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Effect of cell density and nutrient deprivation on hydrogen production by unicellular green alga *Scenedesmus* sp. KMITL-OVG1

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Abstract

Hydrogen is considered as one of the energy carriers for the near future. H_2 production by green algae is catalyzed by hydrogenase activity using electrons from photosynthetic process under the light and from accumulated carbohydrate catabolism in the dark. This research aimed to investigate the effect of cell density and nutrient deprivation on H_2 production by *Scenedesmus* sp. KMITL-OVG1 isolated in Thailand. The result showed that cell culture with the optical density at 750 nm of 0.8 gave the highest H_2 production rate. Interestingly, the highest H_2 production rate of 1.957 ± 0.100 mL L^{-1} h^{-1} and hydrogenase activity of 0.031 ± 0.001 ml L^{-1} min^{-1} were found in cells incubated under potassium deprivation. H_2 production rate was approximately 3 folds higher than that of cells incubated in normal TAP medium. The increased H_2 production rate and hydrogenase activity might be involved in the reduction of starch accumulation. Moreover, the deprivation of potassium combined with other nutrients did not enhance H_2 production rate by *Scenedesmus* sp. KMITL-OVG1.

Keywords: hydrogen production, nutrient deprivation, green algae, *Scenedesmus* sp.

1. Introduction

Biophotolysis is one of the promising concepts for clean hydrogen production. Molecular hydrogen (H_2) produced by microalgae is considered as an attractive energy carrier due to the potential for a sustainable production system [1]. In 1939, Gaffron discovered the ability of unicellular green alga *Scenedesmus obliquus* to produce H_2 upon illumination [2]. Under anaerobic condition, *S. obliquus* can either use H_2 as electron donor in the CO_2 fixation in the dark [3], or evolve H_2 in the light [4]. Under light condition, energy in form of ATP and the reducing power NAD(P)H obtained from the light reaction of photosynthesis are utilized as energy and electron sources, respectively, to produce H_2 [5].

H_2 production by microalgae is divided into two main processes, direct photolysis and indirect photolysis. In a direct photolysis, H_2 is produced by electrons obtained from the water-splitting of photosynthetic pathway whereas in an indirect photolysis, H_2 is produced by electrons obtained from the degradation of storage carbohydrate [6 & 7]. However, Oxygen, an inhibitor of hydrogenase enzyme, which is generated by photosystem II (PSII) activity in photosynthesis, is a great obstacle for H_2 production by microalgae [8]. Therefore, two-stage cell culture system (system separating H_2 production phase from growth phase) is exploited for efficiently enhancing productivity of H_2 [9]. In the growth phase, algal cells are grown autotrophically or heterotrophically in the medium under the presence of O_2 to accumulate biomass. In the H_2 production phase, cells are incubated in the nutrient deprived medium for period of time under anaerobic condition in order to induce H_2 production.

Because sulfur and nitrogen are major constituents of important biomolecules in the cells, the deprivation of these elements in cells leads to a damage of protein structure, enzyme function and cellular metabolism [10]. Under sulfur deprivation, green algae *Chlorella* and *Chlamydomonas* stopped dividing cells, changed their morphology, altered photosynthesis and cellular metabolism and accumulated endogenous proteins and starches for survival [11 & 12]. In addition, a process of water splitting in the light of *Chlamydomonas reinhardtii* was blocked under sulfur deprivation, resulting in the lower rate of photosynthetic oxygen evolution than rate of mitochondrial respiration. As a result, the environmental system of algal culture became anaerobiosis, thereby activating hydrogenase activity and H_2 production [13]. Under nitrogen deprivation, *C. reinhardtii* lost an impairment of D1 protein, a key subunit of PSII in oxygenic photosynthetic organisms, leading to a decrease of O_2 in cells and this finally enhanced H_2 production [14].

Phosphorus and potassium are also essential and important elements for a cellular metabolism. They are cofactors of many enzymes involving in carbohydrate metabolism and cellular metabolism [15]. The deprivation of phosphorus and potassium is likely to influence H_2 production of green algae [16 & 17]. It has been reported that the phosphorus depletion led to the inhibition of O_2-evolving activity in *C. reinhardtii* cells. This caused the metabolic changes that were favorable for H_2 production [13 & 18]. In addition, starch was accumulated in algal cells during phosphorus deprivation and degraded to obtain a large amount of electron sources for H_2 production [18]. In case of potassium deficiency stress, the green alga *S. obliquus* showed high H_2 production due to the establishment of anoxic condition by the PS II activity diminishment from impairing the D1 protein replacement [17].

