

Research article

Capability of Phytoplankton (*Chlorella vulgaris*) Cultivation in Wastewater from Grease Trap

Suwat Chunharat¹, Prepawarin Wongkeetee², Sumalee Saipan²,
Panlop Pommee² and Chayarat Srisunont^{2*}

¹Faculty of Sports and Health Science, Thailand National Sports University, Krabi Campus,
Krabi Province, Thailand

²Environmental Science and Technology Program, Faculty of Science and Technology,
Bansomdejchaopraya Rajabhat University, Bangkok, Thailand

Received: 19 August 2025, Revised: 21 November 2025, Accepted: 27 December 2025, Published: 13 July 2026

Abstract

The aim of this research was to treat wastewater from grease traps by cultivating *Chlorella vulgaris*, a valuable commercial phytoplankton in biodiesel production and aquatic animal feeding. However, there is lack of information about its cultivation capability. Therefore, the most suitable medium and optimal inoculum cell density for its cultivation were examined. *Chlorella vulgaris* was cultured in pre-treated grease trap wastewater that had undergone sterilization and sedimentation with the addition of three different medium formulations. Then, the initial cell densities were varied to 5×10^4 , 5×10^5 , and 5×10^6 cells/mL. Cell density analysis was conducted daily for 7 days. Water quality parameters such as pH, temperature, dissolved oxygen (DO), chemical oxygen demand (COD), nitrate, and phosphate were measured before and after cultivation. The results showed that the medium with the addition of calcium hydroxide 1 g/L supported the highest algal specific growth rate at 0.318 day^{-1} . An initial quantity of 5×10^6 cells/mL yielded the highest algal growth and density. During cultivation in all experiments, the water temperature remained stable at 25°C . DO concentrations and pH ranged from 5.82-9.82 mg/L and 5.49-8.82, respectively. After the cultivation period, COD decreased from an initial range of 228.00-276.80 mg/L to 66.67-212.80 mg/L. Nitrate and phosphate were removed at 91.03% and 75.36% from the initial concentration, respectively. Finally, it can be concluded that to improve the growth efficiency of *C. vulgaris* in wastewater from grease traps, the addition of a nutrient medium consisting of calcium hydroxide with an initial algal density of 5×10^6 cells/mL should be provided. This inoculum condition offers potential applications in microalgal biotechnology and sustainable wastewater treatment.

Keywords: phytoplankton; green algae; culture; wastewater treatment; grease trap

*Corresponding author: E-mail: scchayarat@bsru.ac.th

<https://doi.org/10.55003/cast.2026.268841>

Copyright © 2026 by King Mongkut's Institute of Technology Ladkrabang, Thailand. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Wastewater from grease traps is a significant pollution load originating from food and restaurant industries. This becomes a critical environmental issue, with profound impacts on natural aquatic ecosystems. Grease traps are designed to remove fats, oils, and grease (FOG) from wastewater (Tchobanoglous et al., 2014). These systems operate on the principle of density separation, in which low-density substances such as oils and grease float to the surface and are retained. The heavier particulates settle to the bottom of the trap (Wang et al., 2010). However, grease traps are limited in their ability to remove other pollutants from wastewater. The physicochemical characteristics of grease-trap wastewater include high organic matter and nutrient concentration, which are accompanied by a lower pH (5-6) condition (Tang et al., 2024). This requires more post-treatment to remove these substances from the wastewater before it is discharged into the body of water. The cultivation of phytoplankton in wastewater is a potential water treatment option, offering benefits in both environmental and economic terms. The use of microalgae has emerged as a promising approach due to their ability to adsorb pollutants, remove organic compounds, and alter wastewater composition, thereby reducing its toxicity.

