic material, which was the ring-shaped hydrophobic acrylic. Regarding 5% (v/v) addition of synthetic and biological materials, the maximum cumulative hydrogen production using immobilizing synthetic material of ball-shaped hydrophobic polyethylene (HBPE) (1,256.5 mL H_2/L wastewater) was a two-fold increase of cumulative hydrogen production when compared to its production using immobilizing biological material of rope-shaped hydrophilic ramie (609.8 mL H_2/L wastewater). SEM observation of immobilized biofilm on a ball-shaped HBPE or a rope-shaped hydrophilic ramie was the rod shape and gathered into group.

Key words: Biohydrogen production, Biofilm, Synthetic material, Biological material, SEM

INTRODUCTION

Due to climate change and global warming resulting from the combustion of fossil fuels, clean energy alternatives are in the agenda of almost every industrialized nation all over the world (Keskin et al. 2011). In particular, fermentation processes that utilize free carbon available in large-volume discharges of agro-industrial wastewater containing carbohydrates can recover available energy as well as purify the effluent. High-temperature conditions are especially conductive to hydrogen-producing reactions because of the thermodynamics of the reaction processes. Thermophilic bacteria can utilize a variety of carbon sources and generate high yields of hydrogen as well as tolerate acidic fermentation processes (Hasyim et al. 2011). However, hydrogen production rates using fermentative microorganisms can be enhanced by developing suitable immobilization methodologies (Basile et al. 2010). Many works have reported about the immobilization of bacteria on supporting materials to improve hydrogen productivity by helping the acclimatization of microbes, decreasing the lag phase of bacterial cultivation (Bai et al. 2009), and increasing the density of consortia (Wu et al. 2003). Several synthetic materials prepared from activated carbon (Zhang et al. 2008), expanded clay (Barros et al. 2010), glass bead (Zhang et al. 2007), polystyrene, and PET (Barros and Silva 2012) and biological materials such as luffa sponge (Chang et al. 2002), coir, rice straw, and bagasse (Kumar and Das 2001) have been used for immobilization for biohydrogen production.
In this study, the objectives were to investigate the thermophilic biohydrogen production from

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vermicelli processing wastewater by immobilizing synthetic materials at the ratios of medium volume to wastewater volume (0-6%, v/v) and to comparatively evaluate the biohydrogen production using immobilized synthetic and biological materials under thermophilic condition.

MATERIAL AND METHODS

Preparation of feedstock and supporting materials

Wastewater was collected from a rice vermicelli factory, located in Nakhonpathom province, Thailand by a water sampler (grab sampling method) and used as substrate for fermentative hydrogen production. The physical and chemical characteristics of the wastewater were pH 5.0, COD 7,220 mg/L, BOD 5,400 mg/L, TKN 68 mg/L, and TS 5,325 mg/L (APHA 2005). Anaerobic sludge was taken from the Bio–fertilizer plant in Nonthaburi province, Thailand. Seed sludge was screened with a sieve (2.0 mm) to eliminate the large particulate materials and was heated at 90ºC for 10 min to inhibit hydrogen–consuming bacteria and facilitating the growth of spore–forming bacteria (Valdez-Vazquez et al. 2009). Three types of synthetic materials (acrylic, polyethylene, and polyvinylchloride) with ball, wheel and ring-shaped hydrophobic materials (ring-shaped materials, i.e., hydrophobic acrylic, HBA, and polyvinylchloride, HBPVC; ball-shaped hydrophobic polyethylene, HBPE and wheel-shaped HBPE) were used. Two types of biological materials (ramie and loofah) with rope and cubic-shaped hydrophilic materials, which were the rope-shaped hydrophilic ramie and cubic-shaped hydrophilic sponge were also used. All materials were placed in reactor at 0-6% (v/v). Their characteristics are summarized in Table 1.

Table 1 - Characteristics of synthetic and biological materials.

Parameter	Specifications			
	Synthetic material			
Shape	Ring	Ring	Ball	Wheel
Material	Acrylic	Polyvinylchloride	Polyethylene	Polyethylene
Dimension (width x height) (mm^2)	20 x 10	20 x 10	20 x 20	11 x 7
Volume (cm^3)	1.13	1.13	1.00	0.20
Total surface area (m^2/m^3)	432	432	250	850
Parameter	Biological material			
Shape	Rope		Cubic	
Material	Ramie		Loofah (sponge)	
Dimension (width x height) (mm^2)	0.20 x 50		10 x 10	
Volume (cm^3)	NA		1.00	