Besides nutrient deprivation, cell density plays also an impact role in H_2 production by green algae. High density of algal cells causes the disruption of light penetration and thereby reducing the photosynthetic activity [19 & 20]. It has been previously shown that once the light intensity enters the system, the optimal cell density of algal cells promotes effectively H_2 production [21].

In this study, H_2 production by unicellular green alga *Scenedesmus* sp. KMITL-OVG1 which has been shown as one of high potential H_2 producers from a primary screening of green algal strains in our laboratory [22] was investigated. The objectives of this work were to investigate the effect of initial cell density on H_2 production. Subsequently, the effect of single nutrient and multiple nutrient deprivations on H_2 production by *Scenedesmus* sp. KMITL-OVG1 were studied. The results of this study would provide the potential of H_2 production and the ability of this green algal strain as a good H_2 producer.

2. Materials and methods

2.1 Green algal strain and growth condition

The unicellular green alga *Scenedesmus* sp. KMITL-OVG1 was isolated from a natural pond at King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand. It was identified as *Scenedesmus* sp. by morphological characteristics and molecular analysis using 18S rDNA sequencing. The nucleotide sequences of 18S rDNA were deposited in GenBank under accession number MH979037. *Scenedesmus* sp. KMITL-OVG1 was grown in a 250-mL Erlenmeyer flask containing 100 mL of autoclave-sterilized Tris acetate phosphate (TAP) medium (pH 7.2) [23] with shaking at 120 rpm under cool-white fluorescent lamps with a light intensity of 30 μmol photons m^{-2} s^{-1} at 30 ^oC for 36 h.

2.2 Effect of initial cell density on H_2 production

Scenedesmus sp. KMITL-OVG1 grown as previously described for 36 h was harvested by centrifugation at 7,000 rpm at 4 ^oC for 10 min, washed twice and resuspended in a fresh TAP medium. The green algal culture was adjusted to the optical density at wavelength 750 nm of 0.2, 0.4, 0.6, 0.8, 1.0 which corresponds to the cell dry weight of 0.027, 0.056, 0.091, 0.132 and 0.179 g_{CDW} L^{-1}, respectively. Five mL of cell suspension were transferred to a 12-mL gas-tight vial. The vial was sealed with a rubber stopper, purged with argon gas for 10 min to enter an anaerobic environment. The cell suspension was further incubated at 30 ^oC with shaking at 120 rpm under the light intensity of 30 μmol photons m^{-2} s^{-1} for 24 h before measuring H_2 by Gas Chromatograph.

2.3 Effect of nutrient deprivation on H_2 production

For investigation of single nutrient deprivation, *Scenedesmus* sp. KMITL-OVG1 grown for 36 h was harvested by centrifugation, washed twice and resuspended in five different kinds of TAP media; sulfur-deprived TAP (TAP-S), nitrogen-deprived TAP (TAP-N), phosphorus-deprived TAP (TAP-P), potassium-deprived TAP (TAP-K) and normal TAP as a control medium. To remove sulfur in TAP-S, $MgSO_4.6H_2O$, $FeSO_4.7H_2O$, $ZnSO_4.7H_2O$ and $CuSO_4.5H_2O$ were removed from the medium but the metal ions Mg^{2+}, Fe^{2+}, Zn^{2+} and Cu^{2+} were replaced by adding $MgCl_2$, $FeCl_2$, $ZnCl_2$ and $CuCl_2$, respectively, with the same ion

concentrations. In TAP-N, NH_4Cl was removed from the medium. In TAP-P, KH_2PO_4 and K_2HPO_4 were removed from the media but K^+ was replaced by an addition of KCl. In TAP-K, KH_2PO_4 and K_2HPO_4 were removed from the medium but PO_4^{3-} was replaced by an addition of NaH_2PO_4 and Na_2HPO_4, respectively. The displacement and replacement of these compounds in all different TAP media are summarized in Table 1. Then, cell suspension was adjusted to reach the optimal optical density. Five mL of cell suspension were transferred to a glass vial and purged with argon for 10 min to enter an anaerobic condition. The vial was shaken at 120 rpm under the light intensity of 30 μmol photons m^{-2} s^{-1} at 30 ºC for 24 h before analyzing H_2 production by Gas Chromatograph [24]. In case of multiple nutrient deprivation study, *Scenedesmus* sp. KMITL-OVG1 grown for 36 h was harvested by centrifugation, washed twice and resuspended in multiple nutrient deprived TAP media including TAP-KS, TAP-KN, TAP-KP, TAP-KPN, TAP-KNS and TAP-KPS media.