Generally, there is absent of phytoplankton in grease trap wastewater. However, the wastewater quality may support phytoplankton growth. Green algae, *Chlorella vulgaris*, is the best alternative option for treating wastewater due to its rapid growth and tolerance of wastewater environments with varying pH levels and organic content. Several studies reported the successful cultivation of *C. vulgaris* in various types of industrial wastewater, including effluents from livestock farms and manufacturing plants (Wang et al., 2010; Vovk et al., 2020). This alga shows strong potential for wastewater treatment due to its ability to remove nitrates, phosphates, and heavy metals from contaminated water sources. Additionally, *C. vulgaris* is a sustainable and economically valuable bioresource. This alga can perform photosynthesis, which captures atmospheric carbon dioxide and contributes to effective climate change mitigation. Its growth also produces valuable biomass rich in proteins, lipids, and carbohydrates, which can be used for aquatic animal feed and biodiesel production, thereby adding economic value to the treatment process (Mata et al., 2010; Wang et al., 2010). Its biochemical composition is rich and diverse, with protein content ranging from 50-60% dry weight, and includes essential amino acids, vitamins, minerals, and antioxidant compounds such as chlorophyll, carotenoids, and phycobilins (Becker, 2007). These properties have made *C. vulgaris* a popular raw material for dietary supplements, functional foods, and animal feed. In the field of bioenergy, *C. vulgaris* is recognized as a viable source for biodiesel production due to its ability to accumulate high levels of intracellular lipids, particularly long-chain fatty acids, which are suitable for the synthesis of high-quality biodiesel under optimized conditions (Chisti, 2007). This presents an essential alternative to fossil fuels and contributes to greenhouse gas reduction. Therefore, integrating the cultivation of *C. vulgaris* into wastewater not only improves water quality by removing nutrients and organic matter but also provides high value for use in the aquaculture and biodiesel industries. However, limited research has been conducted on its cultivation potential in grease trap wastewater, which may require further adjustment to support the suitable growth conditions of this microbial species.

The cultivation of *C. vulgaris* has gained considerable interest in both laboratory and industrial-scale research due to its broad potential in various fields, including nutrition, bioenergy, environmental remediation, and biotechnology (Al-Hammadi & Güngörmüşler, 2024). However, there has been limited information on the algal production efficiency

cultivated in treated or untreated grease trap wastewater. The basic requirement for *C. vulgaris* cultivation has been documented in many studies (Farooq et al., 2022; Peter et al., 2023). The addition of glucose 20 g/L as a carbon source and nitrate 0.5 g/L as a nitrogen source into the cultivation medium resulted in the best *C. vulgaris* production. Reduction in algal growth was observed at low nitrogen concentrations of less than 0.37 g/L (Farooq et al., 2022). Furthermore, *C. vulgaris* requires alkaline conditions for better production (Peter et al., 2023). An acidic environment weakens electrostatic forces between microalgae and flocculants, thereby reducing biomass production capacity. The highest yield of *C. vulgaris* was obtained in an alkaline medium (pH 11), and it could be grown at pH 8-10 (Al-Hammadi & Güngörmüşler, 2024). In phytoplankton cultivation, pH can be adjusted with sodium hydroxide or calcium hydroxide. These chemical substances are commonly used in industrial wastewater plants (Mehrpour et al., 2023). Therefore, these chemical substances are readily available to treat wastewater for algae cultivation. Using wastewater in algae cultivation instead of pure water effectively reduces water use, resulting in a smaller water footprint. This supports the sustainability of responsible consumption and production (SDG 12). Although *C. vulgaris* has been extensively studied in various wastewaters, few studies have examined its growth, nutrient removal, and biomass production in grease trap wastewater, which contains high concentrations of fats, oils, grease, and organic matter. Enhancing the growth of *C. vulgaris* in this type of wastewater requires nutrient supplementation and pH adjustment. Recently, there has been a lack of information about these cultivation details.

Therefore, the ability of *C. vulgaris* cultivation in wastewater collected from grease traps was investigated. The objective of this study was to determine the optimal nutrient formulation and inoculum density for effectively cultivating *C. vulgaris* in this type of wastewater. The findings offer insights into efficient microalgal cultivation strategies that enable the use of grease trap wastewater. This can help reduce aquatic pollution and mitigate the environmental and public health impacts of untreated wastewater discharges into natural water bodies. Finally, a dual benefit in both environmental remediation and simultaneous algal biomass production can be successfully achieved.

2. Materials and Methods

2.1 Microalgae preparation

The green microalga *Chlorella vulgaris* TISTR 8580 was obtained from the Thailand Institute of Scientific and Technological Research (TISTR). The algal pure culture was propagated using BG-11 medium at 10% of the inoculum volume. All equipment used during the cultivation process was sterilized using an autoclave. Aseptic techniques were applied throughout the process. Subculturing was performed every seven days using a 1:10 ratio of inoculum to culture volume. Distilled water sterilized at 121°C for 20 min via autoclaving was used in all cultivation procedures.

