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Research Article

Possibility of Hydrogen Production from Glutamate-Acetate Medium by *Rhodopseudomonas palustris* TN1 under a Closed-Light System Combined with the Solar Light Energy Systems

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ABSTRACT

Hydrogen productions by photosynthetic bacteria (PSB) have been widely studied as it is a clean energy with high energy content potentials. PSB is a popular microorganism that utilizes solar energy to produce hydrogen which could lead to reduction in production costs. However, production that relies on solar energy has many limitations including; instability of weather, daylight limitation, and season changes. The main objective of this study was to produce hydrogen gas using *Rhodopseudomonas palustris* TN1 under 4 light systems. In this study, Glutamate-Acetate (GA) was used as the medium. Results show that the highest hydrogen production of 352.18±37.12 mL/L was achieved after 72 h of cultivation using a tungsten lamp as a light source. The solar indoor system, and the combination of solar indoor light used during daytime with tungsten lamp used at night produced the second and third highest volumes of hydrogen gas of 206.76±41.72 and 175.35±20.74 mL/L after 60 and 96 h of cultivation, respectively. However, there was no significant difference ($p > 0.05$) between these results. Based on hydrogen productivity, tungsten lamp system and the combination of solar indoor light and tungsten lamp resulted in the highest volumes of 9.04 and 8.40 mL/L/h, respectively. These results were accounted for 91-96% of the produced hydrogen. As per the electricity costs, tungsten lamp system and the combination of solar indoor light and tungsten lamp system required 0.30 and 0.36 baht/mL respectively. On the other hand, the solar indoor light system did not incur any electricity cost.

Keywords: hydrogen production, *Rhodopseudomonas palustris* TN1, glutamate-acetate medium, solar energy, closed-light system

1. INTRODUCTION

Hydrogen gas is a proven clean energy as its combustion provides only water vapor and heat energy. According to previous research, the combustion of a mole of hydrogen gas produced 122 kJ/g of energy [1-4]. Hydrogen energy consumption is rapidly increasing due to concerns over environmental problems. Fossil fuel combustion produces carbon dioxide (CO_2) which causes the global warming [5]. In the past 50 years, atmospheric CO_2 concentration and surface temperature of the earth were increased by 18% and 0.64 ºC, respectively [5].

Hydrogen gas productions by photosynthetic bacteria (PSB) has been studied and improved in the past decades. In order to increase the production's efficiency, previous research was focused on; reactor designs (such as flat and tubular bioreactors) [6], light sources [3], light intensities [2], bacterial strains [7], carbon sources, nitrogen sources [8], various initial pH [2] and temperatures [7]. The most important design parameters has been the size and geometry of the bioreactor, its position and orientation [6]. While surface to volume ratio could be increased by a flat bioreactor, irradiated light energy was found to be efficiently used in a tubular reactor [6].

Cost is one of the concerned economic aspects for hydrogen gas production. Productions that rely on solar energy faces many challenges such as season changes, variable day light hours, UV irradiation [9], and unstable light intensity and wavelength [10]. Changes of light intensity and its wavelength can negatively affect PSB's growth, resulting in irregular growth patterns and reduction of hydrogen gas production [11]. However, there are many advantages of solar energy. For instance, the energy is freely available and unlimited [11]. On the other hand, using lamps in production attracts unavoidable costs including, electricity cost, lamp purchase, and loss of light energy [3]. Rectangular design of the light-covered structure was proven to be a suitable structure for tungsten lamp-based hydrogen production at night by *Rhodopseudomonas palustris* TN1 [3]. Moreover, parameters such as light sources (tungsten, light-emitting diode (LED), and fluorescent) were also investigated in order to increase hydrogen production efficiency [2-3, 9, 12-14] while maintaining lower costs of electricity consumptions by the lamps [3]. LED is well known as an energy saving lamp with longer use life, lower heat production, and higher efficiency of electricity conversion [9]. On the other hand, tungsten lamp is a high energy consumption lamp. After 96 h of hydrogen production by *Rps. palustris* TN1, using LED lamp resulted in the lowest electricity costs, compared to those of fluorescent and tungsten lamps. However, the electricity cost per unit of a produced hydrogen gas using LED lamp attracted more expensive electricity cost when compared to the experiment set that used tungsten lamp [3]. When different light sources were presented, each species of PSB has specific activity, depending on their bacteriochlorophylls [3, 12-13. 15], For examples, long wavelength of LED light was proven to be a suitable light source for *Rhodobacter* sp. strain KUPB1 [15], while halogen lamp was suitable for *Rhodopseudomonas palustris* WP3-5 [16] and *Rhodobacter* sp-RV [14], and tungsten lamp was a suitable light source for *Rps. palustris* TN1 [3]. Thus, the studies of combinations of lamp (night production) and solar light (day production) may reveal answers on how the 50% reduction of electricity costs associated with hydrogen production by *Rps. palustris* TN1 could be achieved.

