Effect of Light Intensities and Atmospheric Gas Conditions on Biohydrogen Production of Microalgae Isolated from Fisheries Wastewater

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ABSTRACT

Recently, the fishery farming industry has been developed rapidly due to increasing demand and consumption as well as the depletion of wild fish resources. Production processes in the industry usually generate large amounts of wastewater containing high nutrients, posing a threat to downstream water. However, phytoplankton removal techniques commonly used to counteract the threat, though appearing to have low efficiency, are timeconsuming and less sustainable. Microalgae are photosynthetic microorganisms that convert solar energy into hydrogen. Using the isolated algae from fish farms as a source of renewable energy production could be a promising choice for handling fisheries wastewater in a more efficient manner. However, hydrogen production processes from algae still need more studies as their efficiencies vary between algae species and growth factors. In this work, the efficiency of hydrogen production from *Scenedesmus accuminatus* and *Arthrospira platensis* harvested from fish farms under three different light intensity conditions and three atmospheric gas conditions was determined. The results showed that the best conditions for hydrogen production from both species included 24 h darkness and carbon dioxide addition. Under the atmospheric gas combination of 99% argon and 1% carbon dioxide, *S. accuminatus* could produce hydrogen gas as high as 0.572 μmol H_2/mgCh h within 12 h, while the highest hydrogen production (0.348 μmol H_2/mgCh h) obtained from *A. platensis* was found under the atmospheric gas mixture of 98% argon and 2% carbon dioxide. Interestingly, *S. accuminatus* appeared to produce more hydrogen than *A. platensis* under the same conditions.

1. INTRODUCTION

Recently, the fish farming industry has grown rapidly due to population growth and the increasing demand for healthy food choices. The process of raising fish commercially, however, requires large amounts of fresh water, which then causes high amounts of wastewater after the harvesting process, leading to the rapid growth of phytoplankton due to the microscopic dimensions of microalgae (0.5-30 μm). Moreover, phytoplankton removal techniques used commonly used appear to be time consuming and unsustainable while having low efficiency (Uduman et al., 2009; Alfafara et al., 2002). Therefore, turning what is considered a problem into a raw material for producing a high value-added product such as bioenergy could be a promising approach for effectively handling fisheries wastewater.

Microalgae are photosynthetic microorganisms that naturally convert solar energy into hydrogen via respiration under dark aerobic conditions and via fermentation under anaerobic conditions. Compared with conventional hydrogen production, biological hydrogen production process using fermentative microorganisms (Kosourov et al., 2005; Wang et al., 2003) photosynthetic bacteria, or algae involves less energy and is considered to be more environmental friendly (Das and Veziroglu, 2001; Levin et al.,

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2004). Previous reports showed that photosynthetic microorganisms that were cyanobacteria and green algae are of interest in hydrogen production from solar energy and water (Dasgupta et al., 2010; Tamagnini et al., 2007; Maneeruttanarungroj et al., 2010; Eroglu and Melis, 2011).

The hydrogen production processes from algae still require more in-depth studies as their efficiency levels vary between algae species and growth factors. Light is known as the primary energy source for the algae growth and enables it to carry out metabolic processes including hydrogen production (Dubinsky et al., 1995; Markou and Georgakakis, 2011). Different algae species have different light harvesting antenna pigments, light saturation for photosynthesis characteristics, and levels of solar energy conversion efficiency (Eroglu and Melis, 2011). As light intensity increases, chlorophylls and some biomass components such as lipid, fatty acids, and protein contents generally decrease (Tedesco and Duerr, 1928), while starch and polysaccharide contents increase (Friedman et al., 1991). Besides, other cultivation factors such as atmospheric gas composition, carbon source, nutrients, pH, and temperature also have some effects on biohydrogen production.

This work aims to study the effect of two growth factors including light intensity and atmospheric gas condition on the hydrogen production by microalgae harvested from fisheries wastewater. The findings of this study hopefully provide an alternative sustainable choice for fisheries wastewater management by biohydrogen production.

