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Photohydrogen production using purple nonsulfur bacteria with hydrogen fermentation reactor effluent

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Abstract

Hydrogen production from waste using photosynthetic bacteria is an attractive methodology. A combination of purple nonsulfur photosynthetic bacteria and anaerobic bacteria is ideal for the efficient conversion of wastewater into hydrogen. In this paper, photohydrogen production using effluent from different hydrogen fermentation reactors was carried out using two strains of photosynthetic purple nonsulfur bacteria. The results indicated that the effluent from the hydrogen fermentation reactors could be used directly for photohydrogen production without aeration or dilution pretreatment. Effluent from the carbohydrate fed hydrogen fermentation reactors is more suitable for photohydrogen production than effluent from a peptone fed reactor. Among the initial dark hydrogen fermentation stage effluents from the three carbohydrate fed reactors (CSTR, ASBR, UASB), CSTR effluent was the most suitable for photohydrogen production.

Keywords: Hydrogen fermentation reactor; Gas pressure; Hydrogen gas; Purple nonsulfur photosynthetic bacteria

1. Introduction

Today global energy requirements are dependent primarily on fossil fuels. The utilization of fossil fuels is causing global climate changes due to pollutant emissions such as CO_X, NO_X, SO_X, C_XH_X, soot, ash and other organic compounds. These pollutants are released into the atmosphere as a result of combustion. To avoid this contamination, hydrogen has been suggested as the energy carrier of the future. When combusted, water is the main product, thus, hydrogen is regarded as a clean nonpolluting fuel. Compared to other gaseous fuels like methane, hydrogen is harmless to humans and the environment [1].

Phototrophic bacteria are indicated in the current literature as the most promising microbial system for the biological production of hydrogen. This is mainly because of their: (1) high theoretical conversion yields. (2) lack of O_2-evolving activity which causes O_2 inactivation problems in different biological systems. (3) the ability to use wide spectral light energy and, (4) the ability to consume organic substrates derived from wastes in association with wastewater treatment [2].

Hydrogen production from waste using photosynthetic bacteria is attractive because energy can be recovered from wastes derived from renewable resources [3]. Photosynthetic bacteria can produce hydrogen from organic acids [4]. The effluent from anaerobic hydrogen fermentation reactors contains high concentrations of organic acids [5]. The possibility of utilizing these initial dark hydrogen fermentation stage effluents for photohydrogen production using purple nonsulfur bacteria has not yet been elucidated.

In a previous work, two purple nonsulfur photosynthetic bacteria (WP2-5, WP3-5) were selected from a swine wastewater treatment system. Strain WP2-5 was identified as *Rhodopseudomonas palustris* by methods based on 16S rDNA gene sequence, but strain WP3-5 was not yet identified. Both strains could utilize simple organic acids with light energy to produce hydrogen gas [6]. In this study, photohydrogen production using the effluent from different dark hydrogen fermentation reactors was carried out using two strains of photosynthetic purple nonsulfur bacteria (WP2-5, WP3-5). A combination of purple nonsulfur photosynthetic

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bacteria and anaerobic bacteria was used for efficient conversion of wastewater into hydrogen. The potential use of these bacteria with effluent from dark anaerobic hydrogen production for photohydrogen production will be discussed.