An ability to utilize carbon source is specific to each PSB strains [8]. *Rps. palustris* TN1 is one of the most active photosynthetic bacteria that has been studied. The strain might be suitable for hydrogen production as it can; produce a high quantity of hydrogen gas (> 90%) [3], discharge neutral pH post-production effluent [8], and grow on volatile fatty acids-based substrates (VFAs) such as acetate, propionate and butyrate [8]. According to the previous studies, *Rps. palustris* TN1 could utilized volatile fatty acids (acetate)

containing in pickle bamboo shoot wastewater (PBSW) to produce hydrogen [2], but it failed to use a high molecules carbon sources such as starch containing in rice vermicelli mill effluent (RVME) as substrates. The said results were also similar to results from the original works [8].

In term of economic advantage, hydrogen production by PSB using solar light energy [6, 10, 17] coupled with the abundant agro-industrial wastewater should be studied concurrently to improve the hydrogen production efficiency as well as reducing the production costs [18]. To develop the light source-based production process, experiments were conducted by using the basic synthetic medium. This pilot process provides informative data that can be utilized in the actual agro-industry for which wastewater is used as the substrate.

The objective of this study was to determine the optimal conditions for hydrogen production by *Rps. palustris* TN1 incubated under 4 various light systems where glutamate-acetate (GA) was used as a cultivation medium and to determine the cost of electricity in each experiment. To the best of our knowledge, this was the first study on the hydrogen production under the combinations of close-light system (rectangular structure with tungsten lamps) and solar light systems.

2. MATERIALS AND METHODS

2.1 Bacteria and Culture Medium Preparation

Rhodopseudomonas palustris TN1 was provided by the Environmental Biotechnology Laboratory, Faculty of Agro-Industry, Prince of Songkla University. The strain was isolated from Songkhla lagoon in southern Thailand, by using neutral modified GA medium. The isolation was conducted at room temperature (30±2ºC) under anaerobic-tungsten light conditions with the light intensity of 3,000 lux [8]. *Rps. palustris* TN1 was activated anaerobically 3 times by sub-culturing under the mentioned conditions.

A modified GA medium, a basal medium which was modified by adding 5 mM glutamate as a nitrogen source and 20 mM acetate as a carbon source [2-3, 8] was used as the activation medium. For anaerobic modification, GA medium was flushed for 30 seconds using 0.5 L/min argon gas. Then, the media was sterilized in an autoclave (Iwaki, ACV-3167N, Japan) at 121ºC for 15 min [3].

Bacterial starter was prepared by; pouring 72 mL of the working GA medium into a 100 mL vial bottles, adding 10% (v/v) of cell inoculum which was prepared under 600 nm optical density of 0.5. The culture was then incubated for 24 hours.

2.2 Experimental Design

2.2.1 Reactor and the closed-light system design

Bioreactors for the cultivation of photosynthetic bacteria were constructed using a transparent acrylic cylinder and plates with the thickness of 6 mm (Figure 1A). The length and height of these bioreactors were 230 x 200 mm, and the diameter of 200 mm which resulted in an effective volume of 5.5 L. There were one feed inlet and one feed outlet of 6 mm diameter attached to the left and right side of the bioreactor. Air in and out ports with the same diameter were also attached to the top of the bioreactor. A sampling valve was mounted at the bottom of the bioreactor as shown in Figure 1A.

Experiments were carried out by batch process, which initiated by feeding 4.5 L of GA medium to the acrylic cylinder reactors and 10% (v/v) of cell inoculum (Figure 1A). The closed-light systems were fitted with rectangular structures as described by Botthong et al [3] (Figure 1B). Tungsten lamp was acquired from Sylvania, Thailand, with the specifications of 100 watt and 1,250 lm.

2.2.2 Effect of various light systems

To investigate the effects of various light systems on total biogas production, hydrogen production, cell growth and pH change, four experiments were conducted. These experiment sets consisted of; 1) a tungsten lamp only with the optimal 3,000 lux of light intensity (X_1), 2) solar

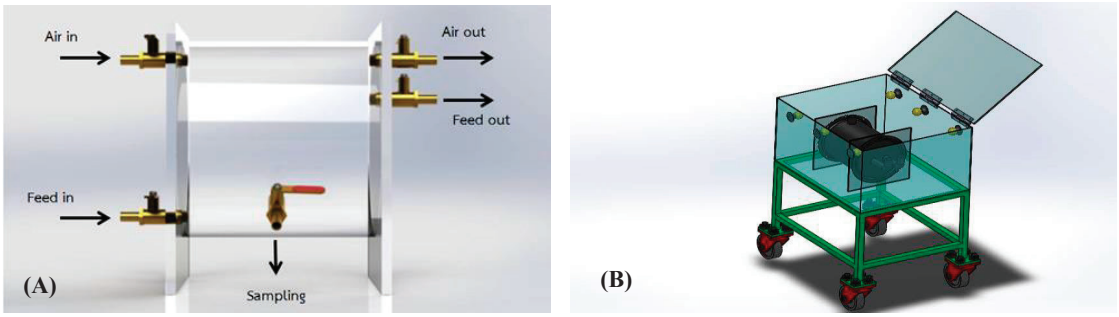

Figure 1. Schematic diagram of the 5.5 L bioreactor (A), and the rectangular structure of the closed-light systems (B) to produce hydrogen gas by *Rhodopseudomonas palustris* TN1.