2. METHODOLOGY

2.1 Morphological classification

Morphological classification of algal samples was based on their appearances under a light microscope Morphologies of cyanobacteria were recorded using photomicrographs. Techniques as described by Desikachary (1959), Anagnostidis and Komarek (1988) , Hoffmann (1988) and Hegewald (1990) were used for the classification using an Olympus CH30RF200 compound microscope.

2.2 Sample cultivation and isolation

Water samples were filtered with plankton net (mesh size 5 um). Pick cell method was used for algal isolation (APHA, 1992). The unialgal culture was examined under a light microscope. The successful cultures were inoculated into flasks containing BG-11 medium (for *S.* cultivation) or Zarrouck medium (for *Arthrospira* cultivation) . Streak plate dilutions or whole filament isolations were conducted on agar-solidified plates using the same medium for culture purification. All tubes, flasks, and plates were incubated in photochambers at room temperature under fluorescent lamps (~3,000 lux) (Castenholz, 1988).

2.3 Strain of microalgae and growth conditions

The dominant species of microalgae from one of the fish farms in Chiang Mai were isolated and identified as described above. Two dominant species, *S. accuminatus* (green algae) and *A. platensis* (cyanobacteria), were selected for the study of H_2 production efficiency. The green algae were cultivated on BG-11 medium, while the cyanobacteria strain was grown on ZN medium under white fluorescence lamps (80 $\mu E/m^2$ s) at room temperature (Table 1). Cells grown as above were inoculated with an initial optical density of 0.01 and grown for 1 week at room temperature under a light intensity of 80 $\mu E/m^2$ s until the optical density reached the OD_{730} of 0.672 for *A. platensis* and the OD_{750} of 0.755 for *S. accuminatus*. During cultivation, pH was maintained at 10 and 7 for *A. platensis* and *S. accuminatus*, respectively. Subsequently, cells were harvested by centrifugation at 5000 rpm at 4 ^oC for 15 min. before adding to the photoreactors.

2.4 Photobioreactors setup

To identify the efficiency of both microalgae on H_2 production under different light intensities and atmospheric gas conditions, the batch experiments were conducted in 500 mL gas-tight photoreactors (Figure 1). A 350 mL of cell suspensions was transferred to the photoreactors, which were then tightly capped and flushed with argon gas for 10 min. to eliminate atmospheric oxygen in the headspace. Initial pH for the growth of *A. platensis* and *S. accuminatus* was at 10 and 7, respectively. The reactors were incubated at room temperature under different treatment conditions, as shown in Table 2, and manually shaken before each sampling. The gas mixture was allowed to pass through 10%NaOH solution for selective absorption

of carbon dioxide. Gas samples were collected every 12 h interval using a sterile glass syringe for composition analysis. Moreover, the algae cells and chlorophyll concentrations were also measured before and at the end of each experiment according to Mackinney (1941). All the experimental sets were carried out as triplicates.

Table 1. Composition of BG-11 medium and ZN_0 medium for *S. accuminatus* and *A. platensis*, respectively

BG-11 (N-free medium)		ZN_0 (N-free medium)	
Nutrient	Amount (g/L)	Nutrient	Amount (g/L)
$NaHCO_3$	16.80	H_3BO_3	2.850
K_2HPO_4	0.50	$MnCl_2\ 4H_2O$	1.810
$NaNO_3$	2.50	$ZnSO_4\ 7H_2O$	0.220
K_2SO_4	1.00	$CuSO_4\ 5H_2O$	0.080
NaCl	1.00	MoO_3	0.015
$MgSO_4\ 7H_2O$	0.20		
$CaCl_2\ 2H_2O$	0.04		
$FeSO_4\ 7H_2O$	0.20		
$Na_2EDTA\ 2H_2O$	1.60		

