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Biohydrogen Production from Rice Noodle Processing Wastewater by Immobilizing Hydrophobic Media

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ABSTRACT

Maximum cumulative hydrogen production of the rice noodle processing wastewater (552.29 mL H_2/L wastewater) was observed at initial pH 6.0 under thermophilic condition (55±1°C). Regarding 5% (v/v) addition of Hydrophobic Media (HB), the maximum cumulative hydrogen production using immobilizing ball-shaped HB (1,256.50 mL H_2/L wastewater) was a three-fold increase of cumulative hydrogen production when compared to its production using immobilizing wheel-shaped HB (424.31 mL H_2/L wastewater). The SEM observation of immobilized biofilm on a ball-shaped HB was the rod shape and gathered into groups.

Key words: Biohydrogen production, rice noodle processing wastewater, pH, temperature, immobilizing hydrophobic media

INTRODUCTION

Biohydrogen production by fermentative process is desirable because such methods generate high yields of hydrogen and high rates of bacterial growth with relative low energy inputs, compared to photobiological methods (Hasyim *et al*., 2011). Hydrogen is a clean fuel with Carbon Dioxide (CO_2) emission and can be sustainably produced (Argun *et al*., 2009). The fermentative processes that utilize free carbon available in large-volume discharges of agro-industrial wastewater containing carbohydrates can recover available energy and purify the effluent (Wei *et al*., 2010; Wongthanate *et al*., 2014). The high-carbohydrate wastewaters will be the most useful for industrial hydrogen production (Van Ginkel *et al*., 2005). In addition, biohydrogen production would be accompanied by the acidic metabolites (e.g., acetic acid, butyric acid, etc). A proper control of the culture pH is a critical factor affecting the efficiency of the fermentation (Wang *et al*., 2007). Moreover, temperature is the one of important factors on biohydrogen production, in which mesophilic and thermophilic hydrogen production conditions were compared and in which the thermophilic condition was shown to be superior to the mesophilic condition (Akutsu *et al*., 2009). Thermophilic bacteria can utilize a variety of carbon sources and generate high yields of hydrogen as well as tolerate acidic fermentative conditions (Hasyim *et al*., 2011). Immobilized cell systems have been successfully applied for biohydrogen production in various bioreactors because they are resistant to cell wash-out during operation and can maintain a higher cell density (Singh *et al*., 2013). In general, three types of immobilized cell systems have been applied in biohydrogen production, including surface attachment, self-flocculation and gel entrapment approaches, among which the surface attachment approach was most frequently used for dark hydrogen fermentation (Lin *et al*., 2009). However, many previous studies have reported about immobilized cell on supporting materials to enhance hydrogen production by helping acclimatization of microbes, decreasing lag phase of bacterial cultivation and increasing density of consortia (Wu *et al*., 2003; Cheng *et al*., 2006). Synthesized materials were, for example, activated carbon (Zhang *et al*., 2008), glass bead (Zhang *et al*., 2007), polystyrene, PET (Barros and Silva, 2012).

The objectives of this study were to investigate impacts of fermentative hydrogen production from rice noodle processing wastewater in terms of initial pH (4.5-7.0) under mesophilic (35±1°C) and thermophilic (55±1°C) conditions in a batch reactor and to enhance the biohydrogen production by immobilizing synthetic hydrophobic media (HB) at the ratios of media volume to wastewater volume (0-6%, v/v) under thermophilic condition. Morphology of immobilized cells of HB for maximum hydrogen production from rice noodle processing wastewater was observed by a scanning electron microscope (SEM).

MATERIALS AND METHODS

Substrate and seed sludge: Wastewater was collected from a rice noodle processing plant, located in Nakhonpathom province, Thailand. Anaerobic sludge was taken from the Bio-fertilizer plant in Nonthaburi province, Thailand. Seed sludge was screened with a sieve (2.00 mm) to eliminate the large particulate materials and was heated at 90°C for 10 min to inhibit hydrogen-consuming bacteria and facilitating the growth of spore-forming bacteria (Valdez-Vazquez and Poggi-Varaldo, 2009).

