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Biomass and biohydrogen production by unicellular green alga *Chlorella vulgaris* var. *vulgaris* TISTR 8261 using frozen food industrial wastewater

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Abstract

Biohydrogen production by green algal biomass is promising method for sustainable H_2 production and bioenergy recovery. In this approach, green algae convert organic and inorganic substances (used as the sole source of carbon and electrons) in wastewater into H_2. In this study, biomass and H_2 production from the unicellular green alga *Chlorella vulgaris* var. *vulgaris* TISTR 8261, cultivated in frozen food industrial wastewater, was investigated. The results revealed that growth rate of algal cells cultivated in treated wastewater was significantly higher than that in untreated wastewater but lower than that in the synthetic control Tris acetate phosphate (TAP) medium. In addition, the cells grown in treated wastewater exhibited a high ability to remove nitrate, nitrite, phosphate, and sulfate from the water. Furthermore, algal cells were cultured with various concentrations of sodium acetate (0-17.4 mM); the optical density of the cultures at 750 nm increased with increase in acetate concentration. Cell growth in treated wastewater supplemented with 17.4 mM sodium acetate was similar to that in TAP medium. The highest H_2 production of 12.87 ± 0.58 μmolH_2 mg Chl a^{-1} was observed in cells incubated in treated wastewater supplemented with 17.4 mM sodium acetate; this yield was higher than that obtained from cells incubated in nitrogen-free TAP medium. The results of this study support the potential use of wastewater for biomass and biohydrogen production by *C. vulgaris* var. *vulgaris* TISTR 8261.

Keywords: Biohydrogen, Biomass, *Chlorella vulgaris* var. *vulgaris* TISTR 8261, Food industrial wastewater

1. Introduction

Currently, the world population and economy have been growing rapidly; thus, fossil fuel energy is an unmet demand. Fossil fuels would no longer be sustainable energy sources in future because they are limited in supply, and their combustion causes greenhouse gas emissions, leading to environmental problems [1]. Hydrogen gas, a renewable and environmentally friendly fuel, could be a potential alternative.

H_2 can be produced by many processes, such as thermochemical processes, water electrolysis, and biological processes [2]. Biological H_2 production varies depending on the type of microorganism. There are at least two main H_2 production processes via green algae: H_2 production by photosynthesis, utilizing unlimited sunlight as an energy source and water as an electron source, and by fermentation, utilizing the catabolism of stored organic compounds as electron sources [3]. In addition to the ability of green algae to produce H_2, they can utilize inorganic and organic compounds as carbon sources for their growth and cellular metabolism under photoautotrophic and photoheterotrophic conditions, respectively [4]. Among green algae, the unicellular green alga *Chlorella* is a well-known commercial source of food supplements, animal feeds, and aquaculture feed and

is used for the production of bio-fertilizers and biofuels [5]. In particular, *Chlorella vulgaris* has a high protein content with 41%-58% of its dry weight and thus can be used as a source of single cell protein (SCP) [6].

In general, green algal cultivation requires an enriched medium comprising many organic and inorganic compounds, including trace minerals, for their growth [7]. In order to reduce the cost of the medium for *Chlorella* cultivation, wastewater from the food industry, comprising a large number of organic and inorganic compounds, is used as a culture medium [8]. Many strains of *Chlorella*, such as *Chlorella vulgaris* and *Chlorella zofingiensis*, have been reported to produce high biomass yield through their cultivation in wastewater. *Chlorella vulgaris* generated a biomass yield of 3.50 g L^{-1} when cells were cultivated in brewery wastewater for 5 d [9], whereas *Chlorella zofingiensis* generated a biomass yield of 2.96 g L^{-1} when cells were cultivated in piggery wastewater for 10 d [10]. In addition, *Chlorella vulgaris* cultivated in wastewater resulted in a high H_2 yield of 11.06 mL H_2 L^{-1} under anaerobic conditions [11]. In the green alga *Chlamydomonas reinhardtii*, H_2 production was observed in cells cultivated in olive mill wastewater [12] and sorghum stalk wastewater [13].

The unicellular green alga *Chlorella vulgaris* var. *vulgaris* TISTR 8261 has been shown to grow autotrophically in Bold's basal medium (BBM) as well as BG11 and N8 media and heterotrophically in Tris acetate phosphate (TAP) medium. It also shows potential as an H_2 producer [14]. These media consist of many organic and inorganic substances, including metal ions and minerals. In this study, wastewater from a large export-oriented frozen food industrial factory located in the central part of Thailand was used as the feedstock. The wastewater contains organic and inorganic compounds that serve as carbon and nitrogen sources as well as high concentrations of metal ions and minerals. Green algae can use these substances for their growth and metabolize them for H_2 production. Moreover, algal cultivation is a potential wastewater treatment method. Therefore, this study aimed to use wastewater from a frozen food industrial plant as a culture medium to cultivate *Chlorella vulgaris* var. *vulgaris* TISTR 8261 and investigated biological H_2 production.