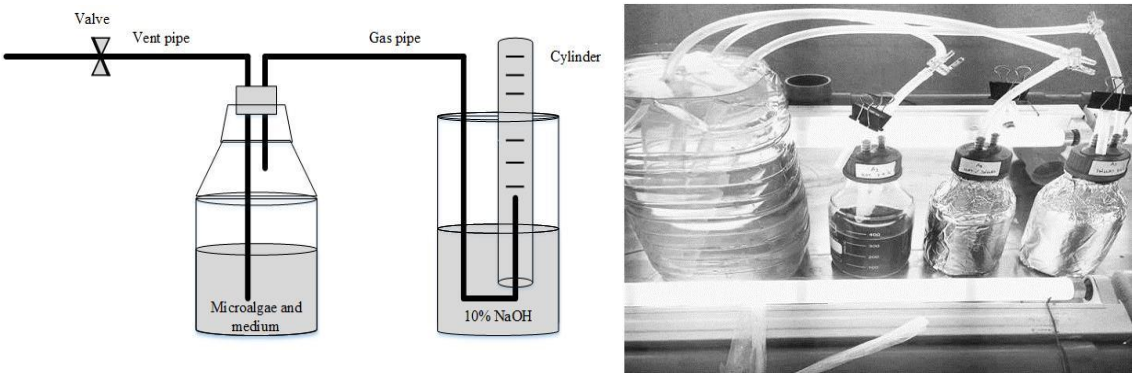

Figure 1. A schematic of the photoreactors for H_2 production from microalgae

2.5 Measurement of hydrogen production

The composition of gas was analyzed by gas chromatography (Agilent 7890A, USA) with TDC using a molecular sieve 5A, 60/80 column. Helium gas was used as the carrier gas under 3.43 psi and 50 mL/min. of flow rate. In analyzing the gas mixture through chromatography, the moles of H_2 present were calculated taking into account that the total pressure shown on the meter was the sum of the partial pressures contributed by the gas present in the headspace of the photobioreactor. According to Dalton's law of partial pressure, the partial pressure of each gas is presented by the following expression.

$$P_H = X_H \times P,$$

Where P_H is the partial pressure of H_2, X_H is the mole fraction in the gas sample obtained from GC, and P is the total pressure. Then, the number of moles of H_2 could be calculated using the ideal gas equation defined as.

$$P_H V = n_H RT$$

After molar fraction and concentration of hydrogen had been calculated, the hydrogen production rate was obtained and reported as a ratio of μmol of H_2 per mg chlorophyll *a* per h (μmol H_2/mgCh h).

2.6 Chlorophyll a concentration

Once the gas production ceased, biomass samples were taken for determination of chlorophyll *a* concentration. Biomass samples were filtered through a glass fiber filter (Whatman GF/F, retention > 0.7 μm), and pigments were extracted overnight at 4 °C with 90% ethanol in the dark. After centrifugation, the absorbance of the supernatant was measured spectrophotometrically (UV-visible spectrophotometer, Spectronic Genesys 5, USA) at 665 nm, before and after acidification with HCl 2 M (Mackinney, 1941). The chlorophyll *a* concentration was calculated as:

Chlorophyll a concentration = 29.6 × (A-B) × v/V, (μg/L)

Where A was the absorbance value before acidification, B was the absorbance value after acidification, v was the sample volume (L) and V was the ethanol volume used (mL).

2.7 Statistical analysis

The effects of light intensities and atmospheric gas conditions on hydrogen production from both algae strains were statistically evaluated using a 2-way ANOVA. All tests were considered at a significance level of 0.05.

Table 2. Treatment conditions used in this study

Light intensities	Atmospheric gas condition
Light condition (80 μE/m^{2}s), 24 h	100% argon 99% argon + 1% CO_2 98% argon + 2% CO_2
Light condition (80 μE/m^{2}s), 12 h Dark condition, 12 h	100% argon 99% argon + 1% CO_2 98% argon + 2% CO_2
Dark condition, 24 h	100% argon 99% argon + 1% CO_2 98% argon + 2% CO_2

