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Contributed Paper

Optimization and Performance of Covered-light Structures for Hydrogen Production by *Rhodopseudomonas palustris* TN1

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ABSTRACT

In recent times, hydrogen production from agro-industry wastewaters using photosynthetic bacteria have been widely studied. Most research has been done with various light sources under uncovered-light structure resulting in light energy losses. Aims of this study are the following parameters; type of light source and shaking rate, and the effect on hydrogen production by *Rhodopseudomonas palustris* TN1. These studies have employed 50 mL vial bottle reactors under experimental conditions of anaerobic-light system, various covered-light structures (rectangular, hexagonal and curved structure), and at room temperature for 96 h via bath process. Glutamate-acetate medium (GA) was also used as a culture medium. Tungsten lamp lighting without shaking rate was found to be the most suitable condition for hydrogen production. Hydrogen production significantly decreased when shaking rate was increased ($p \leq 0.05$). Then, the individual three covered-tungsten light structures with controlled temperature at 30-37 °C was used for hydrogen production. The rectangular structure obtained the highest hydrogen production of $1,100 \pm 47.70$ mL/L. The hydrogen production cost (calculated based on electricity used only) between the uncovered-tungsten light structure with GA medium (0.05 baht/mL H₂ or 0.0016 US dollar/mL H₂) and the covered-tungsten light structure with GA medium (0.04 baht/mL H₂ or 0.0013 US dollar/mL H₂) showed no significant differences ($p > 0.05$).

Keywords: optimization, hydrogen production, *Rhodopseudomonas palustris* TN1, covered-light structure

1. INTRODUCTION

In the past decade, hydrogen has been well known as an environmentally friendly energy gas because the final product of hydrogen combustion is only water with a high energy content of 122 kJ/g. [1-5]. Biological hydrogen productions contain three methods; green algae and cyanobacteria [6], photosynthetic fermentation [6], and dark fermentation [6, 7]. Photosynthetic fermentation using photosynthetic purple non-sulfur (PNS) bacteria are found to be effective bacteria for hydrogen production because of high substrate conversion efficiency and its ability to use a wide variety of substrates to produce hydrogen [8].

PNS bacteria are able to grow in both dark-aerobic and light-anaerobic conditions, but the higher hydrogen production rate by PNS bacteria occurs in light-anaerobic conditions [6]. Thus, electricity was converted to a light energy source via a variety of light sources (tungsten, light-emitting diode (LED) and fluorescent). In addition, sunlight is one of the most effective light energy sources for hydrogen production by PNS bacteria because of its availability [9]. However, limitations of sunlight used are climate changes and its uselessness at night [10], occurring high heat energy and irradiance [11], uncontrollable in wavelength selection [9], and the abundance of ultraviolet (UV) rays affecting cell growth. Moreover, the high level of irradiances of the natural sunlight at noon periods are also affected, resulting in lower hydrogen production and higher poly- β -hydroxybutyrate (PHB) and carotenoid productions as by-products [12]. All light sources generate a variety of wavelengths and intensities, which are the most important parameters because light wavelengths and intensities are absorbed by the specific bacteriochlorophylls (BChls) of PNS bacteria in order to generate energy for cell growth

and hydrogen production [5, 10, 13-14]. Unfortunately, most experiments have been done by turning on the lights without any covering structures [5, 10], resulting in light energy losses to the environment.

Thus, the main objective of this study is to optimize the type of light source and shaking rate for maximum hydrogen production and to design and create the covered-light structures with low electricity costs. To our knowledge, this is the first report on the development of light energy efficiency consumption by using covered-light systems.

2. MATERIALS AND METHODS

2.1 Bacteria and Culture Medium

Pre-cultures of *Rhodospseudomonas palustris* TN1 were grown anaerobically at pH 7.0 and at room temperature (30 ± 2 °C) in a GA medium, which is a basal medium modified by the addition of 5 mM glutamate as a nitrogen source and 20 mM acetate as a carbon source [1]. The GA medium was flushed for 30 seconds by 0.5 L/min argon gas and was sterilized at 121 °C for 15 minutes by an autoclave (Iwaki, ACV-3167N, Japan).