Experimental setup

The batch reactor of 250 mL lab bottle with a working volume of 200 mL was added with 20 mL of heated seed sludge and 180 mL of wastewater. The mixed liquor in the batch reactor was first purged with nitrogen gas for 1 min to ensure an anaerobic condition prior to each run and clogged with a silicone rubber stopper and connected to the three-way stopcock. The bioreactor was connected to airbag to avoid gas leakage from the bottle. The bioreactors were placed in a water bath shaker at 120 rpm. All the experiments were performed in triplicate. The detailed procedures for each serial experiment are explained below. First, to verify the optimal ratio of media volume to wastewater volume from the immobilizing ring-shaped HBA or ring-shaped HBPVC on hydrogen production from vermicelli processing wastewater, the mixed liquor was added to the ring-shaped HBA or HBPVC at the ratios of 2, 3, 4, 5, and 6 % (v/v) in batch reactor under the initial pH 6.0 and 55 ± 1ºC. Then various shaped synthetic materials and biological materials were used at 5% (v/v) under the same environmental conditions. Finally, in order to observe the morphologies of immobilized cells of the ball-shaped HBPE and rope-shaped hydrophilic ramie after the experiment, the biofilms on materials were fixed with 2.5% v/v glutaraldehyde solution at 4ºC for 2 h, rinsed with phosphate buffer saline (PBS 0.1M, pH 7.2), and then dehydrated in a water-ethanol solution of 70% to absolute alcohol for 30 min (Basile et al. 2010). The sample was sputter-coated with a layer of gold under vacuum prior to being subjected to a scanning electron microscope (JEOL JSM-35CF, Japan).

Analytical method

The volume of biogas production was measured daily by a plunger displacement method of glass–tight syringes (Owen et al. 1979). The components of biogas production were analyzed by a gas chromatography (Varian STAR 3400, America), which was equipped with a thermal conductivity detector (TCD). A stainless–steel column was packed (Alltech Molesieve 5A 80/100 10'x 1/8"). Argon was used as the carrier gas for hydrogen (H_2), nitrogen (N_2), and methane (CH_4) analysis. Helium was applied as the carrier gas for carbon dioxide (CO_2) analysis (Selembo et al. 2009). The temperatures of the injector, detector, and column were kept at 80, 90 and 50°C, respectively. Hydrogen gas production was calculated from the headspace measurements of the gas composition and total volume of the biogas produced at each time interval was determined by using the following equation (Eq. 1) (Van Ginkel et al. 2005).

$$V_{H,i} = V_{H,i-1} + C_{H,i}(V_{G,i-1}) + V_H(C_{H,i} + C_{H,i} + C_{H,i-1}) \quad \text{(Eq.1)}$$

where $V_{H,i}$ and $V_{H,i-1}$ are cumulative hydrogen gas volumes at the current (i) and previous (i-1) time intervals, $V_{G,i}$ and $V_{G,i-1}$ are the total gas volumes in the current and previous time intervals, $C_{H,i}$ and $C_{H,i-1}$ are the fractions of hydrogen gas in the headspace of the bottle measured using gas chromatography in the current and previous intervals, and V_H is the total volume of headspace in the reactor.

The following modified Gompertz equation (Eq. 2) was used to calculate the cumulative hydrogen production (Van Ginkel et al. 2005).

$$H = P.exp\left\{-exp\left[\frac{Rm.e}{P}(\lambda - t) + 1\right]\right\} \quad \text{(Eq.2)}$$

where H (mL) is the cumulative hydrogen production, P (mL) is the hydrogen production, R_m (mL/h) is the maximum hydrogen production rate, λ (h) is the lag phase time, t (h) is the incubation time and e = 2.71828.

Liquid samples from the bioreactor were monitored for pH and COD analysis after the experiment.