3. RESULTS AND DISCUSSION

3.1 Effect of light intensity on H_2 production

In this work, the impact of three different light conditions (24 h of light (80 μE/m^2 s), light (12 h) (80 μE/m^2 s) and dark (12 h) (0 μE/m^2 s), and 24 h of dark (0 μE/m^2 s) on hydrogen production from both algae strains were examined. There is no doubt that solar energy conversion efficiency as well as hydrogen production are affected by light intensity. Normally, higher light intensity accelerates the growth of biomass and promotes higher hydrogen production (Maneeruttanarungroj et al., 2010). On the other hand, too much light intensity can also suppress hydrogen production due to high oxygen production (Meli et al., 2000). The results revealed that both *A. platensis* and *S. accuminatus* showed greater hydrogen production under 24 h of the dark condition, which was observed from the first 12 h with the value of 0.348 μmol H_2/mgCh h and 0.572 μmol H_2/mgCh h, respectively (Figure 2(a)). This is in agreement with a previous study by Papazi et al. (2014) demonstrating that the fastest rate and the maximum value of hydrogen production were achieved in the dark conditions. Under 24 h of light conditions, however, there was almost no detectable hydrogen production from either species (data not shown). This probably indicated the result of oxygen produced via photosynthesis that inhibited activities of hydrogenase and nitrogenase (Eroglu and Melis, 2011; Eroglu et al., 2010). The application of green algae for H_2 production must address the effect of oxygen inhibition. In order to prevent the suppression of H_2 production, Hallenbeck and Benemann (2002) recommended that oxygen concentration should be maintained at less than 0.1%.

During the diurnal cycle of 12 h light/ dark condition, however, the result shows that both algae could still produce hydrogen at up to 0.298 μmol H_2/mgCh h and 0.047 μmol H_2/mgCh h under the atmospheric gas condition consisting of 98% argon and 2% CO_2 for *S. accuminatus* and *A. platensis*, respectively (Figure 2(b)). This diurnal light-dark cycle has been reported to help increase the yield of hydrogen production due to the improved stability of nitrogenase activity, which creates greater hydrogen production capacity (Meli et al., 2000; Eroglu et al., 2010). Under dark condition, the results (Figure 2(a) and 2(b)) show high levels of hydrogen observed in the treatments where the carbon source (CO_2) was applied to the atmospheric gas, especially the green algae (*S. accuminatus*). This may be due to the ability of green algae to produce hydrogen by means of carbon fixation from an external carbon source under dark condition (Chen and Johns, 1996).