2.2 Wastewater preparation

Fifty liters of wastewater were collected from a grease trap at the food center of Bansomdejchaopraya Rajabhat University. The wastewater was first filtered through a 50-micron mesh bag. To eliminate other microorganisms that may interfere with *C. vulgaris* growth, chlorine was then added to the sample at 50 mg/L (Jefri et al., 2022), followed by continuous aeration for 3 days to remove residual chlorine and increase dissolved oxygen

levels. To remove suspended solids, aluminum sulfate ($\text{Al}_2(\text{SO}_4)_3$) was added to promote flocculation. The clarified supernatant was decanted and used as the experimental medium. Finally, the pH levels were measured before and after wastewater preparation, with values of 5.93-7.50 and 3.83-5.49, respectively, being recorded.

2.3 Determination of suitable nutrient formulations for *Chlorella vulgaris* cultivation in grease trap wastewater

Cultured *Chlorella vulgaris* cells were inoculated into pre-treated grease trap wastewater at an initial cell density of 5×10^6 cells/mL, with a total working volume of 5 L per sample. The nutrient formulas A, B, and C, which were chosen from the literature, had been effectively used in algal mass culture (Table 1). These media were generally used in clean water and had never been employed in grease trap wastewater studies. Therefore, to identify the most effective nutrient solution for mass culture in grease trap wastewater, these three different nutrient formulations were added separately into designated experimental jars containing *C. vulgaris* and grease trap wastewater. Each treatment was performed in triplicate. The control group consisted of cultivating *C. vulgaris* (5×10^6 cells/mL) in grease trap wastewater without nutrient addition.

Table 1. Nutrient supplementation in the experiment

Formula A ¹		Formula B ²		Formula C ³	
Urea (46-0-0) (g/L)	1.200	Urea (46-0-0) (g/L)	0.005	Urea (46-0-0) (g/L)	0.150
Fertilizer (18-46-0) (g/L)	0.100	Fertilizer (16-20-0) (g/L)	0.015	Fertilizer (16-20-0) (g/L)	0.100
Rice bran (g/L)	0.500	(NH_4) ₂ SO ₄ (g/L)	0.100	KNO ₃ (g/L)	0.200
Ca(OH) ₂ (g/L)	1.000				
C/N (mol/mol)	0.476	C/N (mol/mol)	-	C/N (mol/mol)	-
N/P (mol/mol)	27.411	N/P (mol/mol)	19.091	N/P (mol/mol)	8.310

Note: ¹Thewaratmaneekul et al. (2006); ²Petchmanee (1999); ³Lohalaksanadech et al. (2011)

During the experiment, continuous aeration was provided to ensure water circulation and prevent algal sedimentation. According to the grease trap wastewater temperature at 25-29°C (Tang et al., 2024), all treatments were maintained under fluorescent lighting at a constant room temperature of 25°C for a period of 7 days. Daily sampling of 5 mL was conducted to quantify the algal population using a hemacytometer under a light microscope. Cell density was recorded and calculated as cells per milliliter (cells/mL). Additionally, water parameters—including pH, temperature, and dissolved oxygen (DO)—were measured before and after the cultivation period using a handheld pH meter Cybercan PC 300 (EUTECH Instruments, Singapore) and DO meter Cybercan DO 110 (EUTECH Instruments, Singapore).

2.4 Determination of the optimal inoculum *Chlorella vulgaris* density for cultivation in grease trap wastewater

To determine the optimal inoculum density for *Chlorella vulgaris* cultivation, pre-cultured *C. vulgaris* cells were inoculated into prepared grease trap wastewater at three different initial cell concentrations: 5×10^4 cells/mL (A1), 5×10^5 cells/mL (A2), and 5×10^6 cells/mL (A3), each with a total volume of 5 L and conducted in triplicate. Nutrient supplements were added to each treatment group after inoculation.

Control groups were established as follows: 1) WW was grease trap wastewater without algal inoculation or nutrients; and 2) Controls A1, A2 and A3 were *C. vulgaris* cultivated at 5×10^4 , 5×10^5 , and 5×10^6 cells/mL, respectively, in grease trap wastewater without nutrient addition.