2.2 Experimental Design

Experiments were carried out by 50 mL vial bottles with working volume of 36 mL GA medium and 10% (v/v) cell inoculum prepared by absorbent determination at 660 nm of 0.5.

The structures of the covered-light systems were conducted by a wood matrix and consisted of 6 tungsten lamps, a sensor for temperature detection, an exhaust fan, and a dimmer switch for light adjustment. The inside structure was covered by a plate reflector. The length, width and height of these structures were 80 cm, 75 cm, and 35 cm, respectively (Figure 1 and 4).

Tungsten lamp was Sylvania, Thailand,

specifications of 100 watt and 1,250 lm. LED lamp was Philips, China, specifications of 13 watt and 1,400 lm. Fluorescent lamp was Osram, China, specifications of 18 watt and 1,170 lm.

To investigate the effects of the type of light source, shaking rate, and the three covered-light structures on cell growth and hydrogen production, experiments (Table 1) were conducted as described below;

Table 1. Experimental design by one factor at a time, method at 3 factors and 3 levels for hydrogen production by *Rhodopseudomonas palustris* TN1 under light-anaerobic condition at pH 7.0 and a controlled temperature of 30-37 °C.

Factors	Levels
1. Type of light sources	Tungsten LED Fluorescent
2. Shaking rates (rpm)	0 100 200
3. Covered-light structures	Rectangular Hexagonal Curved

2.2.1 Effect on type of light sources

The GA medium was prepared at the initial pH of 7.0 under 36 mL working volume of vial bottle reactors via bath process. The GA medium was sterilized at 121 °C for 15 minutes by an autoclave. After that, 4 mL of starter (10% v/v and adjusted $OD_{660} = 0.5$) was added and then cultured under 3,000 lx of the various light sources (tungsten, LED and fluorescent) at a temperature of 30-37 °C for 96 h. The shaking rate of this study was 0 rpm and sampling was every 12 h for hydrogen, dry cell weight

(DCW), and pH determinations.

2.2.2 Effect of shaking rates

The GA medium was prepared as described in section 2.2.1. After that, 4 mL of starter was added and then cultured under the optimal light source (obtained from section 2.2.1) with various shaking rates of 0, 100 and 200 rpm at controlled temperatures of 30-37 °C for 96 h. Sampling was conducted every 12 h for hydrogen, DCW, and pH determinations.

2.2.3 Effect of covered-light structures

Firstly, we designed the three covered-light system structures as shown in Figure 1: rectangular (a), hexagonal (b), and curved (c) structures. The GA medium was then prepared as described in section 2.2.1. After that, 4 mL of starter was added and then cultured under the optimal light source (obtained from section 2.2.1) with the optimal shaking rate (obtained from section 2.2.2) at a controlled temperature of 30-37 °C for 96 h under these three structures (Figure 1). Sampling was conducted every 12 h for hydrogen, DCW, and pH determinations.

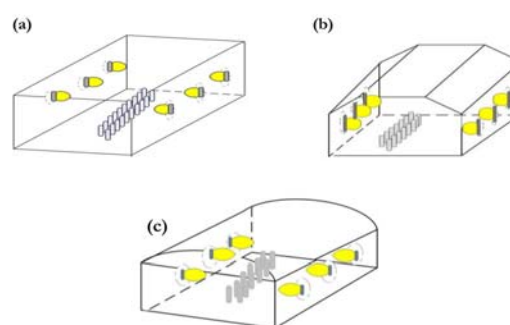


Figure 1. Schematic diagram of the three covered-light structures for hydrogen production by *Rhodopseudomonas palustris* TN1: (a) rectangular, (b) hexagonal, and (c) curved.

2.3 Analytical Methods

The cumulative hydrogen yields were measured by a syringe technique for each test [1]. The hydrogen content of the biogas was measured using an Oldham MX-2100 gas detector (Cambridge Sensotec Ltd., England) [1].