2. Materials and methods

2.1 Collection and analysis of wastewater

The wastewater used in this study was randomly collected from a frozen food industrial factory in Phra Nakhon Si Ayutthaya province, Thailand. This plant is a large export-oriented frozen food factory located in the central part of Thailand. Two types of wastewater were collected: (1) untreated wastewater obtained from the pretreatment process through filtration in order to remove or reduce coarse materials, and (2) treated wastewater obtained from the secondary treatment process using a biological method with an activated sludge process and a chemical method using a dissolved air flotation system. The measurement of pH and dissolved oxygen (DO) in the wastewater was immediately performed using a portable multi-parameter meter (Hach, HQ40D, USA). Wastewater was then filtered to remove sediments using filter paper No. 3 (90 mm diameter) (Whatman, UK). Biochemical oxygen demand (BOD) was measured using a BOD analyzer (Lovibond, OxiDirect, Germany). The chemical oxygen demand (COD) and total nitrogen (TN) content were determined according to the American Public Health Association (APHA) 5220 C and 4500-N C, respectively [15]. The total carbon (TC), total inorganic carbon (TIC), and total organic carbon (TOC) contents were analyzed using a TOC analyzer (TOC-VCSH, Shimadzu, Japan). The total phosphorus (TP), total sulfur (TS), and metal ion levels were measured using inductively coupled plasma-optical emission spectrometry (ICP-OES) (PerkinElmer, Optima 7300 DV, USA). The concentrations of nitrate, nitrite, phosphate, and sulfate were determined using ion chromatography (Thermo Fisher Scientific, Dionex ICS-5000^+ DP, USA).

2.2 Green algal strain and cultivation

The unicellular green alga *Chlorella vulgaris* var. *vulgaris* TISTR 8261 was obtained from the Thailand Institute of Scientific and Technological Research (TISTR), Pathum Thani province, Thailand. To use wastewater as a culture medium, the initial pH of wastewater from the filtrate was adjusted to pH 7.2 with 2 N NaOH before sterilization by autoclaving. To prepare a starter, *Chlorella vulgaris* var. *vulgaris* TISTR 8261 was cultivated in a 250 mL Erlenmeyer flask containing 100 mL of TAP medium [16]. The culture was shaken at 120 rpm under a light intensity of 30 µmol photon m^{-2} s^{-1} at 25 ℃ for 36 h. Cells were harvested by centrifugation at 7,000 × *g* at 20 ℃ for 10 min. The cell pellet was washed twice in untreated and treated wastewater and resuspended in these wastewaters. The initial cell concentration was adjusted to an optical density at 750 nm (OD_{750}) of approximately 0.1. A 100 mL aliquot of the culture was transferred to a 250 mL Erlenmeyer flask. The cell culture was shaken under the previously described conditions for 14 days. After cultivation, the concentrations of nitrate, nitrite, phosphate, and sulfate in wastewater were analyzed, and their removal efficiency by *Chlorella vulgaris* var. *vulgaris* TISTR 8261 were calculated.

2.3 Growth, dry weight, and chlorophyll content determination

Algal growth was determined by measuring OD_{750} using a spectrophotometer (Shimadzu, UV-1800, Japan). Dry cell weight was measured by filtration of 10 mL of cell culture through a GF/C glass microfiber filter (47 mm diameter; Whatman, UK). The filter containing cells was washed twice with distilled water, dried at 85 °C in an oven for 16 h, and placed in a desiccator for 1 h before weighing. For chlorophyll determination, algal cells were harvested by centrifugation at 7,000 × *g* at 20 °C for 10 min. Chlorophyll was extracted from the cell pellet with 90% (v/v) methanol at 70 °C under darkness for 4 h. The chlorophyll concentration was determined by measuring absorbance of the chlorophyll extracts at 665 and 660 nm using a spectrophotometer (Shimadzu, UV-1800, Japan) and calculated according to a method described by Lee and Shen [17].