RESULTS AND DISCUSSION

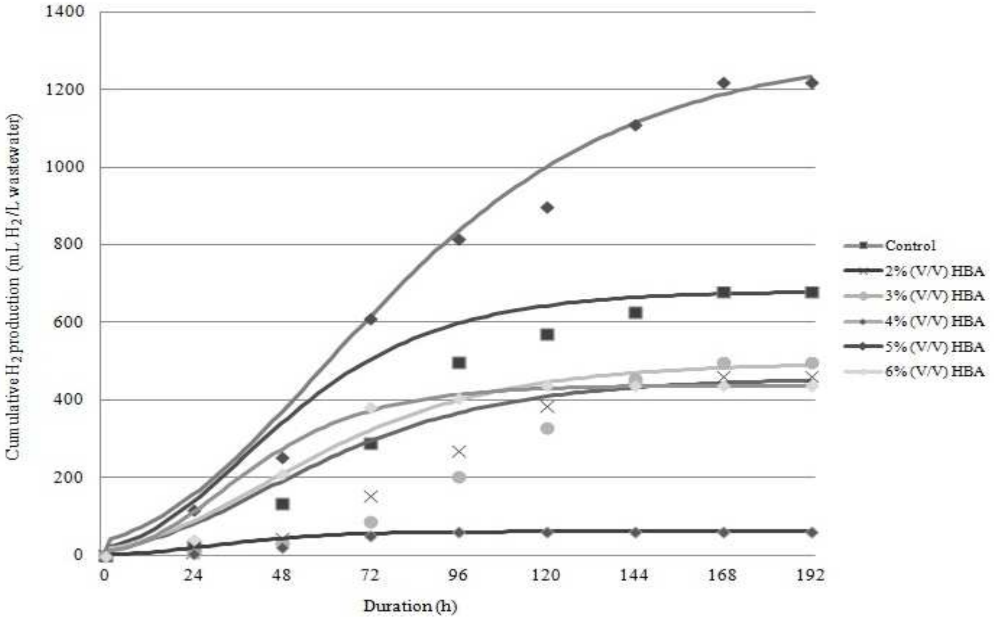
Hydrogen production with immobilized biofilm on the ring-shaped HBA and HBPVC

Results showed that maximum cumulative hydrogen production occurred in thermophilic immobilized fermentation (Fig. 1A). The lag phase of thermophilic fermentation was around 8-12 h and pH profile dropped from 6.0 to 4.5 during fermentation. The COD removal was about 34.38-43.75%. It could be due to the possibility that immobilization helped acclimatize bacteria and decrease the lag phase. These results were similar to other studies, which utilized immobilization technology for microorganism to resist the inhibition from the toxic substrate and to decrease the lag phase (Prieto et al. 2002; Wongthanate et al. 2014).

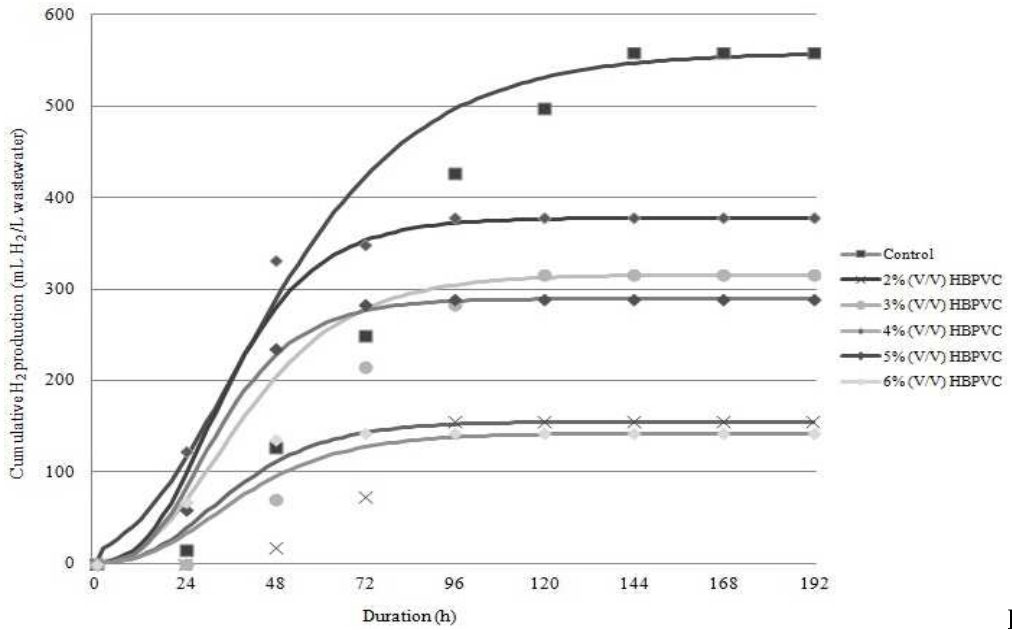
Cumulative hydrogen production was decreased from the control when the amount of immobilizing ring-shaped HBA was increased from 2 to 6% (v/v), except at 5% (v/v) that resulted the highest production as 1,210 mL H_2/L wastewater. Other results were about 459.3, 495.5, 60.4 and 437.4 mL H_2/L wastewater with 2, 3, 4 and 6% (v/v), respectively. These results could be due to the inefficient mass transfer arising from the improper immobilized cell loading amount and excessive amount of bio-carrier in the bioreactor, which would have reduced the synthetic medium movement or contact between the microflora and wastewater (Singh et al. 2013).