Cell growth was determined by dry cell weight (DCW) [5]. The initial cell concentration was adjusted to 0.5 at 660 nm absorbance, and then converted to dry cell weight using a relationship curve between absorbance values and dry cell weights.

The light intensity was measured using a digital lx-meter (LX-1010BS, China) [5]. The pH value was measured with a calibrated pH meter (Inolab pH, Germany) [5].

All experiments were studied in triplicate and data was expressed in average values. Statistical values were analyzed using ANOVA (SPSS statistic software version 16, USA) and the Duncan's new multiple range test (DMRT) was used to analyze the significance of the values ($p \leq 0.05$).

3. RESULTS AND DISCUSSION

3.1 Effect on Type of Light Sources

Rhodopseudomonas palustris TN1, the photosynthetic bacteria, was screened and selected under anaerobic-light conditions (tungsten lamp) from Songkhla Lake, Thailand [1]. However, there are not any works that use other lamps for hydrogen production for this strain. Thus, LED and fluorescent lamps were used as light sources for hydrogen production for this strain and compared to the tungsten lamp.

The experiments allowed us to conclude that tungsten lamp was the best light source for the highest hydrogen production of $1,604.17 \pm 364.12$ mL/L by *Rps. palustris* TN1 for 96 h cultivation. Hydrogen production under LED and fluorescent lamps as the light sources obtained hydrogen accumulations of 188.33 ± 27.54 and 190.00 ± 30.42 mL/L, respectively (Figure 2B). Fortunately, hydrogen content was very high at 97.44 ± 0.63 , 99.12 ± 0.65 and 97.43 ± 2.97 %, respectively, based on the total gas. The amount of hydrogen gas produced under a tungsten lamp was approximately 8-times higher than that of both LED and fluorescent lamps because the difference in light sources generated the different light wavelengths and intensities which affected cell growth and hydrogen production. The tungsten lamp generates a variety of light wavelengths covering the whole absorption spectrum of photosynthetic bacteria (PSB) [14] and would be a more suitable light source for the reaction of bacteriochlorophylls (BChls) (adsorb at 590-880 nm) [14] than red LED and fluorescent lamps which provide a light wavelength range of 700-850 nm [10] and 450-650 nm [15], respectively.

Considering on electric used and hydrogen production cost, it was found that tungsten lamp has much more electricity used than LED and fluorescent lamp, but tungsten lamp has lower hydrogen production cost than others (Table 2) due to a lot of hydrogen gas produced in a short time. Therefore, tungsten lamp was selected for the next experiment.

Table 2. Comparison of the electricity costs of hydrogen productions under GM medium between tungsten, LED and fluorescent lamps by *Rhodospseudomonas palustris* TN1.

Factors	Tungsten	LED	Fluorescent
1. Total hydrogen gas (mL/L)	1,604.17±364.12	188.33±27.54	190.00±30.42
2. Number of lamp (piece)	6	6	6
3. Cultivation time (h)	72	96	96
4. Unit of electric used (unit)*	43.2	7.4	10.4
5. Electric cost (Baht)	106.25	13.79	19.38
6. The cost of hydrogen production (baht/mL) (US dollar/mL)**	0.05 (0.0016)	0.07 (0.0022)	0.10 (0.0032)

Notes: Electric used (unite) = $\frac{W \times n \times t}{1,000}$,

where W refers to power of lamp (watt), n refers to number of lamp (piece), and t refers to total used time (h). Watt of tungsten, LED and fluorescent lamps were 100, 13 and 18 watt. Total used times were 72, 96 and 96 h, respectively. Units of electricity used were calculated as follows:

1-15 unit was multiplied by 1.8632

16-25 unit was multiplied by 2.5026

26-35 unit was multiplied by 2.7549

36-100 unit was multiplied by 3.1381

** Exchange rate 31.157 baht is 1 US dollar on March 15, 2018.

Zhang et al. [16] reported that the best specific wavelength of a monochromatic LED lamp was 590 nm with a specific light intensity of 6.75 W/m² to produce hydrogen gas by *Rps. palustris* CQK01. Kawagoshi et al [10] reported that a long-wavelength light

emitting diode (LW-LED) (770-920 nm) has a maximum wavelength of 850 nm with a light intensity of 2,000 lx on the surface of the reactor and is considered a suitable light source for cell growth and hydrogen production by isolated photosynthetic bacteria; ht-PSB stain. Adessi and Philippis [14] demonstrated that the wavelengths of 590, 800, 850 and 880 nm were absorbed by BChls, whereas carotenoids absorb the light wavelength between 450-550 nm. Riansa-ngawong et al. [5] reported that the optimal conditions for hydrogen production by *Rps. palustris* TN1 was a light intensity of 3,000 lx using a tungsten lamp. In addition, carotenoid content acted as an important role in photo-protection when exposed to intense light, resulting in a decrease in BChls [13] and consequently the decrease of hydrogen production. Thus, both of these light wavelengths and intensities should be in the optimal absorption range of BChls in order to avoid carotenoids evolution.

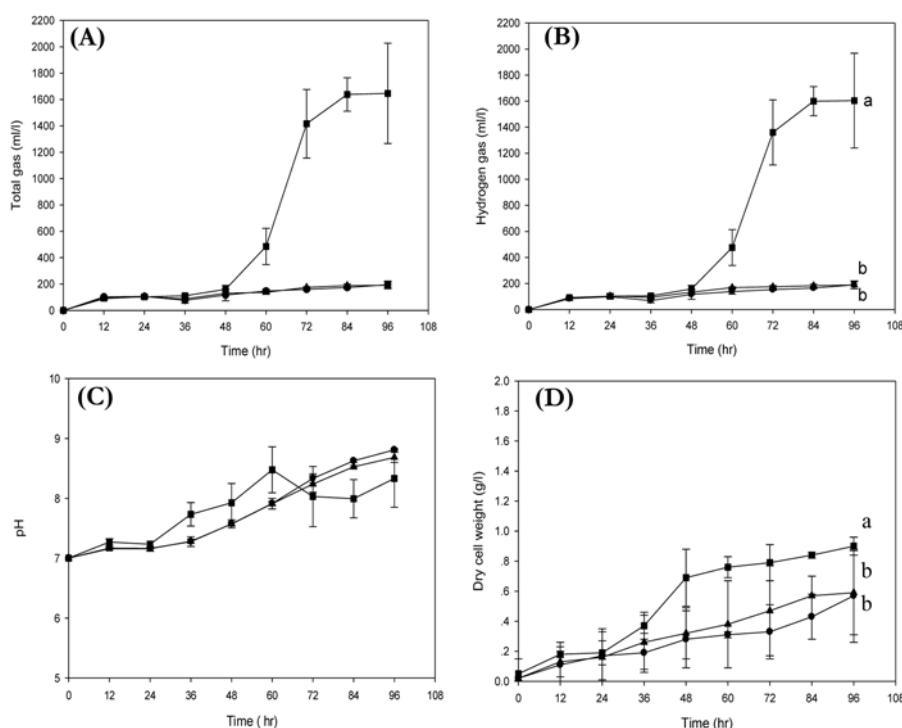


Figure 2. Hydrogen production by *Rhodospseudomonas palustris* TN1 in the GA medium at a controlled temperature of 30-37 °C pH 7.0 under anaerobic-light conditions of 3,000 lx light intensity with various artificial lamps, ■ tungsten, ▲ LED, and ● fluorescent: (A) total gas, (B) hydrogen gas, (C) pH, and (D) dry cell weight.

Note : a and b refer to the significant difference ($p \leq 0.05$) calculated by DMRT.