2.4 H_2 production measurement

Cells grown in TAP medium for 36 h were harvested, washed, and resuspended in 100 mL of TAP and nitrogen-deprived TAP (TAP-N), whereas cells grown in optimal wastewater for 36 h were harvested, washed, and resuspended in 100 mL of wastewater and optimal wastewater. The cell suspension (100 mL) was transferred to a 250 mL Erlenmeyer flask and further incubated in light for 24 h. Subsequently, cells were harvested, washed, and resuspended in 5 mL of fresh medium with an initial pH of 7.2, and transferred to a 10 mL glass vial. The glass vial was closed with a rubber stopper, purged with argon for 10 min, and incubated under a light intensity of 40 µmol photon m^{-2} s^{-1} at 25 °C. H_2 production was determined by headspace gas phase analysis using a gas chromatograph with a thermal conductivity detector (Hewlett-Packard, HP5890A, Japan) [18]. A molecular sieve 5 A 60/80 mesh packed column (2 m length and 6 mm diameter) was used. The injector and detector temperatures were set at 100 °C, whereas the oven temperature was maintained at 50 °C. Argon gas was used as carrier gas. H_2 production yield was calculated as the amount of H_2 evolved per chlorophyll content (µmolH_2 mg Chl a^{-1}) or H_2 evolved per volume of medium (mL H_2 L^{-1}), whereas the H_2 production rate was calculated as a term of H_2 evolved per chlorophyll content per hour (µmolH_2 mg Chl a^{-1} h^{-1}) or H_2 evolved per volume of medium per hour (mL H_2 L^{-1} h^{-1}).

2.5 Effect of acetate on growth, biomass production, and H_2 production

TAP medium contains a sufficient amount of sodium acetate as a carbon source at a concentration of 17.4 mM [16]. To improve algal growth, sodium acetate at final concentrations of 0, 1.74, 3.48, 8.7, and 17.4 mM was added to the treated wastewater. The initial cell concentration was adjusted to an OD_{750} of 0.1. A 100 mL aliquot of the culture was transferred to a 250 mL Erlenmeyer flask. Growth and biomass yield were determined by OD_{750} and dry cell weight measurement, respectively, every day for seven days. For H_2 production, cells were grown in TAP and wastewater for 36 h, harvested, washed, and resuspended in 100 mL of TAP, TAP-N, wastewater, and acetate-containing wastewater. Cells were incubated in the light for 24 h before harvesting and transferred to a 10 mL glass vial. H_2 production was determined using gas chromatography, as previously described.

3. Results

3.1 Chemical characteristics of frozen food industrial wastewater

The chemical characteristics of the untreated and treated wastewater are shown in Table 1. The pH of untreated wastewater was neutral (pH 7.0), whereas that of treated wastewater was moderately alkaline (Table 1). The DO value of untreated wastewater was significantly lower than that of treated wastewater, whereas the BOD, COD, TC, TIC, and TOC values of untreated wastewater were significantly higher than those of treated wastewater (Table 1), indicating a decrease in organic and inorganic matter after treatment. There was no significant difference in TN, TP, and TS concentrations between the untreated and treated wastewater (Table 1). However, the concentrations of some nutrients, such as nitrate, phosphate, and sulfate, in treated wastewater were higher than those in untreated wastewater (Table 1). In addition, both untreated and treated wastewater contained a large amount of minerals, such as calcium, potassium, magnesium, and sodium (Table 1).

Table 1 Chemical characteristics of wastewater from frozen food plant.

Parameters	Untreated wastewater	Treated wastewater
pH	6.78 ± 0.01	8.01 ± 0.02
DO (mgO_2 L^{-1})	0.38 ± 0.02	7.97 ± 0.01
BOD (mgO_2 L^{-1})	148.00 ± 3.10	56.00 ± 2.20
COD (mgO_2 L^{-1})	320.00 ± 1.41	186.20 ± 1.83
TC (mg L^{-1})	68.79 ± 2.36	32.50 ± 2.85
TIC (mg L^{-1})	42.08 ± 4.08	22.84 ± 3.25
TOC (mg L^{-1})	26.71 ± 1.72	9.66 ± 0.39
TN (mg L^{-1})	7.25 ± 0.04	26.90 ± 0.35
TP (mg L^{-1})	2.26 ± 0.01	3.50 ± 0.004
TS (mg L^{-1})	0.30 ± 0.01	0.55 ± 0.001
NO_3^- (mg L^{-1})	0.62 ± 0.04	26.09 ± 0.30
NO_2^- (mg L^{-1})	0.34 ± 0.12	0.52 ± 0.04
PO_4^{3-} (mg L^{-1})	0.87 ± 0.05	6.83 ± 0.16
SO_4^{2-} (mg L^{-1})	0.67 ± 0.05	12.28 ± 0.07
Ca^{2+} (mg L^{-1})	21.96 ± 0.23	22.82 ± 0.04
Cu^{2+} (mg L^{-1})	0.007±0.001	0.006±0.001
Fe (total dissolved) (mg L^{-1})	0.15 ± 0.004	0.05 ± 0.01
K^+ (mg L^{-1})	9.38 ± 0.02	11.44 ± 0.04
Mg^{2+} (mg L^{-1})	5.44 ± 0.05	4.76 ± 0.002
Mn^{2+} (mg L^{-1})	0.016±0.001	0.009±0.001
Na^+ (mg L^{-1})	68.77 ± 0.31	46.26 ± 0.05
Zn^{2+} (mg L^{-1})	0.08 ± 0.02	0.07 ± 0.001