Table 1 Culture medium content of normal TAP, single nutrient deprived TAP media including TAP-S, TAP-N, TAP-P, TAP-K, and multiple nutrient deprived TAP media including TAP-KS, TAP-KN, TAP-KP, TAP-KPN, TAP-KPS and TAP-KNS. Presence (+) and Absence (-) show the availability and non-availability of each chemical compound in the culture medium, respectively. In some cases, salts are replaced by an addition of other chemical compounds.

Compound	Type of medium										
	TAP	TAP-S	TAP-N	TAP-P	TAP-K	TAP-KS	TAP-KN	TAP-KP	TAP-KPN	TAP-KPS	TAP-KNS
NH_4Cl	+	+	-	+	+	+	-	+	-	+	-
$MgSO_4.6H_2O$	+	$MgCl_2$	+	+	+	$MgCl_2$	+	+	+	$MgCl_2$	$MgCl_2$
$FeSO_4.7H_2O$	+	$FeCl_3$	+	+	+	$FeCl_3$	+	+	+	$FeCl_3$	$FeCl_3$
$ZnSO_4.7H_2O$	+	$ZnCl_2$	+	+	+	$ZnCl_2$	+	+	+	$ZnCl_2$	$ZnCl_2$
$CuSO_4.5H_2O$	+	$CuCl_2$	+	+	+	$CuCl_2$	+	+	+	$CuCl_2$	$CuCl_2$
KH_2PO_4	+	+	+	KCl	NaH_2PO_4	NaH_2PO_4	NaH_2PO_4	-	-	-	NaH_2PO_4
K_2HPO_4	+	+	+	KCl	Na_2HPO_4	NaH_2PO_4	NaH_2PO_4	-	-	-	NaH_2PO_4

2.4 Measurement of H_2 production

H_2 was determined by analyzing 500 μL of headspace gas by Gas Chromatograph (Hewlett-Packard HP5890A, Japan) using a thermal conductivity detector (TCD) and argon as a carrier gas. The injector, detector and column temperatures were set at 100, 100 and 50 ºC, respectively [25]. H_2 production rate was calculated as the amount of produced H_2 in unit of mL per L of cell culture in an hour. H_2 production rate was determined after anaerobic incubation for 24 h. All experiments were performed in triplicate.

2.5 Measurement of hydrogenase activity

The green alga *Scenedesmus* sp. KMITL-OVG1 grown for 36 h was harvested by centrifugation, washed twice and resuspended in different fresh media. The algal cell suspension was adjusted to reach the optimal OD_{750}. Five mL of cell suspension were transferred to a gas-tight vial and shaken at 120 rpm at 30 ºC under light intensity of 30 μmol photons m^{-2} s^{-1} for 24 h. Hydrogenase activity was measured in the presence of methyl viologen and sodium dithionite. Two mL of reaction mixture contained 1 mL of algal suspension and 1 mL of 12.5 mM phosphate buffer solution (pH 7.0) containing 10 mM methyl viologen and 40 mM sodium dithionite. The reaction was performed at 30 ºC under darkness for 20 min [25]. The amount of H_2 was determined by analyzing 500 μL of headspace gas by Gas Chromatograph with previously described conditions [25].

2.6 Measurement of starch concentration

Starch concentration was determined by reducing sugar concentration measurement after enzymatic hydrolysis by a protocol modified from Gfeller and Gibbs [26]. Briefly, 1 ml of cell culture was centrifuged at 6,000 xg at 4 ºC for 5 min. To remove chlorophyll from algal cell pellet, chlorophyll was extracted twice with 1 mL of methanol. The obtained cell pellet was then resuspended in 1.7 ml of 100 mM Na-acetate buffer (pH 4.5). The cell suspension samples were then sonicated for 5 min, subsequently centrifuged at 6,000 xg at 4 ºC for 5 min. The supernatant was heated by autoclaving at 110 ºC for 15 min to solubilize the starch. After autoclaving 2.2 units of amyloglucosidase were added to the samples and reaction mixtures were incubated in a water bath at 55 ºC for overnight. The volume of samples was adjusted to 2.0 mL with H_2O and centrifuged again to collect a clear supernatant fraction. Glucose concentration was determined from supernatant by DNS method [27].