2. Materials and methods

2.1. The hydrogen producing ability of strains WP2-5 and WP3-5 using effluent from hydrogen fermentation reactors

The microorganisms obtained from the liquid culture were used for a batch study. The liquid medium was a *Rhodospirillaceae* medium [7]. After centrifugation, the concentrated cells were suspended in 10 ml *Rhodospirillaceae* medium without carbon and nitrogen source. Each serum bottle (total volume: 120 ml) contained microorganisms at an optimal concentration (about 5×10^7 cfu/ml) and different concentrations of effluent from the hydrogen fermentation reactor. After gassing with argon gas to retain 40 ml of medium and sealed with teflon/silicon stoppers, the serum bottles were placed into a climatic room at 35°C providing 155 μmol/m^2/s of illumination (tungsten filament lamp). The compositions of the *Rhodospirillaceae* medium for these experiments were (in g/l): K_2HPO_4, 0.5; KH_2PO_4, 0.5; $MgSO_4 \cdot 7H_2O$, 0.2; NaCl, 0.4; $CaCl_2 \cdot 2H_2O$, 0.05; yeast extract, 0.2; Fe-citrate solution, 5 ml/l; vitamin B_{12}, 1 ml/l. To the medium 1 ml of the trace element solution was added containing the following (in mg/l): $ZnCl_2$, 70; $MnCl_2 \cdot 4H_2O$, 100; H_3BO_3, 60; $CoCl_2 \cdot 6H_2O$, 200; $CuCl_2 \cdot 2H_2O$, 20; $NiCl_2 \cdot 6H_2O$, 20; $NaMoO_4 \cdot 2H_2O$, 40; HCl(25%), 1 ml/l. The final *Rhodospirillaceae* medium pH value was 6.8.

The influence of gas pressure was investigated using the same setup with the exception that the bottle volume was increased from 120 to 260 ml.

2.2. Analytical methods

Samples were collected directly from the reactors with a syringe. After membrane filtration, the ammonia concentration was measured using the indophenol blue method [8]. Gas samples for hydrogen, carbon dioxide and methane analysis were injected into a gas chromatograph (GC) equipped with a thermal conductivity detector (TCD). GC was performed with a HP 6890 system equipped with a stainless-steel column containing Hap Sep Q. The oven, injector and detector temperatures were 80°C, 150°C and 200°C, respectively. Argon gas was supplied as the carrier gas and the flow rate was 20 ml/min. Nitrogenase activity was measured using the acetylene reduction method [9]. Total volatile organic acids were measured using the Dilallo and Alberstson direct titration method [10]. The OD was measured using a Spectrophotometer (Beckman Du® 530) at 660 nm.

3. Results and discussion

3.1. The influence of the initial dark hydrogen fermentation effluent pretreatment on purple nonsulfur bacteria growth

In the anaerobic hydrogen producing process, methanogens may compete for the hydrogen gas. To avoid interference from methane-forming bacteria in the experiment, the influence of the initial dark hydrogen fermentation stage effluent pretreatment on the growth of strain WP2-5 was tested. Fig. 1 shows the cell dry weight variations for strain WP2-5 with different effluent pretreatment methods. From this figure, it can be seen that strain WP2-5 had the maximum cell dry weight with nonaerated or diluted effluent. With aerated effluent, the volatile organic acid escaped quickly and the pH of the effluent also increased, reaching 9.5. The high pH value and low substrate concentration were not suitable for the growth of strain WP2-5. In the following experiments the effluent was used directly without any pretreatment or dilution.

3.2. Photohydrogen production using purple nonsulfur bacteria with effluent from a peptone fed hydrogen fermentation reactor

The effluent from a continuous stirred tank reactor (CSTR) using peptone as the substrate for anaerobic hydrogen production included a large amount of organic acid and ammonia. When this effluent was used as the substrate, strains WP2-5 and WP3-5 could not utilize it to produce hydrogen gas (data not shown). One possible reason was that ammonia from peptone degradation inhibited the nitrogenase activity of the purple nonsulfur photosynthetic bacteria. The effluent from the peptone fed hydrogen