Table 1. Completely Randomized Design (CRD) of various light systems to determine cell growth and hydrogen production by *Rhodopseudomonas palustris* TN1.

Various light systems	Total biogas production (Y_1)	Hydrogen production (Y_2)	Cell growth (Y_3)	pH change (Y_4)
1. Tungsten lamp (X_1)	X_1Y_1	X_1Y_2	X_1Y_3	X_1Y_4
2. Solar light (Indoor) (X_2)	X_2Y_1	X_2Y_2	X_2Y_3	X_2Y_4
3. Solar light (Outdoor) (X_3)	X_3Y_1	X_3Y_2	X_3Y_3	X_3Y_4
4. Tungsten lamp + Solar light (X_4)	X_4Y_1	X_4Y_2	X_4Y_3	X_4Y_4

indoor light only without controlled light intensity and controlled temperature at 30±2 ºC (X_2), 3) solar outdoor light only without controlled light intensity. However, temperature of the medium was controlled at room temperature (30±2 ºC) by using a cooling unit machine with water circulation (X_3), and 4) tungsten lamp for nighttime experiment (light was switched on at 6.00 pm) combined with solar light (tungsten light was switched off at 6.00 am) that was collected during daylight hours (X_4). The treatment X_4, solar lights, indoor or outdoor, was used as a reference for the highest possible volume of hydrogen gas that was produced. All experiments started at 6.00 pm. The experiments were conducted as per the Completely Randomized Design (CRD) (Table 1).

GA medium was prepared by a batch culture process using an acrylic cylinder reactor with working volume of 4.5 L. under the initial pH of 7.0. Then, 10% v/v of starter was added to the media and cultivated for 96 hours under the above said light systems (Table 1), at room temperature of 30±2 ºC. Sampling was done every 12 hours to determine the total biogas, hydrogen gas, dry cell weight (DCW), and pH change.

After that, electricity costs of each experiments were calculated as described by Botthong et al [3].

2.3 Analytical Methods

2.3.1 Total biogas and hydrogen gas

The cumulative total biogas and hydrogen gas were measured by a 100 mL syringe technique combined with vial bottle containing water and 1.0 N NaOH, respectively. Hydrogen content was continually measured using an Oldham MX-2100 gas detector (Cambridge Sensotec Ltd., England) [2-3], [8].

2.3.2 Dry cell weight (DCW)

Cell growth was determined from the content of Dry Cell Weight (DCW) [2-3]. The initial cell concentration was adjusted to 0.5 at 660 nm absorbance, whilst the dry cell weight was calculated from a standard curve showing the relationship between absorbance values and dry cell weights.

2.3.3 pH changes

The calibrated pH meter (Inolab pH, Germany) was used to measure pH values of the above-mentioned systems [2-3].

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

3. Results and Discussions

3.1 Effect of Various Light Systems

Rps. palustris TN1 is one of the effective PSB strains for hydrogen production under a close-tungsten light system. However, the production costs associated with this system is still quite high (0.04 baht/mL or 0.0013 US dollar/mL) [3]. In order to reduce the costs, free, readily available, and unlimited, solar energy could potentially be used during day time to replace the lamp light [11].

Physical characteristic of cells growths and pigment productions in GA medium with various light systems were shown in Figure 2-4. Cells growth and pigment production under the tungsten lamp light system (X_1) were rapidly increased and investigated within 24 h of the cultivation (Figure 2). Results obtained from using the solar indoor light system alone (X_2) and the combinations of tungsten lamp and solar indoor light system (X_4) were investigated at 84 h and 36 h of cultivations, respectively (Figure 3 and 4). On the other hand, there was no differentiation of cells growth and pigment production under the solar outdoor light system (X_3) (data not shown).

The experiments demonstrated that the tungsten light system (X_1) was the best light source that resulted in the highest productions of total biogas and total hydrogen of 387.05±42.26 and 352.18±37.12 mL/L, respectively. These results, which were achieved after 72 h of cultivation time (Figure 5A and 5B), were significantly difference from the others 3 experimental sets (X_2-X_4) ($p \leq 0.05$). As per the hydrogen gas content calculations, 91.0 % of the gas was obtained which was considered a high percentage. The second highest results were obtained from the solar indoor light system (X_2) which produced the total biogas and total hydrogen productions of 224.57±8.87 and 206.76±41.72 mL/L, respectively. This system required a shorter cultivation time of 60 h and yielded 92.07 % of hydrogen gas content. On the other hand, there was no bacterial growth, pH change, nor hydrogen production under the solar outdoor light system (X_3). Thus, the solar indoor light was selected and used in experiment X_4. The tungsten lamp combined with solar indoor light system (X_4) produced the total biogas and total hydrogen gas of 181.12±16.62 and 175.35±20.74 mL/L, respectively. The cultivation time for this system was 96 h which resulted in hydrogen content of 96.81 %. There was no significant difference between X_2 and X_4 ($p > 0.05$), meaning that hydrogen production by *Rps. palustris* TN1 using solar indoor light energy as a light energy source, could reasonably be used to replace the tungsten lamps.