2.7 Statistical analysis

H_2 production rate were calculated as means of at least three independent experiments. Error bar indicated the standard deviation. All data were examined by one-way analysis of variance (ANOVA) with a 95% significant confidence level using SPSS 16.0.

3. Results

3.1 Effect of cell density on H_2 production

The effect of cell density on H_2 production rate by *Scenedesmus* sp. KMITL-OVG1 was investigated. It was found that H_2 production rate by *Scenedesmus* sp. KMITL-OVG1 was significantly higher when cell density at OD_{750} was increased. *Scenedesmus* sp. KMITL-OVG1 reached the maximum H_2 production rate of 0.785 ± 0.073 mL L^{-1} h^{-1} and H_2 production yield of 19.138 ± 1.099 mL L^{-1} with cell density at OD_{750} of 0.8 (Figure 1). However, H_2 production rates of cells with cell density at OD_{750} of 0.6 and 0.8 were not significantly different at $P < 0.05$. Higher cell density with OD_{750} than 0.8 decreased obviously H_2 production rate. In this experiment, we chose cell density at OD_{750} of 0.8 for further experiments due to the highest H_2 production rate and H_2 production yield.

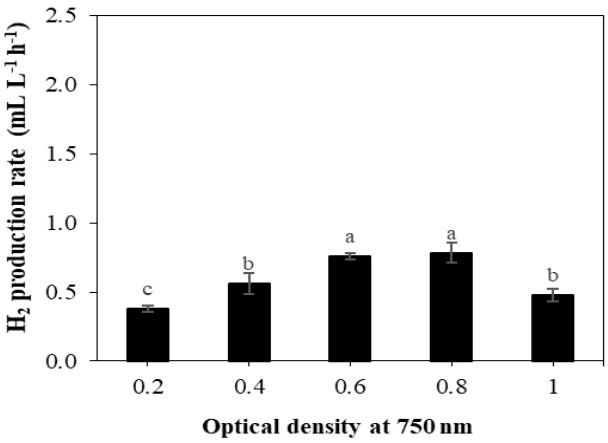

Figure 1 H_2 production rate by *Scenedesmus* sp. KMITL-OVG1 with different cell densities. Data are expressed as means (±SD) of three independent experiments. Different letters indicate significant differences between groups at $P < 0.05$.

3.2 Effect of single nutrient deprivation on H_2 production rate and hydrogenase activity

Under different single nutrient deprivations, the highest H_2 production rate and hydrogenase activity of *Scenedesmus* sp. KMITL-OVG1 with 1.957 ± 0.100 mL L^{-1} h^{-1} and 0.031 ± 0.001 ml L^{-1} min^{-1}, respectively, were found in cells incubated in TAP-K medium (Figure 2A and 2B). H_2 production rate and hydrogenase activity of cells incubated in TAP-K medium were approximately 3 and 2 folds higher than those of cells incubated in normal TAP medium (Figure 2A and 2B). In addition, cells incubated in TAP-S, TAP-N and TAP-P media showed significantly lower H_2 production rate than those in TAP control medium (Figure 2A). It was found H_2 production by cells incubated in each deprived condition was related to their hydrogenase activity (Figure 2A and 2B).