All experimental units were maintained under continuous aeration to ensure adequate water circulation and to prevent algal sedimentation. According to the grease trap wastewater temperature at 25-29°C (Tang et al., 2024), cultures were exposed to fluorescent light at a controlled room temperature of 25°C for 7 days. A daily sample of 5 mL was taken from each unit, and the algal cell density and cell concentrations were determined.

Before and after the cultivation period, water parameters such as temperature, pH, and dissolved oxygen (DO) were monitored. Then, water was collected and analyzed for chemical oxygen demand (COD). Additionally, during the experiment, water samples were taken daily from each treatment to determine ammonia-nitrogen ($\text{NH}_3\text{-N}$), nitrate-nitrogen ($\text{NO}_3\text{-N}$), and phosphate-phosphorus ($\text{PO}_4^{3-}\text{-P}$) concentrations, following the standard methods (APHA, 2012).

2.5 Data analysis

The growth rates of the alga *Chlorella vulgaris* were computed using the following equation.

$$\mu = (\ln N_t - \ln N_0) / t$$

Where: μ is the specific growth rate (day^{-1}), N_t is cell concentration at time t (cells/ml), N_0 is initial cell concentration at time 0 (cells/ml), and t is time (days).

Then, statistical analyses, including minimum, maximum, mean, and standard deviation of wastewater parameters and algal growth rates, were computed using Microsoft Excel 365 to evaluate treatment performance and identify trends. Finally, in each experimental design, a paired t-test was employed to compare water parameters before and after the experiment, with a significant level of 0.01

3. Results and Discussion

3.1 Suitable nutrient formula

As shown in Figure 1, the growth of *Chlorella vulgaris* in grease trap wastewater supplemented with nutrient formula A showed significantly higher algal growth. In contrast, formulas B, C, and the control showed minimal or negative growth trends. At the end of the 7-day cultivation period, the final cell densities of alga cultured in nutrient formulas A, B, and C were $46.37 \pm 10.01 \times 10^6$, $5.17 \pm 0.78 \times 10^6$, and $1.68 \pm 0.65 \times 10^6$ cells/mL, respectively. The highest specific growth rate was observed in nutrient medium A, at 0.318

day⁻¹, followed by media B and C, with growth rates of 0.005 and -0.156 day⁻¹, respectively. Although these nutrient formulas had been used for algal cultivation (Petchmanee, 1999; Thewaratmaneekul et al., 2006; Lohalaksanadech et al., 2011), the results from this study demonstrated that nutrient formula A was the most suitable for *C. vulgaris* cultivation in grease trap wastewater. The superior performance of medium A was attributed to its higher nutrient content compared to the other media, which provided *C. vulgaris* with ample essential nutrients necessary for growth. As shown in Table 1, nutrient formula A contains the highest nutrient concentration and the highest N/P ratio (27.411). According to the Redfield ratio, which states that the N/P atomic ratio required for phytoplankton growth is 16, the composition of nitrogen and phosphorus in this medium was suitable for *C. vulgaris* cultivation. This abundance of nutrients likely enhanced the efficiency of photosynthesis and cell division (Stevenson et al., 1996). Meanwhile, the addition of formulas B and C did not promote algal growth in grease trap wastewater (Figure 1). This may be because the lower N/P ratio in nutrient formulas B and C (Table 1) resulted in less capacity for cell enhancement. In this study, background nutrient levels in the grease trap wastewater before culturing were measured. The concentrations of ammonia-nitrogen, nitrate-nitrogen, and phosphate-phosphorus were 0.54 µg/L, 0.16 mg/L, and 0.12 mg/L, respectively. These nitrogen concentrations were sufficient for phytoplankton growth (Mata et al., 2020). However, phosphorus may be limited. The N/P ratio was only 1.783, well below the minimum requirements for phytoplankton element composition (Sharoni & Halevy, 2022). These results suggest that grease trap wastewater may have initially inhibited growth under this nutrient condition. Nutrient supplementation, especially phosphorus, is highly required.