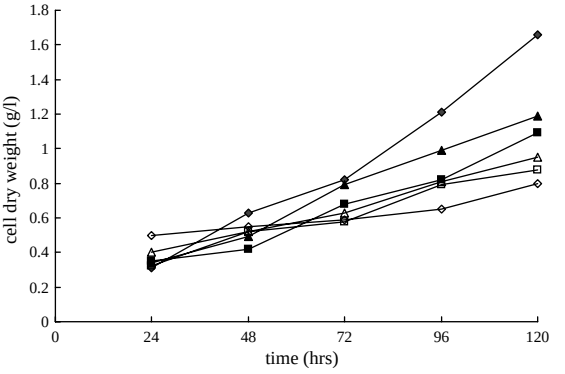

Fig. 1. Cell dry weight variations for strain WP2-5 with different effluent pretreatment methods (light intensity: 155 μmol/m^2/s; temperature: 35°C; headspace: 80 ml) (—◆—, without dilution; —■—, 1:2 dilution; —▲—, 1:5 dilution; —◇—, without dilution + aeration; —□— 1:2 dilution+aeration; —△—, 1:15 dilution+aeration).

fermentation reactor was therefore not suitable for photohydrogen production.

3.3. Photohydrogen production using purple nonsulfur bacteria with effluent from three different sugar fed hydrogen fermentation reactors

An anaerobic sequencing batch reactor (ASBR), upflow anaerobic sludge blanket (UASB) and continuous stirred tank reactor (CSTR) were used for these experiments.

3.4. Effluent from the ASBR

The effluent from a sugar fed hydrogen fermentation ASBR exhibited a high organic acid concentration (about 6000–8000 mg/l of COD) and was composed primarily of butyric acid. In a previous study, strain WP3-5 could utilize 700 mg/l of butyric acid for hydrogen production and the performance was better than utilizing 700 mg/l of propionic acid [11]. It is therefore suggested that strain WP3-5 might utilize the ASBR effluent to produce hydrogen gas. However, the results showed that only an insignificant amount of hydrogen gas could be produced (data not shown). The possible reasons could be the following: (1) The effluent included 80 mg/l of ammonia concentration and this ammonia concentration inhibited nitrogenase production. (2) The effluent also included a high ethanol concentration (about 500–600 mg/l of COD) and ethanol toxicity inhibited the growth of strain WP3-5. At any way, these results show that sugar fed ASBR effluent was not suitable for hydrogen gas production.

3.5. Effluent from the UASB

The effluent from a sugar fed hydrogen fermentation UASB that included high organic acid concentrations (about 3000–4000 mg/l of COD), composed primarily of butyric acid, was used for hydrogen production. The ammonia concentration of this effluent was 147 mg/l. Fig. 2 shows the gas compositions from an 80 ml headspace during photohydrogen production using strains WP2-5 and WP3-5. The amount of hydrogen gas produced was very low; however, the amount of carbon dioxide was much higher, possibly because carbonate was used as the medium buffer in the UASB. Methane gas was detected in this experiment (Fig. 2), indicating that the wastewater from the UASB included methane-forming bacteria.

3.6. The influence of gas pressure

When the bottle headspace was increased, the hydrogen gas produced by strains WP2-5 and WP3-5 also increased (Figs. 2 and 3). The gas pressure reduced with increasing headspace. This indicated that the gas pressure affected the hydrogen gas production and this phenomenon was supported by Cheng's statement [12].

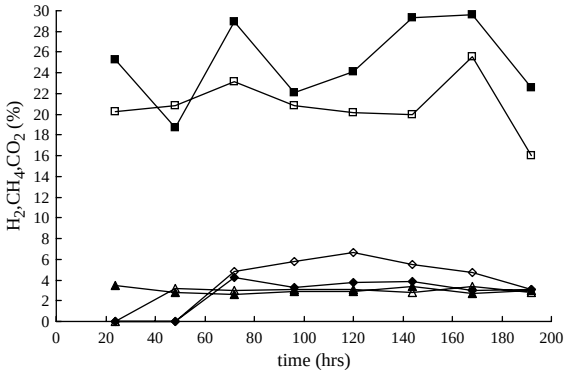

Fig. 2. The gas compositions from an 80 ml headspace during photohydrogen production using strains WP2-5 and WP3-5 with effluent from a sugar fed hydrogen fermentation UASB (light intensity: 155 μmol/m^2/s; temperature: 35°C) (—◆—; WP2-5, H_2; —■—; WP2-5, CO_2; —▲—: WP2-5, CH_4; —◇—: WP3-5, H_2; —□—: WP3-5, CO_2; —△—: WP3-5, CH_4).