The cumulative hydrogen production of vermicelli processing wastewater using immobilizing ring-shaped HBPVC at 0-6 % (v/v) is depicted in Figure 1B. Evidently the cumulative hydrogen production decreased from the control with increasing HBPVC from 2 to 6% (v/v), which was 377.6, 315.5, 289.2, 155 and 142.3 mL H_2/L wastewater with 4, 3, 5, 2 and 6% (v/v), respectively. The results of hydrogen production were consistent with previous studies, which found that the internal mass transfer resistance increased when improper immobilized cell was loaded in the fermentative system (Lee et al. 2003). It could also due to the mechanical surface of synthetic material providing inefficient

bacterial adherence and biofilm formation (Cerca et al. 2005).
It has been shown that the cumulative hydrogen production by immobilized ring-shaped HBA when compared to that by immobilized ring-shaped HBPVC was three-fold higher. This showed that there was a critical amount of immobilized cells needed in the bioreactor for successful fermentative hydrogen production. It was due to enhanced biological activity of hydrogen producing bacteria involved in immobilized form, which was in form of a biofilm (Zhao et al. 2008). On the basis of this result, a subsequent experiment was performed with immobilized materials of 5% (v/v).



A



B

Figure 1 - Cumulative hydrogen production from vermicelli processing wastewater using immobilizing synthetic materials at 0-6% (v/v) addition; (A) ring-shaped HBA and (B) ring-shaped HBPVC.

Comparison of immobilized hydrogen productions with the synthetic and biological materials

Hydrogen-production performances of ball-shaped HBPE and wheel-shaped HBPE, and rope-shaped hydrophilic ramie and cubic-shaped hydrophilic sponge were studied under initial pH 6.0, 55^{o}C and 5% (v/v) addition. Maximum cumulative hydrogen production was about 1,256.5 mL H_2/L wastewater (ball-shaped HBPE) and it was followed by the wheel-shaped HBPE as 498.9 mL H_2/L wastewater (Fig. 2). Zhang et al (2008) used plastic carriers for the immobilization of mixed culture and achieved 2.21 mol H_2/mol glucose hydrogen yields at thermophilic condition. Keskin et al (2011) achieved 4-5 mol H_2/mol sucrose hydrogen yields by pumic stone and ceramic ring packed reactors at thermophilic condition. Among the biological materials, maximum cumulative hydrogen production was 609.8 mL H_2/L wastewater using rope-shaped hydrophilic ramie and was followed by 49.6 mL H_2/L wastewater by cubic-shaped hydrophilic sponge as shown in Figure 2. It was similar to the assessment of loofah sponge as being inefficient for biomass immobilization and hydrogen production in batch mode (Chang et al. 2002). The lag phase of thermophilic fermentation was around 8-48 h and pH profile dropped from 6.0 to 5.0 during fermentation. Also, the COD removal was about 27.4 – 39.3%. These results could be due to different physical characteristics of supporting materials, especially the specific surface area and crevice of ball-shaped HBPE, which provided space for enhancing the biofilm on the surface (Basile et al. 2010). Morphology of the rope-shaped ramie was the filamentous in porous, resulting in improved mixing and mass transfer properties and was beneficial to produce the biofilm on the surface (Zhao et al. 2012). On the basis of this result, the ball-shaped HBPE immobilized cells was found as the best for biohydrogen production.