Their characteristics of wastewater and seed sludge were analyzed; pH, Total Solid (TS), Total Suspended Solid (TSS), Volatile Solid (VS), Volatile Suspended Solid (VSS), Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), Total Kjeldahl Nitrogen (TKN) (APHA., 2005). The physical and chemical characteristics of a rice noodle processing wastewater and seed sludge are shown in Table 1.

Characteristics of synthetic hydrophobic media: Three shapes of synthetic hydrophobic medium with ball, wheel and ring-shaped HB (hydrophobic acrylic, HBA and polyvinylchloride, HBPVC) were used. All materials were placed in reactor at the ratios of media volume to wastewater volume of 0-6% (v/v). Their characteristics are summarized in Table 2.

Table 1: Characteristics of rice noodle processing wastewater and seed sludge

Parameters	Rice noodle processing wastewater	Seed sludge
pH	3.74	8.23
COD (mg L^{-1})	7,220	24,300
BOD (mg L^{-1})	5,400	NA
Total Kjeldal nitrogen (mg L^{-1})	68.70	597
Total solid (mg L^{-1})	5,325	257,860
Total suspended solid (mg L^{-1})	2,820	212,750
Volatile solid (mg L^{-1})	NA	25,370
Volatile suspended solid (mg L^{-1})	NA	14,327

NA: Not analyzed, COD: Chemical oxygen demand, BOD: Biochemical oxygen demand

Table 2: Characteristics of synthetic hydrophobic media

	Synthetic hydrophobic media			
Parameters	A	B	C	D
Shapes	Ring	Ring	Wheel	Ball
Material	Acrylic	Polyvinylchloride	Polyethylene	Polyethylene
Dimension (width×height) (mm^2)	20×10	20×10	11×7	20×20
Volume (cm^3)	1.13	1.13	0.20	1.0
Total surface area (m^2/m^3)	432	432	850	250

Analysis

Experimental setup: The batch reactor of 250 mL lab bottle with a working volume of 200 mL was added with 20 mL heated seed sludge and 180 mL wastewater. The mixed liquor in 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 airbag to avoid gas leakage from the bottle (Wongthanate *et al*., 2014). The bioreactors were placed in a water bath shaker at 120 rounds per minutes (rpm). All experiments were performed in triplicate. The detail procedures for each serial experiment are explained below.

First, to observe the impacts of hydrogen production from rice noodle processing wastewater at initial pH of 4.5, 5.0, 5.5, 6.0, 6.5, or 7.0 end under mesophilic (35±1°C) and thermophilic (55±1°C) conditions. Second, to verify the optimal ratio of media volume to wastewater volume (2, 3, 4, 5, 6%, v/v) on hydrogen production by immobilizing ring-shaped HBA or HBPVC, the mixed liquor was conducted in batch reactor under the optimal initial pH 6.0 and 55±1°C. Then, three shapes (ball, wheel and ring-shaped HB) were used at 5% (v/ v) under the same environmental conditions. Finally, the morphology of immobilized cells of the ball-shaped HB after experiment, the biofilm on ball-shaped HB was fixed with 2.5% v/v glutaraldehyde solution at 4°C in 2 h, rinsed with phosphate buffer saline (PBS 0.1M, pH 7.2) and then dehydrated in water-ethanol solution from 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 methods: The volume of biogas production was daily measured by a plunger displacement method of glass-tight syringes (Owen *et al*., 1979). The components of biogas production were daily analyzed by a gas chromatography (Varian STAR 3400, America), which 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 and helium was applied as the carrier gas for carbon dioxide (CO_2) analysis (Selembo *et al*., 2009). The temperatures of injector, detector and column were kept at 80, 90 and 50°C, respectively.

Hydrogen gas production was calculated from headspace measurements of gas composition and total volume of biogas produced at each time interval by using a following Eq. 1 (Van Ginkel *et al*., 2005):

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

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 interval, $C_{H,i}$ and $C_{H,i-1}$ are the fraction of hydrogen gas in the headspace of the bottle measured using gas chromatography in the current and previous intervals and V_H is total volume of headspace in the reactor.