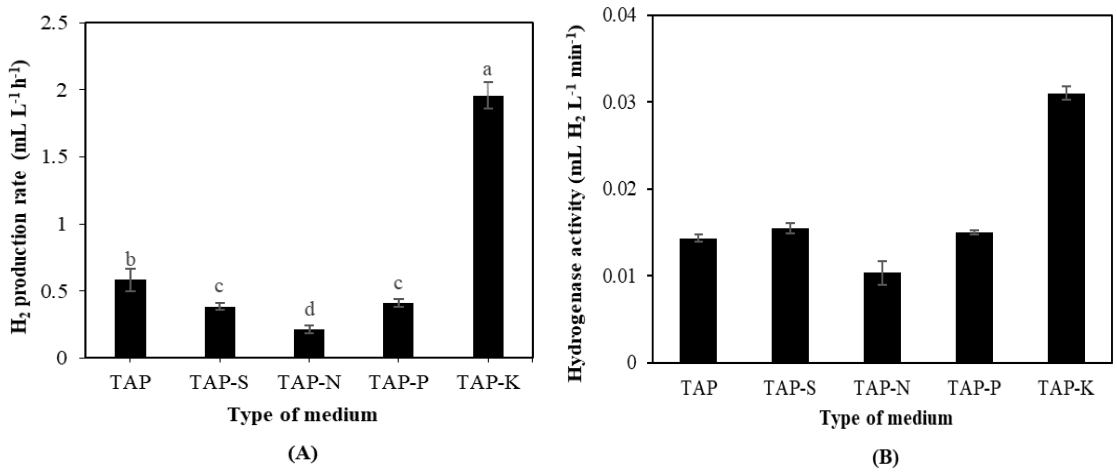

Figure 2 H_2 production rate (A) and hydrogenase activity (B) of *Scenedesmus* sp. KMITL-OVG1 incubated in TAP, TAP-S, TAP-N, TAP-P and TAP-K media. Data are expressed as means (±SD) of three independent experiments. Different letters indicate significant differences between groups at $P < 0.05$.

In this study, starch accumulated in cells incubated in each single nutrient-deprived medium under anaerobic condition for 24 h, was determined. *Scenedesmus* sp. KMITL-OVG1 incubated in TAP-N accumulated the highest starch concentration with 31.428 ± 1.010 μg mL^{-1} whereas cells incubated in TAP-K accumulated the lowest starch concentration with 12.857 ± 1.515 μg mL^{-1} (Table 2). It was clearly demonstrated that H_2 production rate and hydrogenase activity of *Scenedesmus* sp. KMITL-OVG1 cells were inversely proportional to starch accumulation in all treatments under anaerobic condition.

Table 2 H_2 production rate, hydrogenase activity and starch concentration of *Scenedesmus* sp. KMITL-OVG1 under different nutrient deprivation conditions.

Type of medium	Hydrogen production rate (ml L^{-1} h^{-1})	Hydrogenase activity (ml L^{-1} min^{-1})	Starch concentration (μg mL^{-1})
TAP	0.581 ± 0.087	0.014 ± 0.000	13.571 ± 0.001
TAP-S	0.382 ± 0.026	0.015 ± 0.001	20.714 ± 1.515
TAP-N	0.211 ± 0.027	0.010 ± 0.001	31.428 ± 1.010
TAP-P	0.409 ± 0.028	0.015 ± 0.000	17.857 ± 2.525
TAP-K	1.957 ± 0.100	0.031 ± 0.001	12.857 ± 1.515

3.3 Effect of multiple nutrient deprivation on H_2 production rate

From the above results, potassium deprivation could enhance H_2 production rate in *Scenedesmus* sp. KMITL-OVG1. To investigate the effects of potassium deprivation combined with the deprivation of other nutrients on H_2 production rate, cells were incubated in TAP, TAP-K, TAP-KS, TAP-KN, TAP-KP, TAP-KPN, TAP-KNS and TAP-KPS media before H_2 production measurement. The result showed that the highest H_2 production rates with 2.093 ± 0.080 and 2.170 ± 0.050 mL L^{-1} h^{-1} were obtained in cells incubated in TAP-K and TAP-KP media, respectively (Figure 3). Compared to H_2 production rate of cells under potassium deprivation, cells significantly reduced H_2 production rate under the double nutrient deprivations of potassium together with sulfur and nitrogen (Figure 3). In addition, the multiple nutrient deprivations in TAP resulted in the reduction of H_2 production rate by *Scenedesmus* sp. KMITL-OVG1 (Figure 3).