An absence of bacterial growth in the solar outdoor light system (X_3) was due to excessive

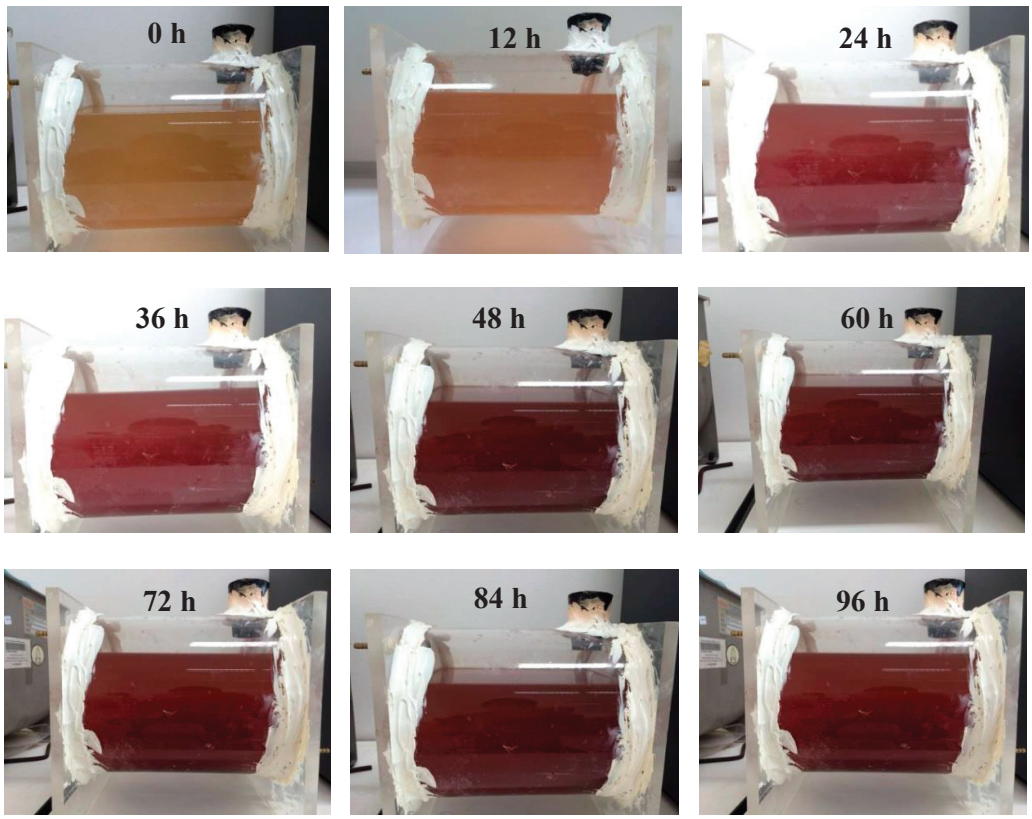

Figure 2. Physical characteristics of cell growth and pigment production under the tungsten lamp light system (X_1) via batch process for 96 h cultivation by *Rhodopseudomonas palustris* TN1.

light intensity and ultraviolet ray (UV) dose on bacterial disinfection [19-20]. The efficiency of UV disinfection depended on the UV dose, exposure time, water or culture medium absorbance, reflection factor [20-21], initial bacterial concentration, water temperature, as well as turbidity of the water [19]. It is well known that UV-A (320-400 nm) is the main parameter for solar inactivation of the bacteria which is also known as a bactericidal effect [19]. PSB can absorb visible wavelengths between 400-950 nm, and it can adapt to photosynthesis under low light intensity. PSB uses large arrays of light harvesting complexes to capture diffuse light energy and conduct it into the reaction center [22]. For hydrogen production by PSB, decrease in light intensity led to an increase in light efficiency [23-24]. From the experiments of light intensity, which was determined during January to March 2018, outdoor light's intensities (X_3) were between 10,000-70,000 lux. Between 6.00 am to 6.00 pm and noon, the maximum intensity was approximately 70,000 lux, while light intensity of the solar indoor light system (X_2) were between 2,000-12,000 lux (Figure 6). Compared to the day light, these indoor light intensities closely resemble the optimal light intensity for this PSB strain (3,000-5,000 lux). The optimal light intensity of TN1 strain at 3,000 lux was reported