A modified Gompertz Eq. 2 was used to calculate cumulative hydrogen data (Van Ginkel *et al*., 2005):

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

where, H (mL) is the cumulative hydrogen production, P (mL) is the hydrogen production, R_m (mL h^{-1}) is the maximum hydrogen production rate, λ (h) is the lag phase time, t (h) is the incubation time and e = 2.71828.

Moreover, liquid samples of bioreactor were monitored for pH and COD analysis after the experiment.

RESULTS AND DISCUSSION

Impacts of initial pH and temperature on biohydrogen production from rice noodle processing wastewater: For Fig. 1, the graphs depicted that hydrogen production was not examined at initial pH of 4.5 and 5.0. Cumulative hydrogen production increased with increasing initial pH in the range of 5.5-6.5 and decreasing at initial pH 7.0, which was highly produced at the beginning and then was slightly stable in an overview. Lag phases of biological hydrogen

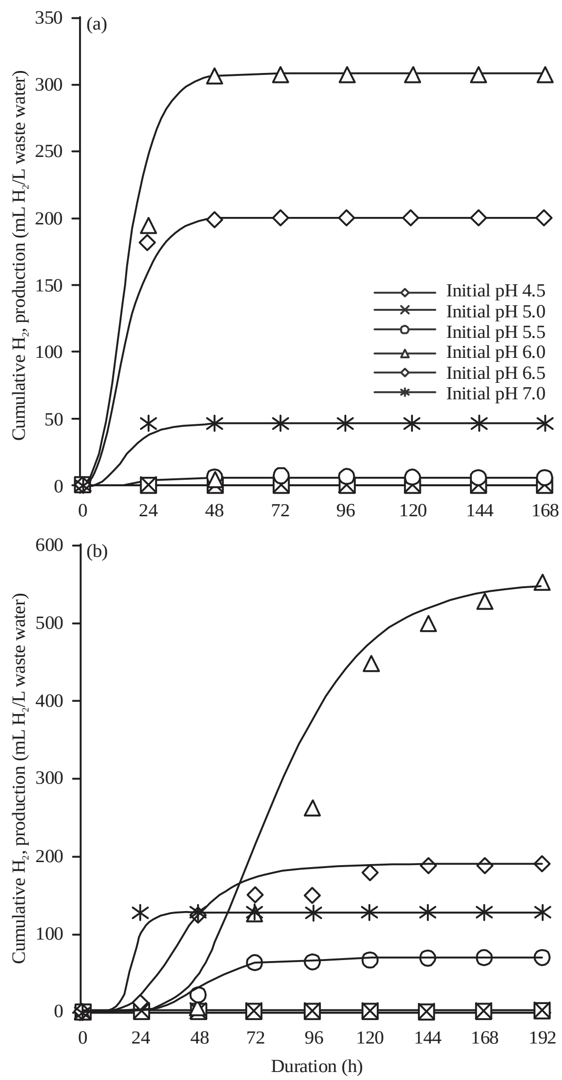

Fig. 1(a-b): Cumulative hydrogen production from rice noodle processing wastewater at initial pH (4.5-7.0) (a) Mesophilic condition (35±1°C) and (b) Thermophilic condition (55±1°C) productions in thermophilic condition (14-44 h) were longer than that of mesophilic condition (4-14 h). These results are not similar to others wherein the lag was relatively longer lasting about 11-13 h at initial pH 6.0 and 55°C (Zhang *et al*., 2003), while some researchers reported biohydrogen production at mesophilic condition to start after a lag phase of approximately 9 h (Baghchehsaraee *et al*., 2010).