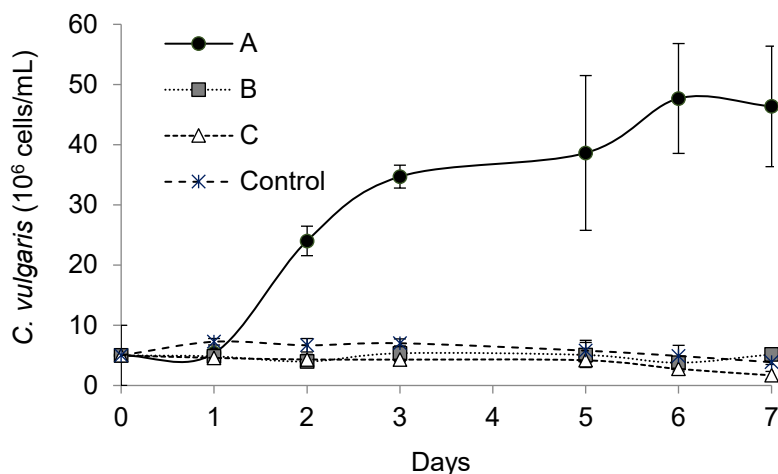


Figure 1. The cell density of *Chlorella vulgaris* in grease trap wastewater supplemented with nutrient formulas A, B, and C compared to the control

The results of the water quality observations are summarized in Table 2. The temperature and dissolved oxygen (DO) of the samples before and after the experiment did not differ significantly. Meanwhile, the paired t-test showed that the pH values differed significantly across treatments (P value < 0.01). The control showed that the grease trap wastewater was initiated with a highly acidic condition ($\text{pH } 3.87 \pm 0.06$). The addition of medium A increased the pH to 6.43 ± 0.03 , whereas media B and C had no significant

effect on pH levels compared to the control (Table 2). This was because medium A was the only formulation containing lime ($\text{Ca}(\text{OH})_2$), which contributed to pH adjustment of the wastewater. This pH elevation resulted in a more alkaline environment, which was favorable for the growth of microalgae. This was consistent with previous studies by Thakwang (2007) and Zhu et al. (2013), who reported that *C. vulgaris* grew optimally at neutral to slightly alkaline pH values, typically between 6.0 and 8.0. The acidity of the aquatic environment plays a crucial role in regulating phytoplankton growth and photosynthetic efficiency. Most phytoplankton species thrive under near-neutral to slightly alkaline conditions (pH 7-8.5), whereas acidic conditions (pH < 6.0) negatively affect carbon fixation and enzymatic activity, thereby reducing growth and biomass production. Moreover, low pH increases the solubility of toxic metals, which can further inhibit cell metabolism and chlorophyll synthesis. Conversely, highly alkaline conditions (pH > 9.0) limit the availability of essential nutrients such as phosphate and nitrogen, leading to growth suppression. These findings highlight that maintaining optimal pH is critical for sustaining phytoplankton populations in both natural waters and wastewater-based cultivation systems (Hinga, 2002).

Table 2. Preliminary water parameters measured for grease trap wastewater supplemented with nutrient formulas A, B, and C before and after the cultivation. (The control had no additional nutrients.)

	Before Experiment			After Experiment		
	Temp (°C)	DO (mg/L)	pH	Temp (°C)	DO (mg/L)	pH
Control	25.00±0.00	4.80±0.20	3.87±0.06	25.00±0.00	6.27±0.23	3.78±0.03
A	25.00±0.00	5.88±0.20	6.43±0.03*	25.00±0.00	5.07±0.06	8.48±0.14*
B	25.00±0.00	4.39±0.21	3.97±0.08*	25.00±0.00	5.42±0.27	4.37±0.13*
C	25.00±0.00	5.79±0.08	3.68±0.02*	25.00±0.00	5.73±0.03	5.77±0.05*

Note: *Significance level of Paired t-test at $P < 0.01$

3.2 The optimum inoculum cell density

The optimal initial cell density for initiating *Chlorella vulgaris* mass culture with the highest growth rate and cell production was investigated in this research. The hypothesis was that with higher initial cell density, phytoplankton could achieve higher growth rates and mass production within the same cultivation period. To determine whether differences in initial cell density resulted in higher or lower specific growth rates and mass production, the growth of *C. vulgaris* at various inoculum densities was investigated. As shown in Figure 2, the initial inoculum densities of 5×10^4 (Control A1), 5×10^5 (Control A2), and 5×10^6 (Control A3) cells/mL resulted in *C. vulgaris* cell densities ranging from 0.01 to 0.07×10^6 , 0.28 to 0.50×10^6 , and 4.40 to 8.10×10^6 cells/mL, respectively. The corresponding specific growth rates were -0.015 , -0.066 , and 0.069 day^{-1} for these inoculum levels.