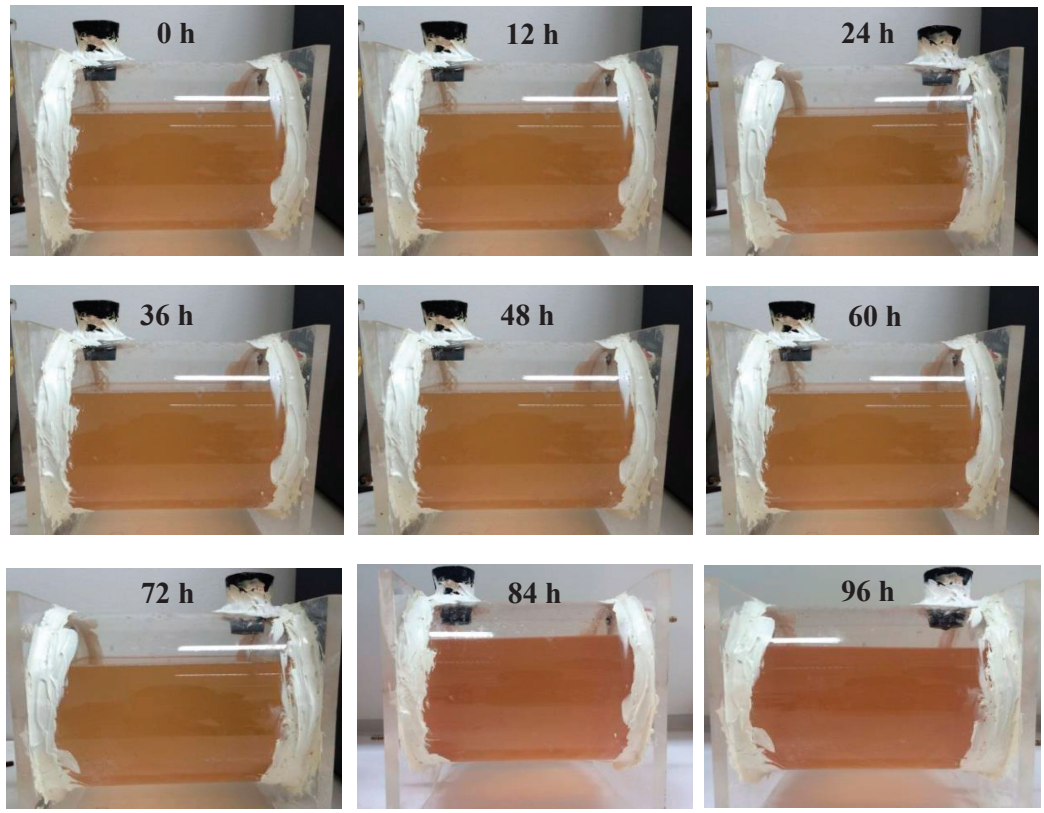

Figure 3. Physical characteristics of cell growth and pigment production under the solar indoor light system (X_2) via batch process for 96 h cultivation by *Rhodopseudomonas palustris* TN1.

by Riansa-ngawong et al [2] and Suwansaard [8] while the optimal light intensity for *Rps. palustris* CQK01 was 5,000 lux [25]. Light intensity is one of the important parameters affecting cell growth, hydrogen production capability, and light efficiency as previously described. Thus, the system of outdoor light was not suitable for TN1 strain growth and hydrogen gas production, however, further studies to improve and develop its efficiency will be required.

The pH values of tungsten lamp system (X_1), solar indoor light system (X_2), and tungsten lamp combined with solar indoor light system (X_4) were increased at the initial fermentation stage from neutral to mild alkali. After 96 h of cultivation, these changed pH were 8.08, 7.62, and 8.21, respectively (Figure 5C). The final pH of X_1 and X_4 were not significantly different ($P>0.05$), but significantly higher than that of X_2 ($P\leq0.05$). Optimal pH was an important parameter that regulated the activity of nitrogenase, the main enzyme found in purple non-sulfur bacteria (PNSB) for which the bacteria utilizes to produce hydrogen gas [26]. In the presence of nitrogen gas, nitrogenase enzyme facilitates production of ammonium. However, under nitrogen deficient conditions, the enzyme produces hydrogen instead. [26-27]. In addition, nitrogenase enzyme is inhibited by the produced