At mesophilic condition, the maximum of cumulative hydrogen production from rice noodle processing wastewater was about 307.97 mL H_2/L wastewater at initial pH 6.0 (Fig. 1a), otherwise it was the maximum hydrogen production of 552.29 mL H_2/L wastewater at the same initial pH under thermophilic condition (Fig. 1b). Results indicated that temperature can affect the activity of hydrogen-producing bacteria by influencing the activity of some essential enzymes, such as hydrogenases for fermentative hydrogen production. In an appropriate range, increasing temperature could increase the ability of hydrogen-producing bacteria to produce hydrogen during fermentative hydrogen production but temperature at much higher levels could decrease it with increasing levels (Wongthanate *et al*., 2014). Moreover, the pH profiles in both conditions after no hydrogen production were dropped finally to pH 4.0-5.0 in fermentative process. Some possible reasons for this may be that hydrogen production occurs in acidification stage of metabolic process and the hydrogen-producing bacteria has a high conversion rate of carbohydrate to hydrogen, then the high concentrations of metabolites may cause the pH to drop to such low level. The bacteria involved could not sustain its metabolic activity at pH values less than 5.0 and complete inhibition was reported in the pH range of 4.0-5.0 (Wongthanate *et al*., 2014). Hence, these results were concluded that the optimal conditions of initial pH of 6.0 and thermophilic temperature were suggested for biohydrogen production from rice noodle processing wastewater.

Optimal ratio of media volume to wastewater volume on hydrogen production by immobilizing ring-shaped HB: Regarding Fig. 2, at 5% (v/v) addition of ring-shaped HBA or HBPVC was revealed that maximum cumulative hydrogen production was occurred in thermophilic fermentation. Lag phase of thermophilic fermentation was around 8-12 h and pH profile was dropped from 6.0-5.0 during fermentation, as well as COD removal of fermentation was about 34.38-43.75%. It may be due to immobilization could help acclimatize bacteria and decrease the lag phase of bacteria cultivation. Results were similar to other researchers that utilized immobilization technology for microorganism to resist the inhibition from the toxic substrate and to decrease the lag phase obviously (Prieto *et al*., 2002). Figure 2a shows the cumulative hydrogen production of rice noodle processing wastewater using ring-shaped HBA at 0-6% (v/v). Cumulative hydrogen production was decreased by increasing amount of ring-shaped HB immobilized cell from 2-6% (v/v), except at 5% (v/v) addition that was highly generation as 748.71 mL H_2/L wastewater. Other results were decreased in order of 366.55 mL H_2/L wastewater (2% (v/v)), 100.46 mL H_2/L wastewater (4% (v/v)) and 40.41 mL H_2/L wastewater (3% (v/v)) as well as 11.18 mL H_2/L wastewater (6% (v/v)), respectively. It could be due to the inefficient mass transfer arising from the improper immobilized cell loading amount (Singh *et al*., 2013). In contrast, cumulative hydrogen production of rice noodle processing wastewater using ring-shaped HBPVC at 0-6% (v/v) was depicted in Fig. 2b. It presented that cumulative hydrogen production was increased by increasing amount of HBPVC from 2-5% (v/v). It was in order of 40.43 mL H_2/L wastewater (2% v/v), 174.41 mL H_2/L wastewater (3% (v/v)) and 197.48 mL H_2/L wastewater (4% v/v). Maximum cumulative hydrogen production was obtained as 224.31 mL H_2/L wastewater at 5% (v/v). It was also decreased to 58.51 mL H_2/L wastewater at 6% (v/v). Results were consistent with research,