The pHs of the culture medium under tungsten, LED and fluorescent lamps have increased from 7.0 to 8.3, 8.6 and 8.8, respectively, at 96 h cultivation (Figure 2C). The pH of the culture medium would be affect the activity of nitrogenase, which is an enzyme concerning hydrogen production by *Rps. palustris* TN1 [17]. The optimal pH for hydrogen production should be 6.5-8.0 [18-21]. The increased pH of the culture medium from neutral value (pH 7.0) to slightly alkaline (pH 8-10) resulted in a decrease in hydrogen evolution and increase of poly- β -hydroxybutyrate (PHB) accumulation in cells [3]. PHB was a by-product of hydrogen production and should be avoided.

The cell concentrations have increased from 0.02 g/L to 0.90, 0.59 and 0.57 g/L,

respectively, at 96 h cultivation. The final cell concentration cultured under a tungsten lamp was 2-fold higher than using LED and fluorescent lamps (Figure 2D) due to the suitable light source and intensity described above.

3.2 Effect of Shaking Rates

To study the various shaking rates, 0, 100 and 200 rpm were used. This experiment was carried out by using a 3,000 lx tungsten lamp, the best light source and intensity obtained by section 3.1, under anaerobic-light conditions at a pH of 7.0 with a controlled temperature of 30-37 °C for 96 h cultivation. It was found that *Rps. palustris* TN1 produced the highest hydrogen gas of 791.51 ± 52.34 mL/L at 0 rpm shaking rate with a calculated hydrogen

content of $98.12 \pm 0.42\%$. Whereas at the shaking rates of 100 and 200 rpm, the amount of hydrogen gas was 628.39 ± 90.47 and 534 ± 72.82 mL/L (Figure 3B), which was

significantly lower than that of non-shaking. Fortunately, hydrogen contents of two higher shaking rates were very high as well: 95.47 ± 1.27 and $97.22 \pm 0.58\%$, respectively.

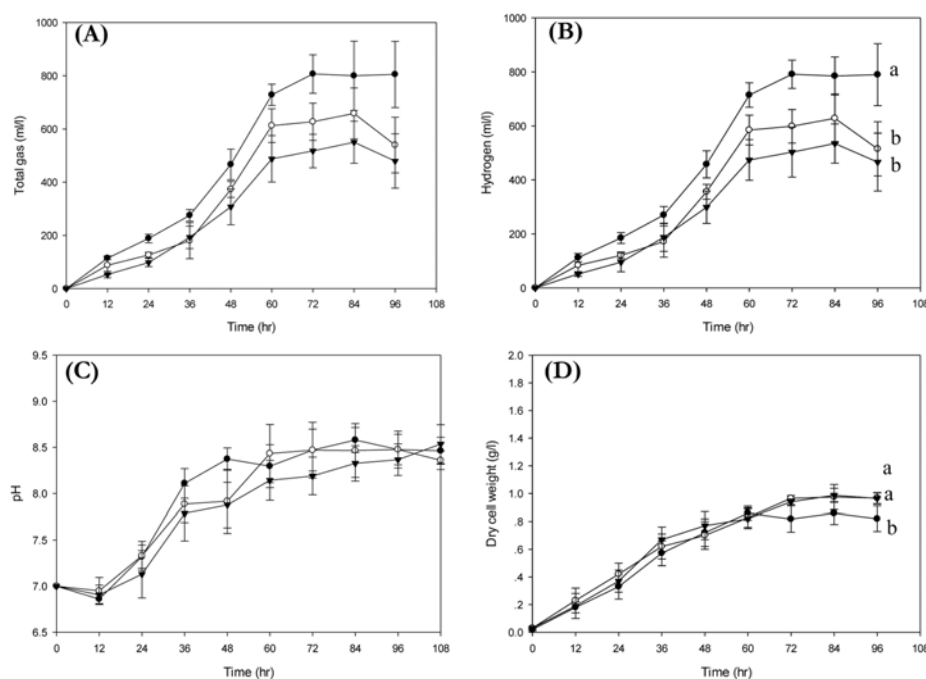


Figure 3. Hydrogen production by *Rhodospseudomonas palustris* TN1 in a GA medium at a controlled temperature of 30-37 °C pH 7.0 under anaerobic-light conditions of 3,000 lx light intensity of a tungsten lamp with various shaking rates, (—●—) 0 rpm, (---○---) 100 rpm, and (—▲—) 200 rpm: (A) total gas, (B) hydrogen gas, (C) pH, and (D) dry cell weight.