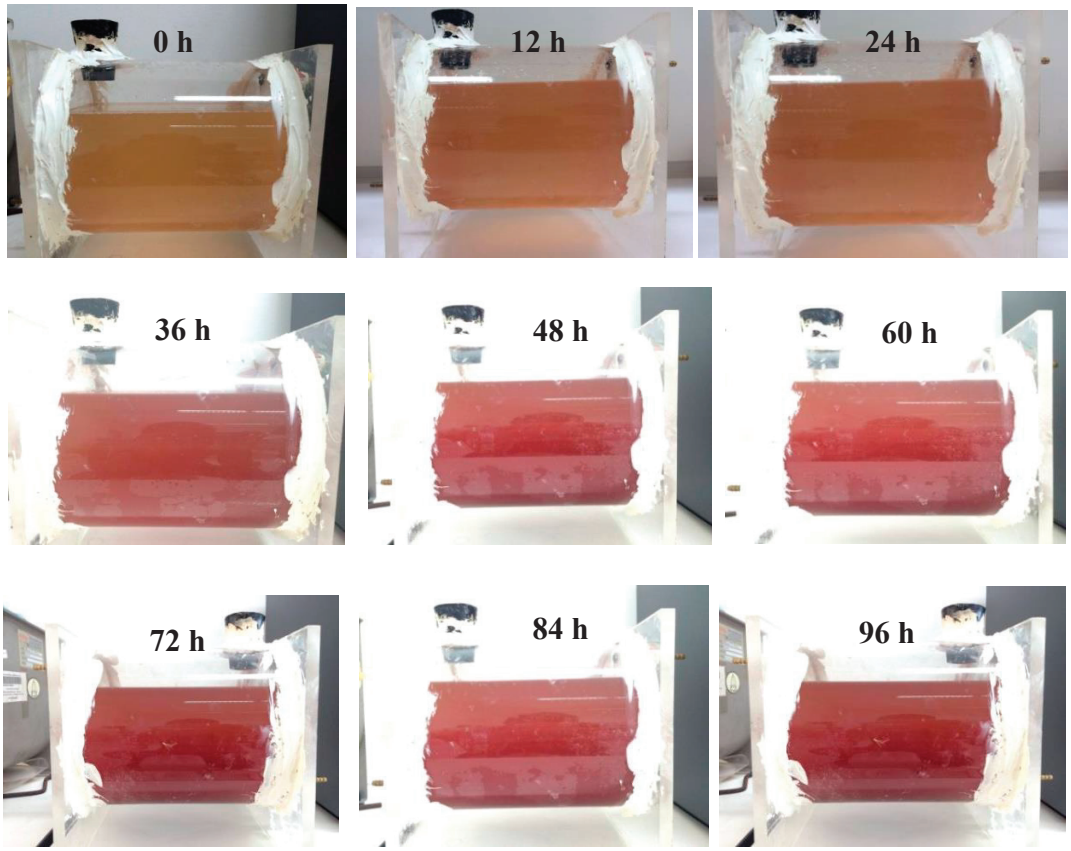

Figure 4. Physical characteristics of cell growth and pigment production under the tungsten lamp combined with solar indoor light system (X_4) via batch process for 96 h cultivation by *Rhodopseudomonas palustris* TN1.

ammonia [18]. Previous studies suggested that the optimal pH of nitrogenase enzyme was between 7.1-7.3 [28]. According to results of this study, the pH mediums were higher than that of nitrogenase's optimal pH after 60 h of cultivation until the end of fermentation process. This phenomenon might be responsible for the lower production of lower hydrogen gas (Figure 5B and 5C). Thus, the pH of culture medium was one of the important parameters and should be considered and evaluated.

In addition, hydrogen productions (mL/L) of this study were converted to hydrogen productivity (mL/L/h), in order to compare the production per a unit of time. Results revealed that the maximum hydrogen productivity obtained from the tungsten lamp system (X_1) and the solar indoor combined with tungsten lamp system (X_4) were 9.04 and 8.40 mL/L/h after 24 and 12 h of cultivation time respectively. There was no significant difference between the production rates of these two systems ($p > 0.05$).

Result obtained from the solar indoor (X_2) system was 3.45 mL/L/h after 60 h of cultivation time (Figure 7), which was lower than those of the other two light systems. Hydrogen production (Figure 5D and 5B) and hydrogen productivity