3.2 Growth and nutrient removal by Chlorella vulgaris var. vulgaris TISTR 8261

Based on OD_{750} measurement, the highest growth of *Chlorella vulgaris* var. *vulgaris* TISTR 8261 was observed when the cells were cultivated in TAP medium (Figure 1). On comparing the cell growth in wastewater, cells cultivated in treated wastewater showed higher growth rate than those cultivated in untreated wastewater (Figure 1). In addition, after cultivation in treated wastewater for 14 days, *Chlorella vulgaris* var. *vulgaris* TISTR 8261 showed high nitrate, nitrite, phosphate, and sulfate removal efficiencies of 98.6%, 96.9%, 97.7%, and 90.9%, respectively.

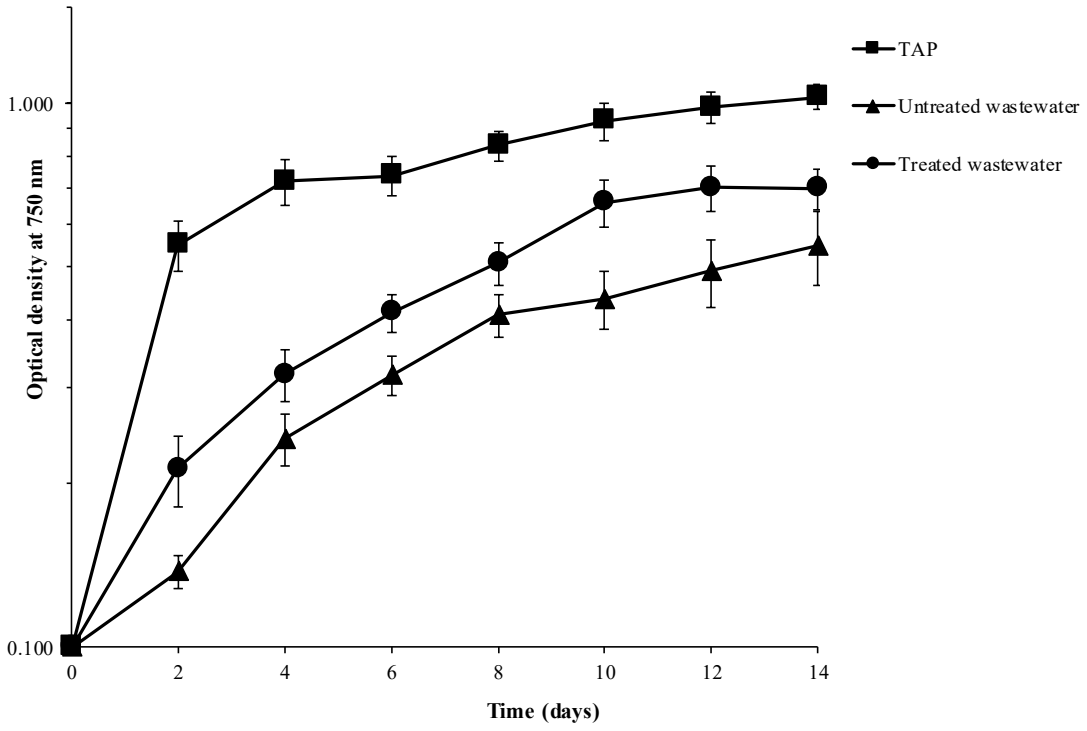

Figure 1 Growth of *Chlorella vulgaris* var. *vulgaris* TISTR 8261 cultivated in TAP medium, untreated wastewater, or treated wastewater, determined through OD_{750} measurement.