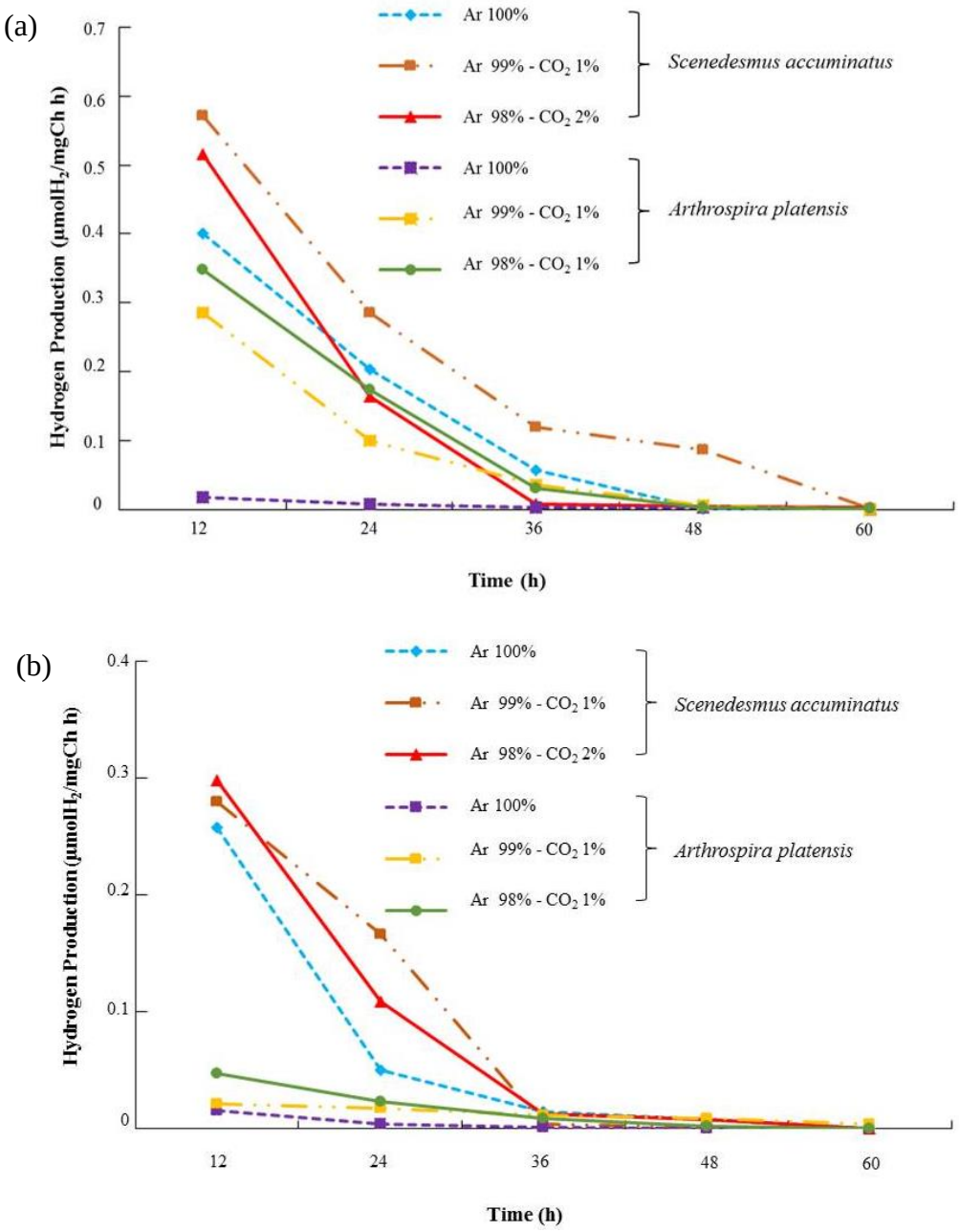

Figure 2. Effect of light intensity on hydrogen production of *A. platensis* and *S. accuminatus* (a) under 24 h dark condition and (b) under 12 h light/12 h dark

3.2 Effect of atmospheric gas conditions on H_2 production

Three atmospheric gas conditions used in the study consisted of 1) 100% argon; 2) 99% argon and 1% CO_2; and 3) 98% argon and 2% CO_2. The results show that greater hydrogen production by both algae strains achieved in the treatments used CO_2 added into the atmospheric gas conditions (Figure 3(a), 3(b) and 3(c)). This indicates that the carbon source, one of the photosynthetically metabolic intermediates, affected the efficiency of hydrogen production. Dutta et al. (2005) suggested that the carbon source affected nitrogenase and reversible hydrogenase activity in the hydrogen production process. Moreover, it was shown that *A. platensis* achieved the highest hydrogen production (0. 348 µmol H_2/mgCh h) in the gas mixture of 98% argon and 2 CO_2 (Figure 3(c)), while *S. accuminatus* tended to produce greater hydrogen (0.572 µmol H_2/mgCh h) in the atmospheric gas mixture of 99%

argon and 1% CO_2 (Figure 3(b)). This is due to the microalgae being photoautotrophic organisms and the carbon source (both inorganic and organic forms) being one of the indispensable nutrients for their cultivation (Vonshak, 1990). Apparently, the carbon source affects their carbon metabolisms (photo-autotrophy, heterotrophy, photoheterotrophy and mixotrophy), biomass productivity and biomass composition (Markou and Georgakakis, 2011). In cyanobacteria, there are two different enzymes capable of producing molecular hydrogen, nitrogenase, and bidirectional hydrogenase (Ananyev et al., 2008). It is tempting to speculate that this strain is capable of high levels of net hydrogen production, especially under nitrogen-fixing condition (in the dark) and even in the presence of hydrogenase (in the daytime). As increasing CO_2 is related to photosynthesis, it has been reported that cyanobacteria has the ability to grow and use CO_2 at a higher rate (De Morais and Costa, 2007), meaning that hydrogen production becomes higher where photosynthetic activity is higher. However, it is notable that biomass productivity could be enhanced by the addition of CO_2 but each alga species responds to different CO_2 concentrations (Ho et al., 2011; Florin et al., 2001; Suali and Sarbatly, 2012).