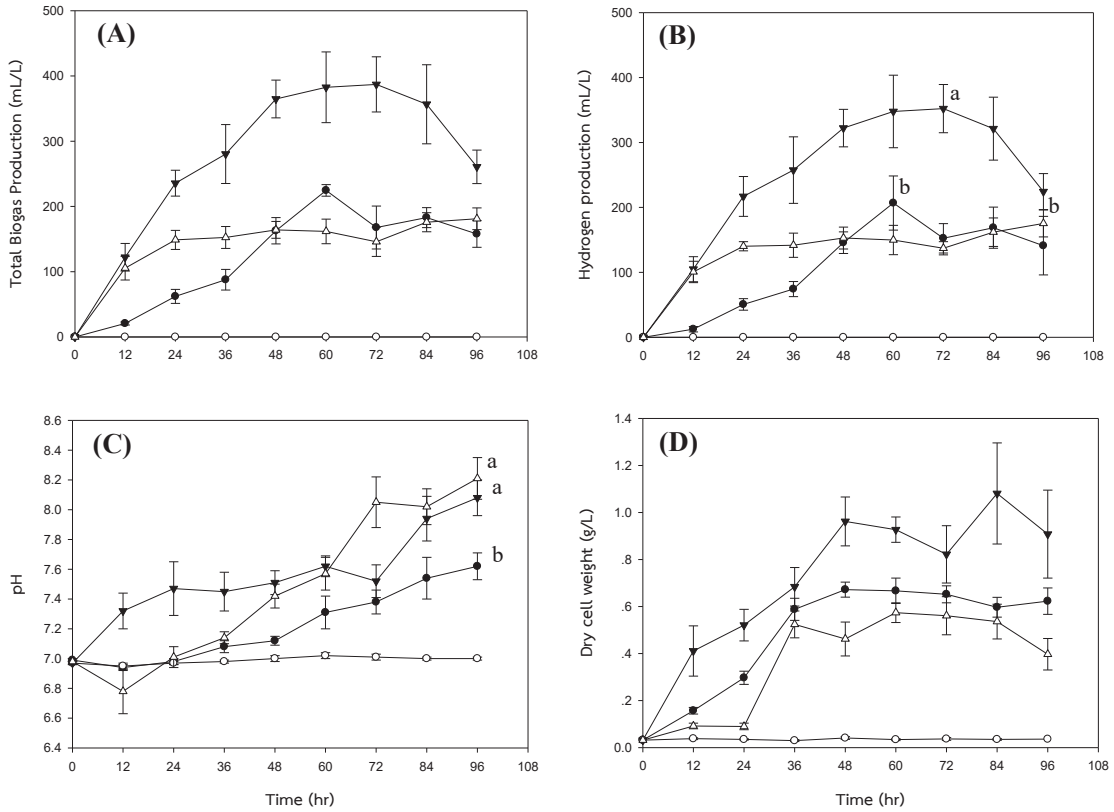

Figure 5. Total biogas (A), hydrogen gas (B), pH (C), and dry cell weight (D) of *Rhodopseudomonas palustris* TN1 anaerobically cultivated in a 5.5 L bioreactor using GA medium with the initial pH of 7.0, and 30±2ºC of controlled room temperature under various light systems; tungsten lamp (–▼–), solar indoor system (—●—), solar outdoor system (—○—), and tungsten lamp combined with solar indoor (–△–).

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

(Figure 7) produced by *Rps. palustris* TN1 cultured under the solar indoor system (X_2) was slightly increased from 12-24 h of cultivation. This was due to varying light intensities during the day which induced *Rps. palustris* TN1's adaptation to the environment (lag phase) [14]. The result was observed when switching phases between daytime and night time, which led to a decrease of photosynthesis efficiency. These findings were similar to the study by Adessi et al [11], which found that hydrogen accumulation by *Rps. palustris* was halted every night for five consecutive days of each experiments. The study also reported that the amounts of dissolved hydrogen in the culture medium were decreased as well. Majority of hydrogen is produced by nitrogenase process while the minority of the gas is produced by hydrogenase process. However, uptake-hydrogenase can also consume hydrogen to produce ATP, H^+, and electrons during the dark period, in the presence of a small amount of oxygen [8, 11, 28-29]. Thus, a number of hours in the morning are

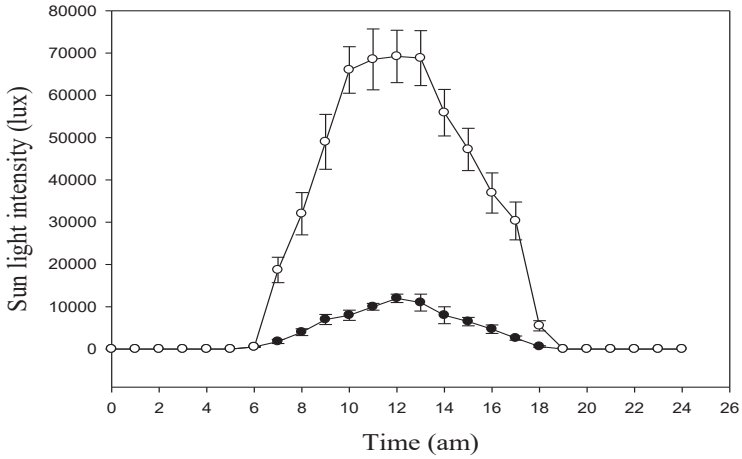

Figure 6. Measurement of solar outdoor intensity (—○—) and solar indoor intensity (—●—) at the Faculty of Agro-Industry, KMUTNB, Prachinburi campus, during January to March 2018.