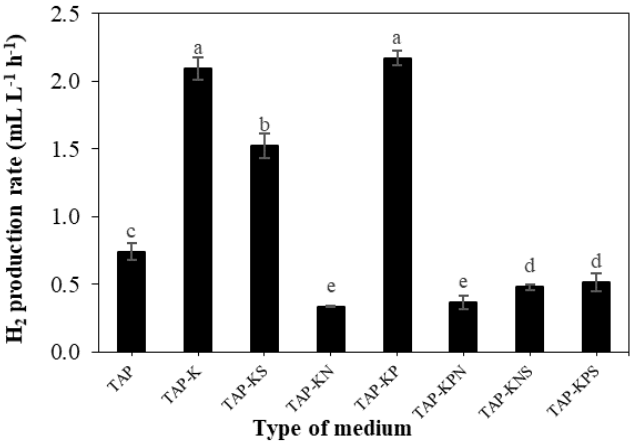

Figure 3 H_2 production rate by *Scenedesmus* sp. KMITL-OVG1 incubated in TAP, TAP-K, TAP-KS, TAP-KN, TAP-KP, TAP-KPN, TAP-KNS and TAP-KPS media. Data are expressed as means (±SD) of three independent experiments. Different letters indicate significant differences between groups at $P < 0.05$.

4. Discussion

In this study, green alga *Scenedesmus* sp. KMITL-OVG1 isolated from a natural pond at King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand shows ability to produce H_2 under anaerobic light condition. Cell density is one of factors influencing H_2 production rate of *Scenedesmus* sp. KMITL-OVG1. From this study, *Scenedesmus* sp. KMITL-OVG1 gave higher H_2 production rate when OD_{750} of cells was increased (Figure 1). It could be suggested that the increased OD_{750} indicated higher cell concentrations, and thus eventually leading to the higher H_2 production rate. However, when OD_{750} of cell culture was increased from 0.8 to 1.0, H_2 production rate was decreased from 0.785 ± 0.073 to 0.477 ± 0.046 mL L^{-1} h^{-1} (Figure 1). It could be explained that too much cell concentrations resulted in the obstruction of the light penetration into the cells, especially cells at the middle in vial. As a result, photosynthetic activity was lower and electrons from the photosynthetic process were reduced. This leads to a decrease in H_2 production rate. It has been reported that high cell density of *C. reinhardtii* caused the light limitation in PSII activity resulting in a decrease of electrons available for H_2 production [28].

In general, H_2 production by green algae is derived from direct photolysis process by hydrogenase activity [6]. Unfortunately, hydrogenase in green algae is sensitive to O_2 and is irreversibly inactivated within few minutes [29]. However, H_2 can be produced through an indirect process in order to produce less O_2. Figure 2 showed that *Scenedesmus* sp. KMITL-OVG1 showed the highest H_2 production rate and hydrogenase activity but lowest accumulated starch concentration when cells were incubated in TAP-K medium. H_2 production rate and hydrogenase activity were approximately 2-fold and 3-fold higher than those in TAP medium, respectively. It might be suggested that potassium depletion leads to the reduction of starch accumulation as shown in Table 2 due to the inhibition of starch synthesis. Therefore, glucose obtained from the photosynthetic pathway provides the high level of electrons, and thus resulting in the induction of electron transfer in plastoquinone (PQ) pool of photosynthetic process. As a result, hydrogenase is highly active and H_2 is increasingly produced as proposed in Figure 4. In *C. reinhardtii*, potassium deprivation has been reported to strongly decrease in the quantity of PSII reaction center protein D_1 and the rate of ATP production, resulting in the retarding of all ATP-dependent processes [14 & 30]. Conversely, potassium deprivation increases plant respiration [31]. Therefore, potassium deprivation might also cause the establishment of O_2 depletion and consequently the higher productivity of H_2 in this strain.

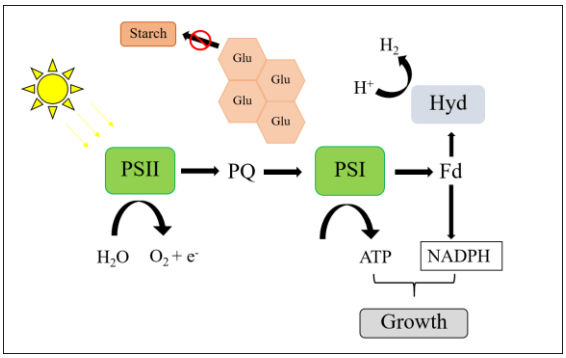

Figure 4 Proposed H_2 production mechanism of *Scenedesmus* sp. KMITL-OVG1 under potassium deprivation.

Normally, sulfur deprivation induces H_2 production in green alga *C. reinhardtii* [32]. Sulfur deprivation reduces the synthesis of amino acids, proteins and other biomolecules, including an inhibition of D_1 protein synthesis and impairment in PSII photochemistry [33]. However, sulfur deprivation could not increase H_2 production and hydrogenase activity in *Scenedesmus* sp. KMITL-OVG1 (Figure 2). It is possible that H_2 metabolism in green algae under sulfur deprivation is distinct depending on the type of green algal species or strains.