Note : a and b refer to the significant difference ($p \leq 0.05$) calculated by DMRT.

The results of these experiments demonstrated that the increasing of shaking rates of 100 and 200 rpm have negatively affected lower hydrogen evolutions. 20.61% and 32.42% of hydrogen gas decreased, when compared to the amount of hydrogen gas produced at 0 rpm shaking rate. This phenomenon might be the hydrogen gas dissolving back into the medium by shaking, resulting in much more hydrogen accumulation. Hydrogenase, an enzyme of PSB that is not only able to produce hydrogen, but also convert hydrogen gas as a substrate under a high hydrogen concentration condition [21], might then be induced by a

high hydrogen concentration, resulting in the decrease of hydrogen gas. However, this experiment was in contrast to others works which found that stirring proved to be an important enhancing factor on not only total hydrogen production but also the conversion efficiency of the substrate hydrogen [22].

After 96 h cultivation, pH values of three shaking rates were increased from a neutral value to 8.4, 8.5 and 8.5 (Figure 3C), respectively, which was not significantly different ($p > 0.05$). Thus, these insignificant differences in pH had no effect on nitrogenase activity.

In the part of cell growth, it was found that cell concentrations of three shaking rates increased to 0.86, 0.97 and 0.98 g/L (Figure 3D), respectively. At non-shaking, the cells concentration was significantly lower than others ($p \leq 0.05$).

Thus, at non-shaking, which obtained the highest hydrogen concentration was selected and used for the next experiment.

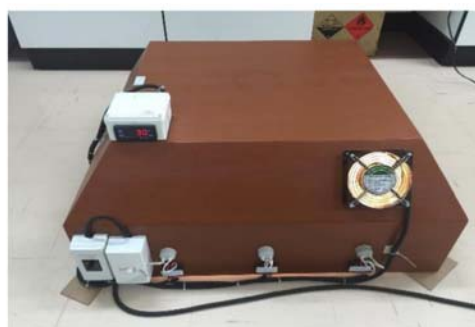
3.3 Effect of Three Covered-light Structures

In preliminary experiments, *Rps. palustris* TN1 was cultured under the three individual covered-light structures, rectangular, hexagonal, and curved, as shown in Figure 4.

After 96 h cultivation, it was found that *Rps. palustris* TN1 could not grow and produce hydrogen gas (Figure 5B and 5D, dark symbols) because of an increased temperature of 45-50 °C. Suwansaard [23] and Wang et al. [8] reported that the optimal temperature of cells growth, *Rps. palustris*, was 30 °C. Watanabe and Fan [24] found that *Rhodopseudomonas gelatinosa* and *Rhodopseudomonas sphaeroides* grew very well at 30-40 °C and was inhibited by a higher temperature at 45 °C. Thus, the cultivation of PSB under three covered-light structures must be tested under a controlled temperature of 30-37 °C by the cooling unit.



(A)



(B)



(C)



(D)

Figure 4. The three covered-light structures for hydrogen production by *Rhodopseudomonas palustris* TN1: (A) rectangular, (B) hexagonal, (C) curved and (D) an inside structure of a hexagonal shape.

After 96 h cultivation with a controlled temperature of 30-37 °C by *Rps. palustris* TN1, the highest hydrogen production of $1,100 \pm 47.70$ mL/L was potentially produced under the rectangular structure, whereas the hexagonal and curved structures obtained a hydrogen gas value of 521.67 ± 62.92 mL/L and 788.33 ± 146.83 mL/L, respectively, which were significantly different in all experiments ($p \leq 0.05$) (Figure 5A and 5B, white symbols). The hydrogen contents of these experiments were 97.63 ± 1.69 , 94.85 ± 2.67 and 99.37 ± 0.13 %, respectively, which were a very high hydrogen content when compared to the total gas production. The amount of produced hydrogen gas under the rectangular structure was 2-folds and 1.4-folds higher than that of the hexagonal

and curved structures. The light intensities at the top surfaces of all vial bottle reactors under three structures of rectangular, hexagonal and curved structures were 3,050, 1,980, and 2,250 lx, respectively, or were 244.50, 158.48, and 180.18 W/m², respectively. It was found that the rectangular structure has a more suitable light reflection area than others that can reflect a higher light loading to the top area of the vial bottle reactor, resulting in an increase of absorbance area of PSB and an increase in hydrogen production (Figure 6).