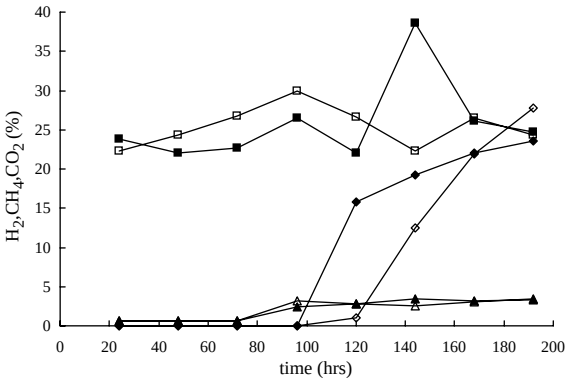

Fig. 3. The gas compositions from a 220 ml headspace during photohydrogen production using strains WP2-5 and WP3-5 with effluent from a sugar fed hydrogen fermentation UASB (light intensity: 155 μmol/m^2/s; temperature: 35°C) (—◆—: WP2-5, H_2; —■—: WP2-5, CO_2; —▲—: WP2-5, CH_4; —◇—: WP3-5, H_2; —□—: WP3-5, CO_2; —△—: WP3-5, CH_4).

3.7. Effluent from the CSTR

Fig. 4 shows the gas composition from a 220 ml headspace during photohydrogen production using strains WP2-5 and WP3-5 with effluent from a sugar fed hydrogen fermentation CSTR. From this figure, the hydrogen gas production begins at 48 h. Hydrogen gas increased steadily with time. However, since the ammonia concentration in the effluent was high, large amounts of hydrogen gas were not produced. From the figure, the hydrogen gas production decreased when the amount of methane gas increased. This suggested that a portion of the hydrogen gas was used to produce methane gas. The residual volatile organic acid

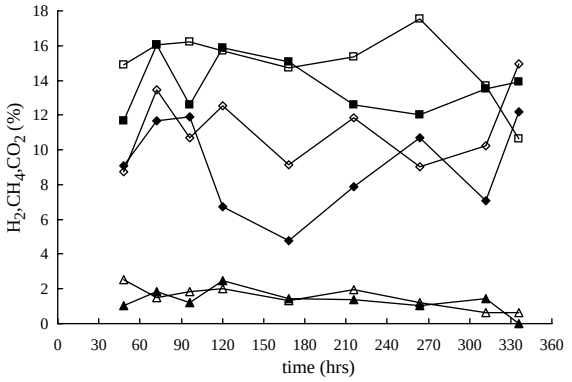

Fig. 4. The gas composition from a 220 ml headspace during photohydrogen production using strains WP2-5 and WP3-5 with effluent from a sugar fed hydrogen fermentation CSTR (light intensity: 155 μmol/m^2/s; temperature: 35°C; the effluent was 1:2 diluted) (—◆—: WP2-5, H_2; —■—: WP2-5, CO_2; —▲—: WP2-5, CH_4; —◇—: WP3-5, H_2; —□—: WP3-5, CO_2; —△—: WP3-5, CH_4).