3.3 Effect of Species on H_2 production

To compare the efficiency of hydrogen production between both alga species, the results showed that *S. accuminatus* had greater ability to produce higher levels of hydrogen than *A. platensis* in every condition. At low light intensity conditions, some have reported that *S. accuminatus* is capable of both uptake and production of hydrogen after anaerobic adaptation (photoreduction of CO_2) or photohydrogen production (Kosourov et al., 2005; Florin et al., 2001), while *A. platensis* is an oxygenic photosynthetic bacteria that can only produce hydrogen by reversible hydrogenase through photosynthesis. At the end of experiments, significant pH changes were observed (from 10.0 to 7.5) in the photobioreactor inoculated with *A. platensis*. On the other hand, the pH value detected from the photobioreactor fed with *S. accuminatus* increased slightly from 7.0 to 7.76, which was still in the suitable range for its growth condition. This could lead to lower biomass and hydrogen production as *A. platensis* is more sensitive to the sudden change in pH due to the formation of hydrogen carbonate. Also, a pH value lower than 9 is not suitable for the growth of *A. platensis*.

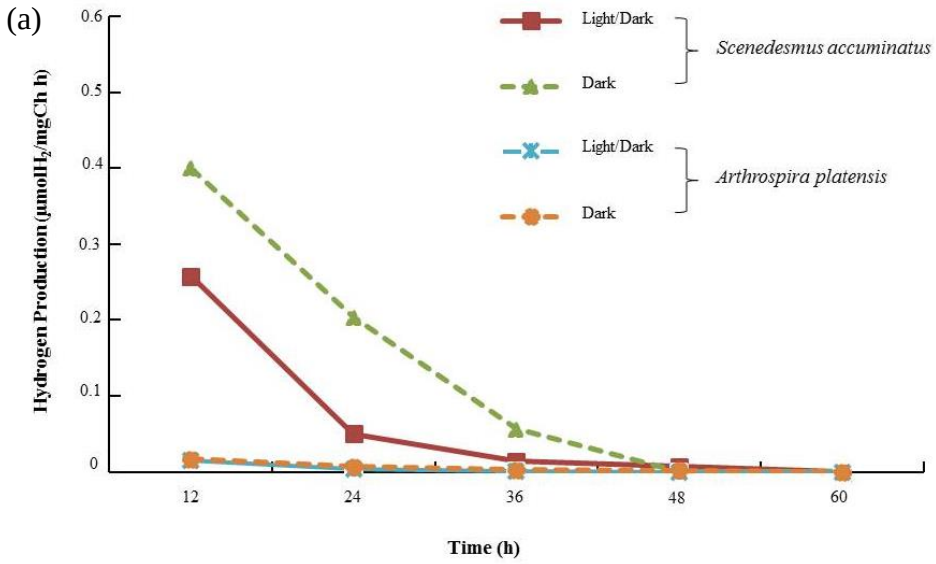

Figure 3. Effect of atmospheric gas conditions on H_2 production: (a) 100% argon; (b) 99% argon + 1% CO_2; and (c) 98% argon + 2% CO_2