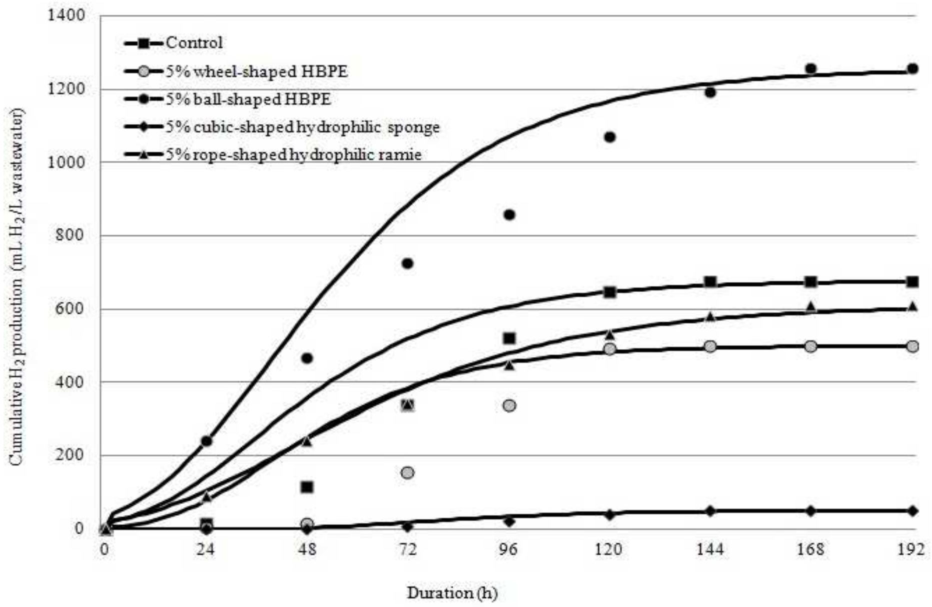
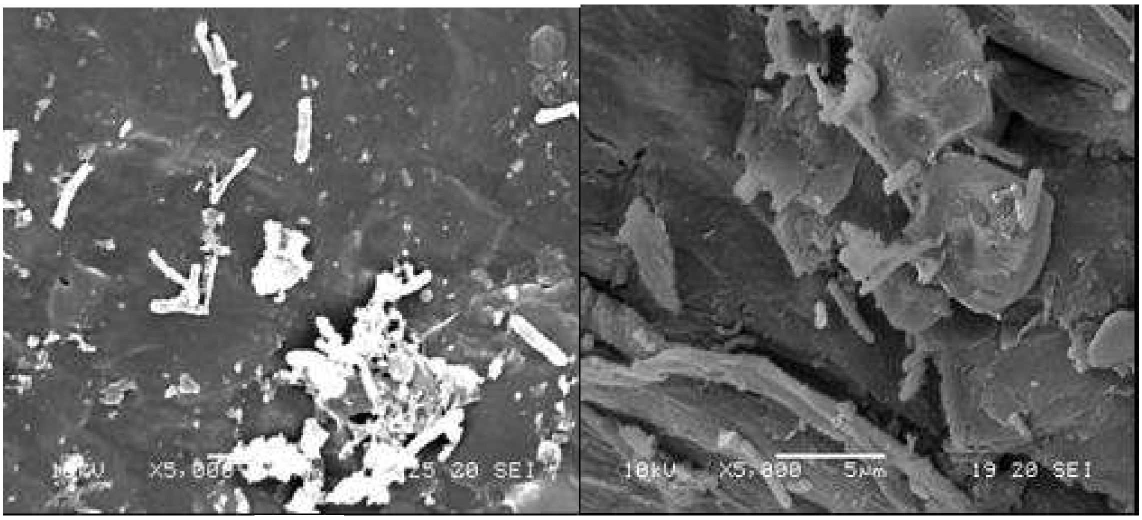


Figure 2 - Cumulative hydrogen production from vermicelli processing wastewater using immobilizing synthetic and biological materials at 5% (v/v) addition.

Morphological observation

Figure 3A showed that the microorganisms were rod-shaped and tended to form flocks on the surface of ball-shaped HBPE, which was an adsorption technique for immobilization of mixed culture. Microorganisms were rod-shaped in the interstice and rough biofilm on the surface of the rope-shaped hydrophilic ramie (Fig. 3B). Morphological properties suggested that microorganisms were similar to rod-shaped bacteria for hydrogen production. These results were consistent with *Clostridium*-like bacteria of LV 10 biofilm (LV composed of PVA solution and latex paint in the volume ratio of 10:90) (Basile et al. 2010) and biological mycelia pellets, which were explored as carriers for the

immobilization of *Clostridium* sp. T2 to improve hydrogen production (Zhao et al. 2012).
Summarizing the shift in biofilm morphology, Basile et al (2010) reported that the seed bacteria wrapped in the biofilm could grow through the surface of biofilm and provided seed bacteria to bulk solution continuously. The bacterial cell mass quantity and variation were not observed on the immobilized biofilm in this study; however, they were also important for immobilized bacterial biohydrogen production. Further studies should be conducted on a continuous bioreactor for a long time and the immobilized bacterial group growth should be observed for identifying and verifying on the bacterial quantity and type.



A B

Figure 3 - Images of bacteria morphologies on (A) immobilized ball-shaped HBPE and (B) immobilized rope-shaped hydrophilic ramie after biohydrogen production at 5 μm bars.

CONCLUSIONS

It was concluded that the characteristics of type and shape of supporting materials and ratio of medium volume to wastewater volume affected the biohydrogen production. At the proper condition, immobilized cells of 5% (v/v) ring-shaped HBA addition appeared to serve as an effective hydrogen-producing bacterial culture in a bioreactor. Among the immobilization material shapes, ball-shaped HBPE at 5 % (v/v) resulted in the maximum cumulative hydrogen production, possibly because of the proper shape for biohydrogen fermentation. Hence, immobilizing ball-shaped HBPE could be a promising technology and a good seeding source for biohydrogen production.

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