3.3 Growth and biomass production under various sodium acetate concentrations

To increase the growth and biomass production of *Chlorella vulgaris* var. *vulgaris* TISTR 8261 using treated wastewater as a culture medium, various concentrations of sodium acetate were added to the treated wastewater. In this study, sodium acetate was used as a carbon source instead of acetic acid because of difficulty in regulating the acidic pH caused by the latter. The results revealed that the growth and biomass production of *Chlorella vulgaris* var. *vulgaris* TISTR 8261 increased with higher sodium acetate concentrations (Figure 2). In addition, cells cultivated in treated wastewater containing 17.4 mM sodium acetate showed similar growth and biomass production as cells cultivated in TAP medium (Figure. 2). The maximum biomass yield with 18.80 ± 0.84 g L^{-1} was found in cells cultivated in treated wastewater containing 17.4 mM sodium acetate for 5 days (Figure 2B). Lower growth rate and biomass production were observed in cells cultivated in treated wastewater containing 0, 1.74 and 3.48 mM sodium acetate (Figure 2).

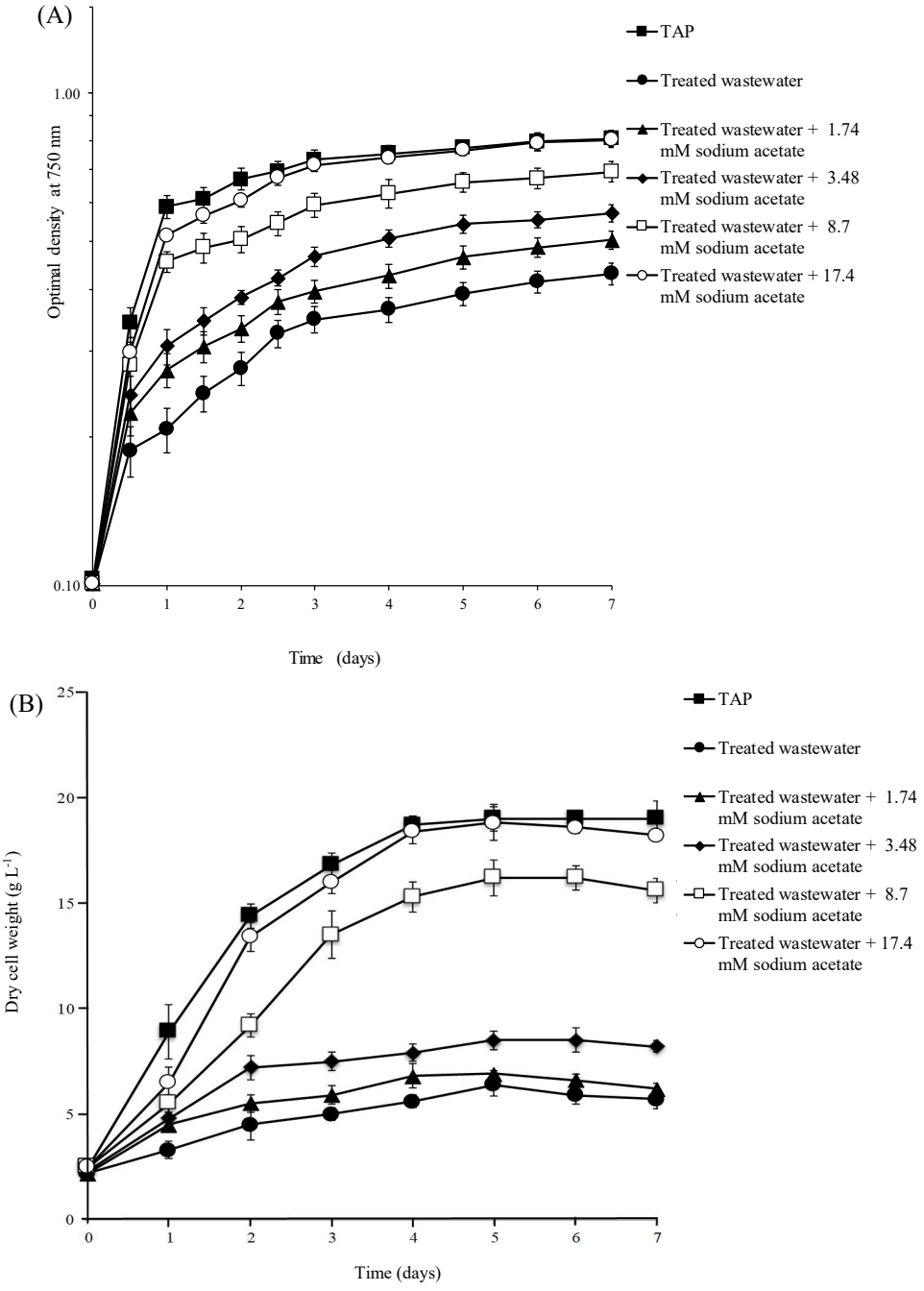

Figure 2 Growth (A) and biomass (B) of *Chlorella vulgaris* var. *vulgaris* TISTR 8261 cultivated in treated wastewater supplemented or not with sodium acetate at various concentrations and in TAP medium.