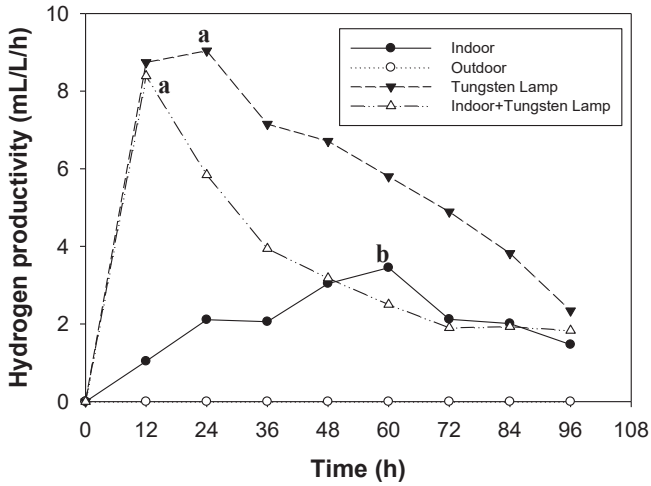

Figure 7. Hydrogen productivity by *Rhodopseudomonas palustris* TN1 in a 5.5 L acrylic cylinder reactor anaerobically cultivated in GA medium with an initial of pH 7.0 under controlled room temperature and different light systems.

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

required to re-saturate the culture medium which could resulted negatively to the total amount of collectable hydrogen gas [11].

Unfortunately, the maximum hydrogen productivities (9.04 and 8.40 mL/L/h) of this study were less than that of the previous work (11.45 mL/L/h) [3], which was conducted under the vials reactor volume of 50 mL with 4.2 cm diameter. This could be due to an oversize diameter of the reactor (20 cm). Our preliminary results (data not shown), show that light intensity behind the reactor was decreased from 2,000 lux to 1,400 and 1,000 lux when diameter of the reactor was increased from 4.2 cm to 7.5 and 10.0 cm, respectively. The experiment was conducted under GA medium condition (Regression coefficient at $R^2 = 0.9843$). Kitajima et al [30] demonstrated that hydrogen production was decreased from 200 to 100 L/m^2 when the diameter of a reactor was increased from 5 to 10 cm. As light energy could not penetrate into the center of the reactor, hydrogen consumption by PSB' uptake-hydrogenase occurred. Thus, the diameter of the reactor should be strongly considered where the highest efficiency of hydrogen production by PSB is concerned.

3.2 Electricity Cost Calculation

After hydrogen productions by *Rps. palustris* TN1, the electricity costs of X_1, X_2, and X_4 experiments were calculated in order to compare the potential and possibility of their applications in the future. The electricity costs of 6-tungsten lamps system (X_1) and solar indoor combined with 6-tungsten lamps system (X_4), which produced the maximum hydrogen gas contents after 72 and 96 h cultivation were compared to that of the solar indoor light system (X_2) after 60 h cultivation. Results show no electricity cost under the solar indoor light system (X_2), whereas, the electricity cost of X_4 (63.44 baht) was cheaper than that

Table 2. Comparison of the electricity costs from hydrogen production by *Rhodopseudomonas palustris* TN1 between X_1, X_2, and X_4 experiments, cultivated in GA medium in 20 cm diameter acrylic cylinder reactors.

Factors	Tungsten lamp (X_1)	Solar indoor (X_2)	Solar indoor + Tungsten lamp (X_4)
1. Total hydrogen gas (mL/L)	352.18±37.12	206.76±41.72	175.35±20.74
2. Number of tungsten lamps (piece)	6	0	6
3. Cultivation time (h)	72	60	96
4. Units of electric used (unit)*	43.2	0	28.8
5. Electricity costs (Baht)	106.25	0	63.44
6. Hydrogen production costs (baht/mL)(US dollar/mL**)	0.30 (0.009**)	0 (0**)	0.36 (0.011**)

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

Where W refers to the power of tungsten lamp (watt), n refers to the number of lamps (piece), and t refers to the total used time giving that resulted in the maximum hydrogen productivity (h). 100 watt tungsten lamps were used in these studies.

* Units of electricity used were calculated as following [3]:

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 on August 4, 2021 was 33.079 baht per 1 US dollar.

of X_1 (106.25 baht) (Table 2). Unfortunately, hydrogen production costs of X_1 (0.30 baht/mL) and X_4 (0.36 mL/L) were higher than that of the previous work (0.04 baht/mL) [3] due to the differences of the bioreactors. As the solar indoor light system (X_2) could reduce electricity costs, the system is highly suitable for the future development. Thus, to undersatand economic aspects of the production, important parameters such as processes developement, light sources, and availability of raw materials should be assessed in order to achieve the lowest possible production costs of hydrogen production.

4. Conclusions

Bio-hydrogen productions by *Rhodopseudomonas palustris* TN1 was studied using 4 different light systems. All of these systems exhibited certain effects on the hydrogen gas production by TN1 stain. Tungsten lamp system provided the highest hydrogen gas content of 352.18±37.12 mL/L and required 0.30 baht/mL of electricity cost. The solar indoor light system resulted in no electricity cost but produced a lower hydrogen gas content (206.76±41.72 mL/L). As a result, the latter system produced 41.29% less hydrogen content than the former one. However, the solar indoor light system is considered a suitable and appropriate system due to its potential to reduce electricity costs.

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

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