The pH values and cell concentrations of these three covered-light structures were increased (pH 9.0, 9.1 and 9.0 (Figure 5C), and cell concentration of 1.01, 0.97 and 0.99 g/L (Figure 5D), respectively), which was not significantly different ($p > 0.05$).

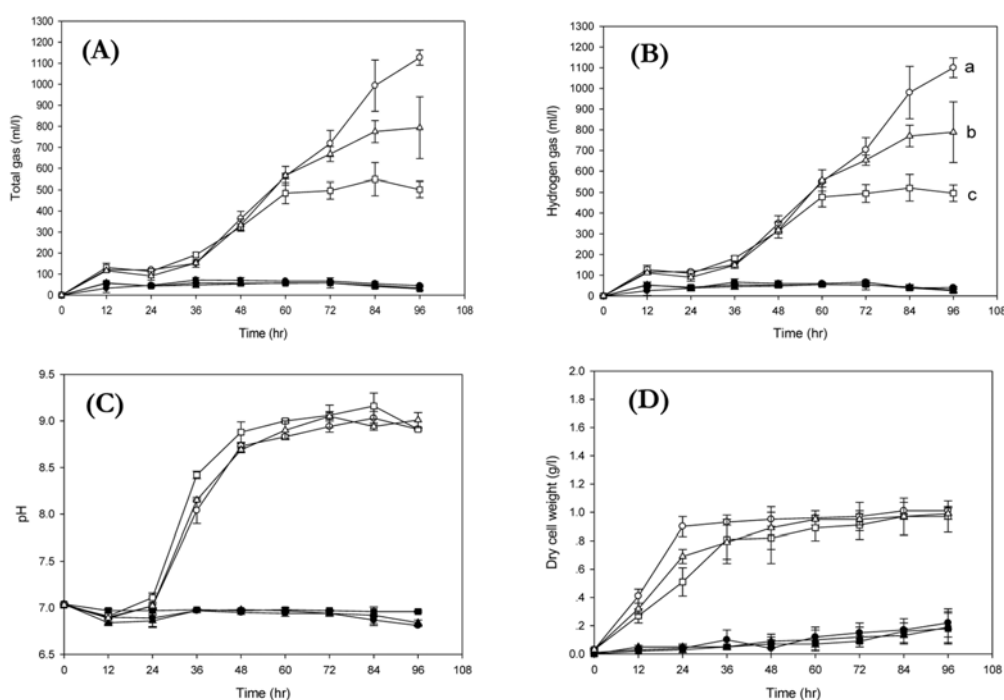


Figure 5. Hydrogen production by *Rhodospseudomonas palustris* TN1 in a GA medium at pH 7.0 under anaerobic-light conditions of 3,000 lx tungsten lamp without a controlled temperature (45-50 °C) (dark symbols) (●) rectangular, (■) hexagonal and (▲) curved, and under controlled temperature (30-37 °C) (white symbols) (○) rectangular, (□) hexagonal and (△) curved: (A) total gas, (B) hydrogen gas, (C) pH and (D) dry cell weight.

Note : a, b and c refer to the significant difference ($p \leq 0.05$) calculated by DMRT.