3.4 H_2 production by Chlorella vulgaris var. vulgaris TISTR 8261 in treated wastewater

The maximum H_2 production of 12.87 ± 0.58 μmolH_2 mg Chl a^{-1} or 16.92 ± 1.52 mL H_2 L^{-1} was detected in cells incubated in treated wastewater supplemented with 17.4 mM sodium acetate (Figure 3). This yield was significantly higher than that obtained from cells incubated in TAP-, TAP-N-, and sodium acetate-free treated wastewater (Figure 3). In addition, the highest H_2 production was observed after 6 h of incubation in treated wastewater supplemented with 17.4 mM sodium acetate under anaerobic light conditions, whereas the highest H_2 production from cells in TAP-N medium was detected after 24 h of incubation (Figure 3). Table 2 shows the maximum H_2 production rate and yield of cells incubated in various types of medium or wastewater under light in terms of H_2 produced per chlorophyll concentration or per medium volume.

Table 2 Maximum H_2 production rate and yield of *Chlorella vulgaris* var. *vulgaris* TISTR 8261 cultivated in different kinds of media.

Type of media	Maximum H_2 production rate		Maximum H_2 production yield	
	(μmolH_2 mg Chl a^{-1} h^{-1})	(mL H_2 L^{-1} h^{-1})	(μmolH_2 mg Chl a^{-1})	(mL H_2 L^{-1})
TAP	1.46 ± 0.26	1.85 ± 0.18	2.99 ± 0.52	4.27 ± 1.30
TAP-N	1.56 ± 0.34	1.91 ± 0.06	11.07 ± 0.61	7.49 ± 0.09
Treated wastewater	0.67 ± 0.23	0.91 ± 0.32	1.54 ± 0.40	2.11 ± 0.59
Treated wastewater +17.4 mM sodium acetate	2.23 ± 0.53	2.93 ± 0.70	12.87 ± 0.58	16.92 ± 1.52

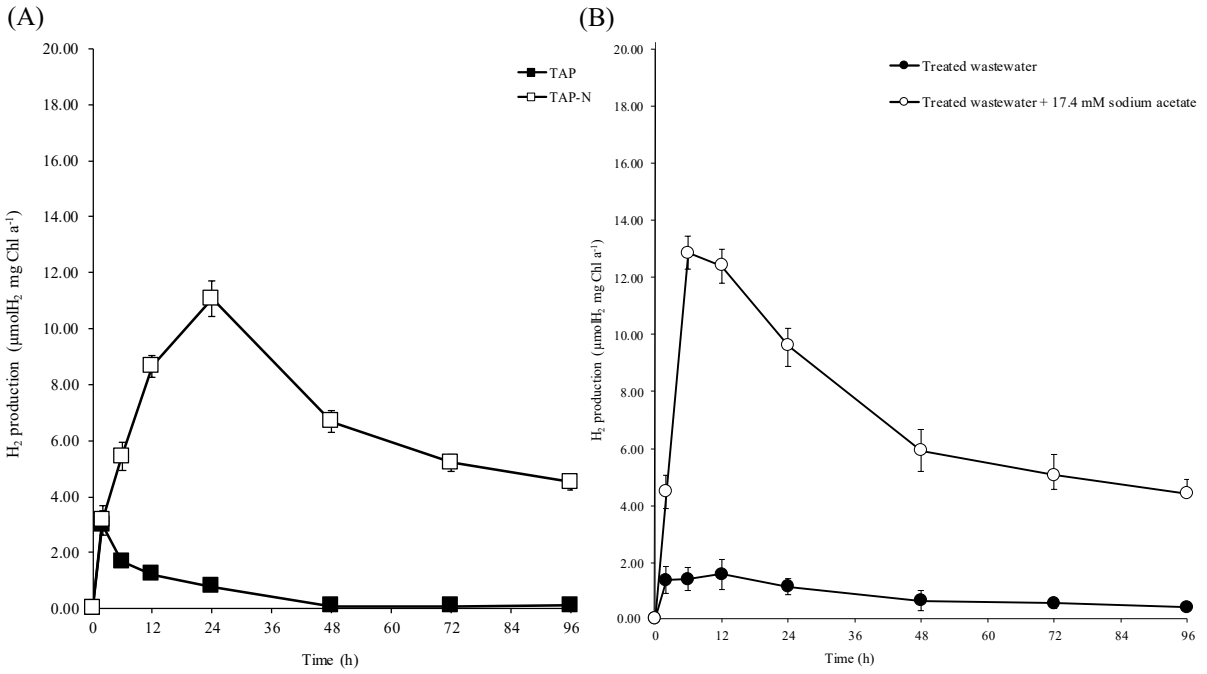

Figure 3 H_2 production by *Chlorella vulgaris* var. *vulgaris* TISTR 8261 incubated in TAP and TAP-N (A) and in treated wastewater with and without supplementation of 17.4 mM sodium acetate (B) under anaerobic condition with light.