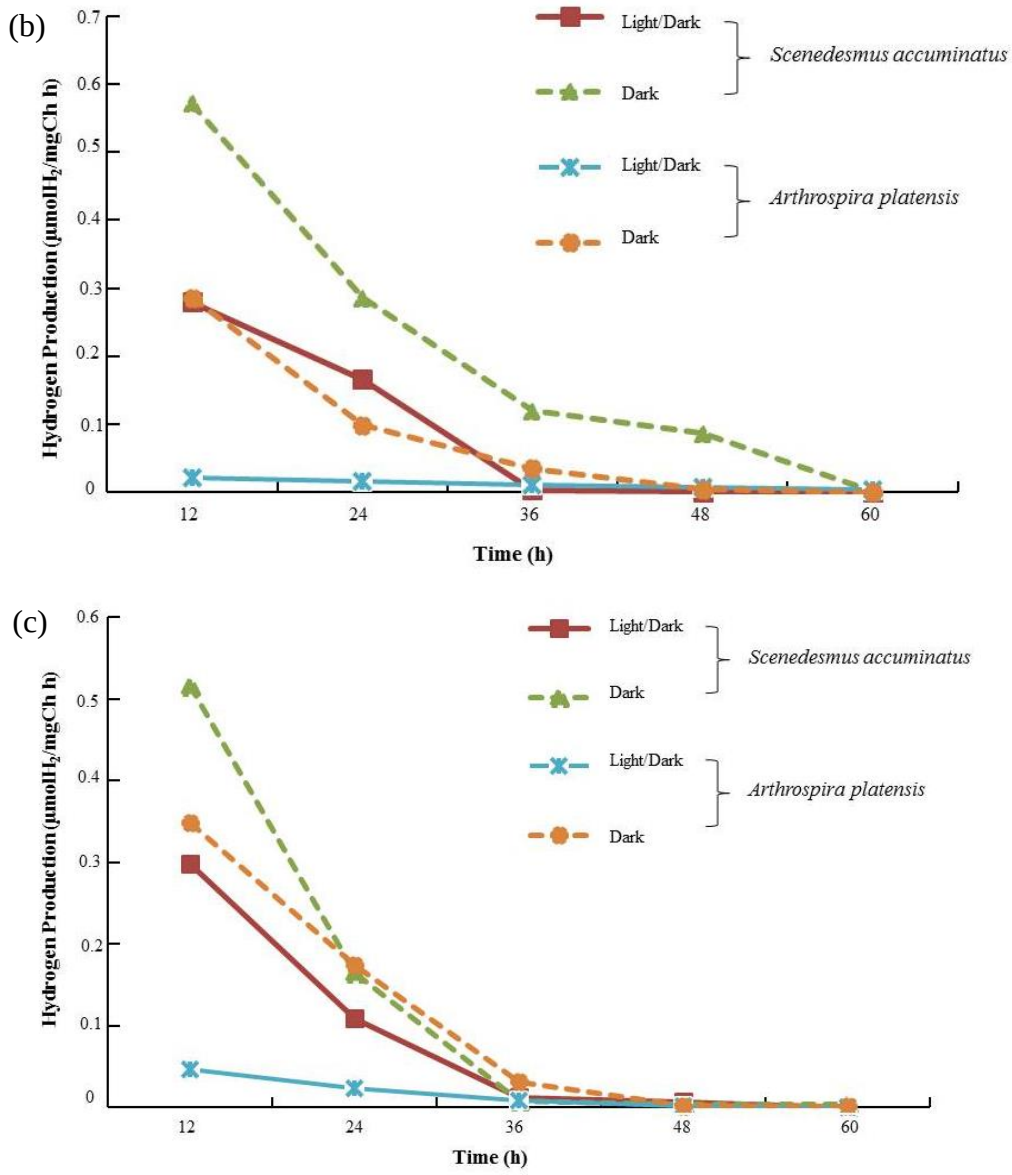

Figure 3. Effect of atmospheric gas conditions on H_2 production: (a) 100% argon; (b) 99% argon + 1% CO_2; and (c) 98% argon + 2% CO_2 (cont.)

4. CONCLUSIONS

Light intensity and atmospheric gas condition had some effects on hydrogen production from microalgae. Both species could not produce any hydrogen under 24 h of light. The best condition for hydrogen production from *S. accuminatus* was in the atmospheric gas condition with a combination of 99% argon and 1% CO_2 under the 24 h dark condition, while *A. platensis* achieved the greatest hydrogen production under the atmospheric gas condition of 98% argon and 2% CO_2 in darkness. In comparison, however, it was found that *S. accuminatus* could produce more hydrogen gas than *A. platensis* under the same condition.

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