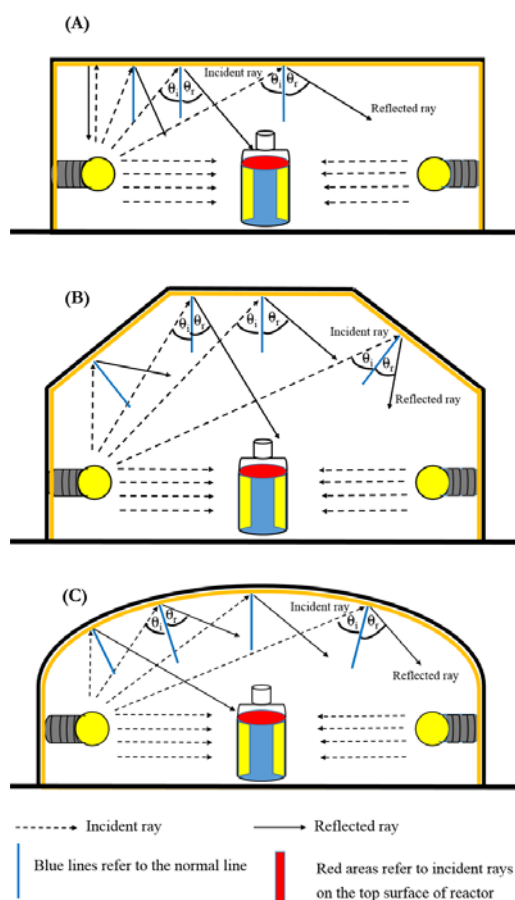


Figure 6. Schematic diagrams of the incident rays and reflected rays of rectangular structure (A) hexagonal structure (B), and curved structure (C).

After 96 h cultivations for hydrogen productions, the cost of hydrogen production calculated based on electric cost were only estimated. The cost of this work, which was done under 4-tungsten lamps, was compared to a previous work, which was done under 6-tungsten lamps without any covered structures [5]. The electric cost was determined at 72 h cultivation, which was a maximum

hydrogen production time. It was found that the electric cost under a covered-light rectangular structure (63.44 baht) was cheaper than that another one (106.25 baht) (Table 3). Unfortunately, the data from Table 3 shows that the electricity costs of both hydrogen productions were not significantly different ($p > 0.05$) because of a lower hydrogen production ($1,100 \pm 47.70$ mL/L).

Table 3. Comparison of the electricity costs of hydrogen productions under GM medium between a covered-light rectangular structure and without a covered-light structure for 72 h cultivation by *Rhodopseudomonas palustris* TN1.

Factors	Without a covered-light structure	Rectangular structure
1. Total hydrogen gas (mL/L)	1,604.17 ± 364.12	1,100 ± 47.70
2. Number of tungsten lamp (piece)	6	4
3. Cultivation time (h)	72	72
4. Unit of electric used (unit)*	43.2	28.8
5. Electric cost (Baht)	106.25	63.44
6. The cost of hydrogen production (baht/mL) (US dollar/mL)**	0.05 (0.0016)	0.04 (0.0013)

Notes: Electric used (unite) = $\frac{W \times n \times t}{1,000}$,

where W refers to power of the tungsten lamp (watt), n refers to number of lamps (piece), and t refers to total used time (h) with a 100 watt tungsten lamp. Total used time was 72 h. Units of electricity used were calculated as follows:

1-15 unit was multiplied by 1.8632

16-25 unit was multiplied by 2.5026

26-35 unit was multiplied by 2.7549

36-100 unit was multiplied by 3.1381

** Exchange rate 31.157 baht is 1 US dollar on March 15, 2018.

4. CONCLUSION

Bio-hydrogen production by photofermentation was investigated using different light sources, shaking rates and covered-light structures. Both the light source and shaking rate affected the hydrogen gas production by *Rps. palustris* TN1. The suitable light source was found to be the tungsten lamp, producing the highest hydrogen gas of 1,604.17 ± 364.12 mL/L whereas no shaking (0 rpm) was the most suitable for cell

growth and hydrogen production. Finally, the suitable covered-light structure for hydrogen production was the rectangular structure, obtaining a hydrogen gas value of 1,100 ± 47.70 mL/L. Unfortunately, hydrogen production under the rectangular structure can only reduce the electricity used but it cannot reduce the cost of hydrogen production (baht/mL).

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