4. Discussion

Three main sources of wastewater have been reported to be used for algal cultivation: municipal, agricultural, and industrial wastewater [5]. In this study, we used wastewater collected from a frozen food industrial factory in Phra Nakhon Si Ayutthaya province, Thailand. Untreated wastewater was obtained from the production process through a pretreatment method, whereas treated wastewater was obtained from the secondary treatment process. DO, BOD, and COD analyses indicated the effectiveness of wastewater treatment in the plant. The treated wastewater showed an alkaline pH, which may have resulted from the addition of sodium hydroxide in the process of chemical wastewater treatment by the dissolved air flotation system. Although untreated and treated wastewater contained a large amount of inorganic and organic substances, only treated wastewater contained high concentrations of nitrate, phosphate, and sulfate (Table 1). Wastewater subjected to a secondary treatment process contains a high amount of nitrate that is generated from organic N

and ammonium by nitrifying bacteria such as *Nitrosomonas, Nitrosococcus, Nitrobacter, and Nitrococcus* [19]. These data indicate that wastewater could be used as a culture medium for *Chlorella* cultivation because some elements in wastewater, such as organic carbon and inorganic nitrogen and phosphorus, can be used as essential components for algal growth [20]. It has been reported that *Chlorella* sp. can grow in many kinds of industrial wastewater, such as wastewater from riboflavin manufacturing industries [21] and brewery wastewater [9].

Analysis of growth of *Chlorella* vulgaris var. vulgaris TISTR 8261 suggested that cells could grow in treated and untreated wastewater, but their growth was significantly lower in wastewater than that in enriched synthetic TAP medium (Figure 1), which comprises a large number of nutrients and minerals essential for algal growth and metabolism [16]. By comparing the cell growth in wastewater, cells cultivated in treated wastewater showed higher growth than cells cultivated in untreated wastewater (Figure 1). This finding could be explained by the higher concentrations of nitrate, phosphate, *and* sulfate in treated wastewater that promoted the growth of *Chlorella vulgaris* var. *vulgaris* TISTR 8261, whereas the lower growth of cells cultivated in untreated wastewater may have resulted from the high solubility of organic compounds in untreated wastewater. This result corroborates with that of a previous study, wherein a high concentration of organic compounds (1,500 mg L^{-1} COD) in wastewater inhibited growth and biomass production in *Chlorella* zofingiensis [10]. In addition, cultivation of *Chlorella vulgaris* var. *vulgaris* TISTR 8261 in treated wastewater for two weeks showed a higher removal efficiency (90%) of nitrate, nitrite, phosphate, and sulfate than result from a previous study [22]; these data support the ability of this algal strain to treat wastewater.

To overcome the decreased growth of cells cultivated in treated wastewater, sodium acetate as a carbon source was added to the wastewater. As expected, a high concentration of sodium acetate enhanced the growth and biomass production of *Chlorella vulgaris* var. *vulgaris* TISTR 8261 (Figure 2). In addition, cells cultivated in treated wastewater supplemented with 17.4 mM sodium acetate showed similar growth and biomass production as those in TAP medium (Figure 2), indicating that sodium acetate can be used as a carbon source for growth and cellular metabolism in *Chlorella vulgaris* var. *vulgaris* TISTR 8261. In frozen food industrial wastewater, acetate might be available at low concentrations because a TC concentration of only 32.50 mg L^{-1} was detected (Table 1). This concentration was not sufficient for cultivation of *Chlorella vulgaris* var. *vulgaris* TISTR 8261 in wastewater. Similarly, acetate concentration in pretreated municipal wastewater was only 20.52 mg L^{-1}, which was below the level suitable for algal growth [23]. Acetate is one of the most common organic carbon sources for heterotrophic cultivation because it is inexpensive and easily oxidized via carbohydrate metabolism in many types of microorganisms [24]. In algal cells, acetate is catabolized by two pathways: tricarboxylic and glyoxylate cycles, both of which provide abundant levels of ATP and NAD(P)H [24]. Acetate has been reported to promote growth and biomass production of *Chlorella vulgaris* cultivated in modified optimized culture medium containing carbon sources, nitrogen sources, and sufficient concentrations of inorganic supplements [25] and in *Chlorella* pyrenoidosa when cultivated in soybean processing wastewater [26].