Nitrogen deprivation is a severe stress condition for all organisms since nitrogen is a major constituent of proteins and nucleic acids. It has been reported that nitrogen deprivation induces H_2 production in some species of green algae such as *Chlorella* sp. ChiS4 and *C. reinhardtii* [14 & 34]. It was explained that H_2 production was increased via a reactivated bidirectional hydrogenase due to electrons obtained from the degradation of accumulated starch under nitrogen starvation [14]. In this study, in *Scenedesmus* sp. KMITL-OVG1, nitrogen deprivation also induces the accumulation of the fermentative starch in cells under light exposure (Table 2). However, nitrogen deprivation seemed to show negative effect on H_2 production and hydrogenase activity in *Scenedusmus* sp. KMITL-OVG1 (Figure 2). It needs further investigation to reveal the explanation for negative effect of nitrogen deprivation on H_2 production in this algal strain.

In this study, phosphate depletion did not induce H_2 production and hydrogenase activity in *Scenedusmus* sp. KMITL-OVG1 (Figure 2). However, in *C. reinhardtii*, phosphate deprivation limited O_2-evolving activity, leading to the anaerobic environmental establishment. This causes metabolic changes in cells and induces H_2 production [18]. Until now, effect of phosphate depletion on H_2 production has been observed only in *C. reinhardtii* Dangeared 137C mt^+ [18].

Figure 3 showed the effect of potassium deprivation combined with other nutrient deprivations on H_2 production in *Scenedesmus* sp. KMITL-OVG1. It is confirmed that potassium deprivation is the main parameter influencing H_2 production in this algal strain. The multiple deprivations of both potassium and phosphate showed no significant differences on H_2 production rate compared with the single potassium deprivation but the multiple deprivation of potassium and sulfur or nitrogen significantly reduced H_2 production rate (Figure 3). Especially deprivation of all potassium, phosphate and sulfur significantly reduced H_2 production rate compared to deprivation of potassium and sulfur or deprivation of potassium and phosphate (Figure 3). It could be suggested that the deprivation of potassium, phosphate and sulfur in cells might have various significant impacts on protein and nucleotide syntheses, intracellular metabolisms such as photosynthesis, cellular respiration, including H_2 metabolism.

Table 3 shows H_2 production rate by *Scenedesmus* sp. KMITL-OVG1 under potassium deprivation compared to that of *S. obliquus* reported so far. H_2 production rate of *Scenedesmus* sp. KMITL-OVG1 under potassium deprivation is higher than that of *S. obliquus* after addition of 2,3-dichlorophenol [35] and slightly lower than that of *Scenedesmus obliquus* D_3 under potassium deprivation [17].

Table 3 Comparison of H_2 production by *Scenedesmus*

Strain	Condition	Maximum H_2 production rate	Ref.
Scenedesmus sp. KMITL-OVG1	K-deprivation	2.093 mL L (culture)$^{-1}$ h^{-1} 41.8 mL L (PCV)$^{-1}$ h^{-1}	This study
Scenedesmus obliquus	Addition of 2,3-dichlorophenol	5.2 mL L (PCV)$^{-1}$ h^{-1}	[35]
Scenedesmus obliquus D_3	K-deprivation	59.4 mL L (PCV)$^{-1}$ h^{-1}	[17]

5. Conclusions

The green alga *Scenedesmus* sp. KMITL-OVG1 shows ability to produce H_2 production. The optimal cell density of cultures for H_2 production is 0.8 of optical density at 750 nm. Potassium depletion promoted H_2 production rate and hydrogenase activity of *Scenedesmus* sp. KMITL-OVG1 by the reduction of starch accumulation. Under potassium-deprived conditions, the maximum H_2 production rate of 1.957 ± 0.100 ml L^{-1} h^{-1}, maximum hydrogenase activity of 0.031 ± 0.001 ml L^{-1} min^{-1} and the lowest starch concentration of 12.857 ± 1.515 µg mL^{-1} were found. The deprivation of either sulfur, nitrogen or phosphorus affected negatively on H_2 production rate in this green algal strain.

6. Acknowledgements

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