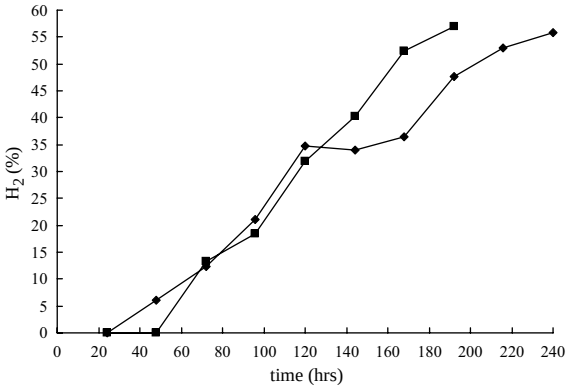

Fig. 5. The gas compositions from an 80 ml headspace during photohydrogen production using strains WP2-5 and WP3-5 with effluent from a glucose and beef fed hydrogen fermentation CSTR (light intensity: 155 μmol/m^2/s; temperature: 35°C; the effluent was 1:2 diluted) (—◆—, WP2-5; —■—, WP3-5).

concentration was about 7000 mg/l (data not shown). This suggests that the amount of hydrogen gas produced could be increased if the effluent ammonia concentration is reduced.

The maximum amount of hydrogen gas produced and the theoretical hydrogen gas conversion yield for strains WP2-5 and WP3-5 with effluent from different anaerobic hydrogen gas fermentation reactors are shown in Table 1. From the table, the effluent from the CSTR was suitable for hydrogen production compared with the UASB when carbohydrates were used as the substrate in the hydrogen fermentation reactor. The amount of hydrogen gas increased with increasing headspace.

3.8. Photohydrogen production using purple nonsulfur bacteria with effluent from a glucose and beef extract fed hydrogen fermentation reactor

Effluent from a CSTR anaerobic hydrogen fermentation reactor was used in this experiment. The ammonia concentration of the effluent was only 22.7 mg/l. Strains WP2-5 and WP3-5 could utilize this wastewater to produce hydrogen gas after 24 h (Fig. 5). The carbon dioxide concentration was very low during this experiment (data not shown) because phosphate was used as the medium buffer in the anaerobic hydrogen gas fermentation reactor.

When the temperature of the CSTR decreased below 43°C, the effluent was not suitable for hydrogen gas production. The reason was that the reactor medium included large amounts of sulfate. When the reactor temperature was below 43°C, sulfur reducing bacteria proliferated quickly, causing an accumulation of hydrogen sulfide in the wastewater. It is possible that hydrogen sulfide was toxic and inhibited the growth of the purple nonsulfur photosynthetic bacteria.

From Figs. 2–5 and Table 1, effluents with low ammonia concentrations were suitable for hydrogen gas production. The effluent from the carbohydrate fed hydrogen fermentation reactor was more suitable for hydrogen gas production compared with effluent from a peptone fed anaerobic hydrogen fermentation reactor. Among the carbohydrate fed hydrogen fermentation reactors, the effluent from

Table 1
The maximum hydrogen gas produced and the theoretical hydrogen gas conversion yield for strains WP2-5 and WP3-5 with effluent from different hydrogen gas fermentation reactors

Reactor (headspace)	Strain	Maximum hydrogen gas production (%)	Theoretical hydrogen gas conversion yield (%)
UASB (80 ml)	WP2-5	6.1	2.2
	WP3-5	7.5	2.7
UASB (220 ml)	WP2-5	23.6	23.3
	WP3-5	27.8	27.4
CSTR (220 ml)	WP2-5	12.2	44.5
	WP3-5	13.5	49.6

the CSTR was the most suitable for photohydrogen production.

4. Conclusions

Effluent from the hydrogen fermentation reactors could be used directly for photohydrogen production by strains WP2-5 and WP3-5 without aeration or dilution pretreatment.

The effluent from the peptone fed hydrogen fermentation reactors was not suitable for photohydrogen production because of its high ammonia concentration. The effluent from the carbohydrate fed reactors, however, was suitable for photohydrogen production.

Among the effluents from the three carbohydrate fed hydrogen fermentation reactors (CSTR, ASBR, UASB), the effluent from the CSTR was the most suitable for photohydrogen production. The highest maximum hydrogen gas production was observed with this effluent.

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