In this study, a lack of nitrogen in TAP medium and an increase in acetate concentration in wastewater enhanced H_2 production by *Chlorella vulgaris* var. *vulgaris* TISTR 8261 (Figure 3, Table 2). In the TAP medium, the nitrogen source was NH_4C_l at approximately 400 mg L^{-1}, whereas in the treated wastewater, the total nitrogen concentration was 26.90 mg L^{-1}. TAP medium contained 15 times higher level of nitrogen sources than treated wastewater. Under nitrogen starvation, H_2 is highly produced due to an increase in the number of electrons obtained from the photosynthetic process and from the accumulated carbohydrate catabolism through fermentation in light [27]. In addition, a high concentration O_2 of sodium acetate promotes starch synthesis and starch accumulation in the cells, leading to an increase in the electron sources for H_2 production under light-independent fermentative processes [28]. Moreover, acetate was found to stimulate O_2 uptake during respiration in *Chlorella* pyrenoidosa, resulting in a decrease in O_2 and the enhancement of H_2 production [29].

Table 3 shows the maximum production of biomass and H_2 by different green algal species cultivated in different types of wastewater compared with those of *Chlorella vulgaris* var. *vulgaris* TISTR 8261. *Chlorella vulgaris* var. *vulgaris* TISTR 8261 produced higher levels of biomass and H_2 compared to other green algal strains, and these levels were similar to those produced by *Chlorella vulgaris* MSU 01 cultivated in BG11 medium supplemented with corn stalk as a carbon source [11]. Interestingly, the unicellular green alga Chlamydomonas reinhardtii showed the lowest biomass production but highest H_2 production [12]. Chlamydomonas reinhardtii showed a higher H_2 production efficiency than *Chlorella vulgaris*. This result can be attributed to the fact that Chlamydomonas reinhardtii could not grow well in olive mill wastewater mixed with TAP medium, resulting in low biomass accumulation. However, this medium might induce stress conditions, such as nitrogen or sulfur deprivation, which are suitable for H_2 production. This study suggests the potential use of *Chlorella vulgaris* var. *vulgaris* TISTR 8261 cultivation for biomass and H_2 production from wastewater, although H_2 production by *Chlorella vulgaris* was lower than that by Chlamydomonas reinhardtii. The optimization of conditions, such as pH, light intensity, incubation temperature, and use of reducing agents, for H_2 production by this strain in frozen food wastewater needs further investigation.

Table 3 Comparison of biomass and H_2 production between *Chlorella vulgaris* var. *vulgaris* TISTR 8261 and other green algal strains.

Green algal strains	Type of wastewater	Maximum biomass production	Maximum H_2 production	References
Chlorella vulgaris var. *vulgaris* TISTR 8261	Frozen food industrial treated wastewater supplemented with 17.4 mM sodium acetate	18.80 ± 0.84 g L^{-1} at 5 days	12.87 ± 0.58 μmolH_2 mg Chl a^{-1} or 16.92 ± 1.52 mL H_2 L^{-1}	This study
Chlorella vulgaris UTX-265	Brewery wastewater supplemented with 10 g L^{-1} glucose	3.20 g L^{-1} at 5 days	-	9
Chlorella zofingiensis	Piggery wastewater with initial COD concentration at 1900 mg L^{-1}	2.96 g L^{-1} at 10 days	-	10
Chlorella vulgaris MSU 01	Corn stalk (4 g L^{-1}) supplemented modified BG11 medium	19 g L^{-1} at 10 days	11.06 mL H_2 L^{-1}	11
Chlamydomonas reinhardtii	Olive mill wastewater in TAP medium (50% dilution)	1.10 g L^{-1} at 70 h	150 mL H_2 L^{-1}	12

5. Conclusion

The unicellular green alga *Chlorella vulgaris* var. *vulgaris* TISTR 8261 could grow in treated and untreated wastewater collected from a frozen food industrial factory in Thailand. However, cell growth in wastewater was lower than that in the enriched TAP medium. Additionally, *Chlorella vulgaris* var. *vulgaris* TISTR 8261 showed a high capability of removal of nitrate, nitrite, phosphate, and sulfate in treated wastewater. In addition, supplementation of 17.4 mM sodium acetate in treated wastewater promoted algal growth and biomass production as well as H_2 production by *Chlorella vulgaris* var. *vulgaris* TISTR 8261. The results of this study suggest that it is possible to use frozen food industrial wastewater as a culture medium for biomass and biohydrogen production by *Chlorella vulgaris* var. *vulgaris* TISTR 8261.

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