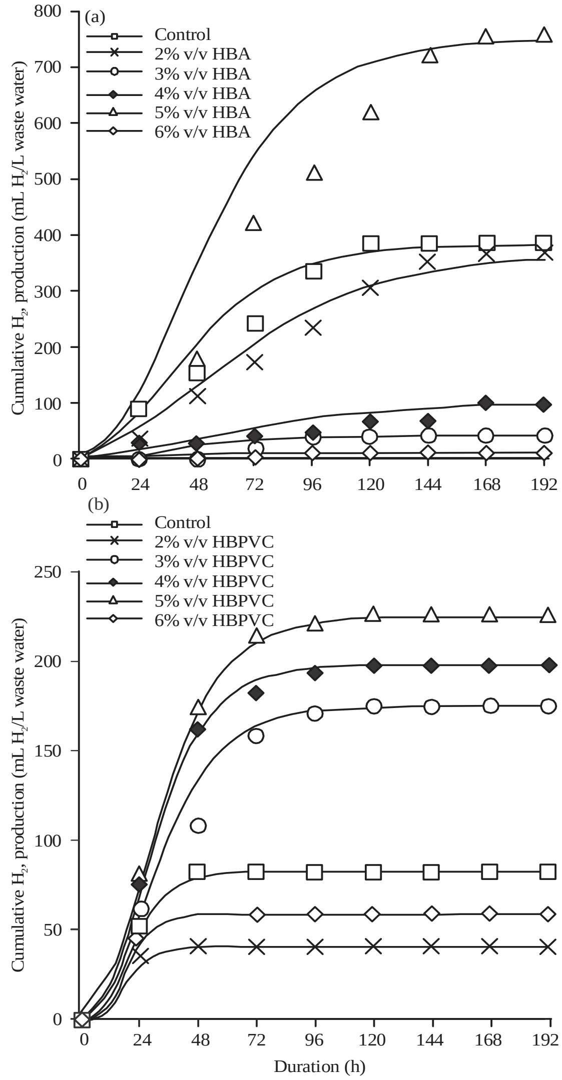

Fig. 2(a-b): Cumulative hydrogen production from rice noodle processing wastewater (initial pH 6.0 and 55±1°C) by immobilizing HB at 0-6% (v/v), (a) Ring-shaped HBA and (b) Ring-shaped HBPVC

which indicated that the internal mass transfer resistance increased when improper immobilized cell loaded in fermentative system (Lee *et al*., 2003). Moreover, excessive amount of bio-carrier in bioreactor may reduce synthetic media movement or contact between microflora and wastewater (Singh *et al*., 2013).

Regarding the mentioned result, it showed that the ring-shaped HBA addition at 5% (v/v) when compared to that of ring-shaped HBPVC had a three-fold increase of cumulative hydrogen production. Consequently, this seems to suggest that there is a critical amount of immobilized cell in bioreactor for successful fermentative hydrogen production. It was due to obtaining a higher cell density of hydrogen producing bacteria, which was a surface attachment or biofilm on media and enhancing the biological activity of bacteria involved in immobilization process (Zhao *et al*., 2012). Thus, it revealed that the maximum cumulative hydrogen production by immobilizing ring-shaped media was optimal ratio of media volume to wastewater volume at 5% (v/v).

Comparison of various shapes of immobilizing HB at 5% (v/v) and hydrogen production at optimal condition: To characterize the hydrogen-producing performance of various shapes of immobilizing HB (ball, ring and wheel), the bioreactors were examined in the influential factors (initial pH 6.0, thermophilic condition and amount of 5% (v/v) addition) under the dark fermentation. The results showed that maximum cumulative hydrogen production was about 1,256.50 mL H_2/L wastewater (ball-shaped HB) and it was followed by ring-shaped HB of 1,116.15 mL H_2/L wastewater. Nevertheless, the lowest cumulative hydrogen production was observed on wheel-shaped HB addition as shown in Fig. 3. Furthermore, lag phase of thermophilic fermentation was around 8-14 h and pH profile was dropped from 6.0-5.0 during fermentation as well as COD removal of fermentation was about 27.42-39.29%. It may be owing to the different physical characteristics of HB, especially specific surface area and crevice of ball-shaped HB, which could provide space for cell growth and the biofilm surface was covered by bacteria cell (Bai *et al*., 2009). However, this result was complied with other studies which used plastic carriers for immobilization of mixed culture and achieved 2.21 mol H_2/mol glucose hydrogen yields at thermophilic condition (Zhang *et al*., 2008) and achieved 4-5 mol H_2/mol sucrose hydrogen yields by pumice stone and ceramic ring packed reactors for immobilization at thermophilic condition (Keskin *et al*., 2012). On this basis of this result, the proper ball-shaped HB of immobilized cells could enhance the biohydrogen production.

Morphological observation: The shape and size of ball-shaped HB are shown in Fig. 4a. Microorganisms are the rod shape and tend to form flocks on surface of ball-shaped HB, which is an adsorption technique for immobilization of mixed culture (Fig. 4b). Morphological properties suggested that it was similar to rod-shaped bacteria for hydrogen production. This result was consistent with *Clostridium*-like bacteria of LV 10 biofilm (LV is composing of PVA solution and latex paint in the volume ratio of 10:90) (Bai *et al*., 2009). However, the bacteria variation was not observed on the immobilized biofilm. Summarizing the shift in biofilm morphology, the seed bacteria wrapped in the biofilm could grow through the surface of biofilm and provided seed bacteria to bulk solution continuously (Bai *et al*., 2009).

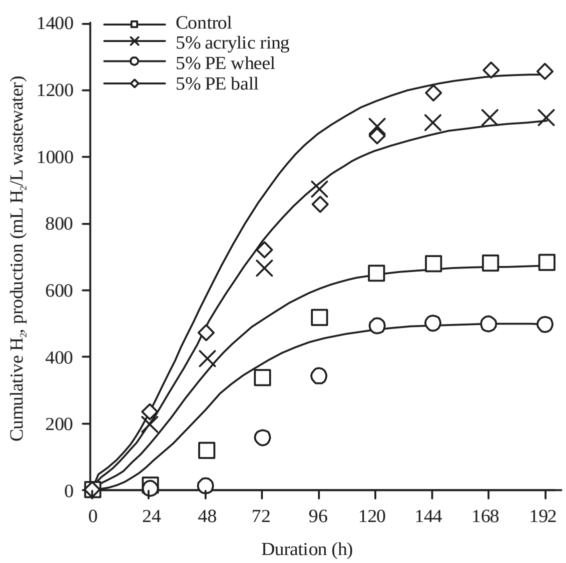

Fig. 3: Cumulative hydrogen production from rice noodle processing wastewater (initial pH 6.0 and 55±1°C) with various shapes of immobilizing HB at 5% (v/v)

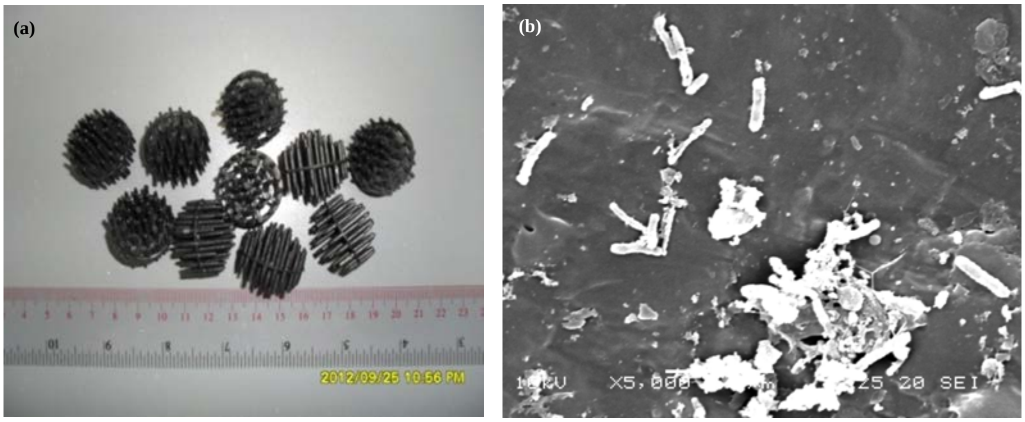

Fig. 4(a-b): Images of (a) Shape and size of ball-shaped HB and (b) Immobilized ball-shaped HB after biohydrogen production at 5 μm bars

CONCLUSIONS

Maximum cumulative hydrogen production was generated from rice noodle processing wastewater at initial pH 6.0 under thermophilic condition. At proper ratio of 5% (v/v) (ring-shaped HB) addition, it appeared to serve as effective hydrogen-producing bacteria culture in bioreactor. Among the immobilized HB shapes, ball-shaped HB at 5% (v/v) could be resulted in the maximum cumulative hydrogen production, possibly because of the higher biomass attachment capacity of ball-shaped HB. Hence, it is concluded that factors of initial pH, temperature and supporting media affected the biohydrogen production. Also, immobilized biofilm could be a promising immobilization technology and a good seeding source for biohydrogen production.

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