When grease trap wastewater supplemented with nutrients was used, and *C. vulgaris* was inoculated at initial densities of 5×10^4 (A1), 5×10^5 (A2), and 5×10^6 (A3) cells/mL, the final cell densities reached the ranges of 0.01 to 0.05×10^6 , 0.21 to 0.50×10^6 , and 3.57 to 14.43×10^6 cells/mL, respectively. The specific growth rates under these conditions were -0.271 , -0.126 , and 0.151 day^{-1} for inoculum densities A1, A2, and A3, respectively.

These results indicated that grease trap wastewater supplemented with nutrients and inoculated with *C. vulgaris* at 5×10^6 cells/mL supports significantly higher algal growth compared to lower inoculum densities. This was likely due to the sufficiently high initial cell density, which allowed for rapid population increase before degradation by contaminant bacteria could occur. Although the wastewater in this experiment was pre-treated with chlorine and sedimentation using aluminum sulfate, bacteria and other microorganisms may remain in the water. By observing phytoplankton density under a compound light microscope, contamination with bacteria was found in all treatments. Bacteria play a crucial role in regulating phytoplankton growth by inhibiting their proliferation through multiple mechanisms. One primary mechanism is nutrient competition, particularly for nitrogen and phosphorus, which are essential for photosynthesis and cell division in phytoplankton (Thingstad, 2000; Seymour et al., 2017). Additionally, certain bacteria can produce toxic or allelopathic compounds that reduce photosynthetic efficiency and damage the cell membranes of phytoplankton (Mayali & Azam, 2004). Bacteria can also create unfavorable microenvironments for algal growth as they may release organic acids, or gases like CO_2 and H_2S , which alter pH and dissolved oxygen levels, and they may form biofilms on surrounding surfaces that interfere with nutrient and light availability. Collectively, these interactions can significantly reduce cell division rates and the population growth of phytoplankton (Amin et al., 2012; Dang & Lovell, 2016).

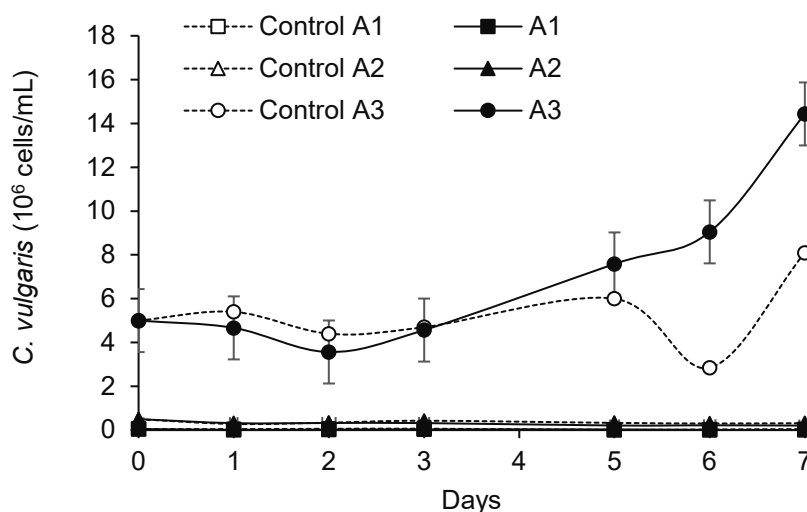


Figure 2. Cell density of *Chlorella vulgaris* in grease trap wastewater inoculation at initial concentrations of 5×10^4 (A1), 5×10^5 (A2), and 5×10^6 (A3) cells/mL

Furthermore, the results of this study revealed that grease trap wastewater supplemented with nutrient medium and inoculated with high initial densities of *C. vulgaris* exhibited greater algal growth. This result was consistent with other research, which found that an initial cell inoculum of *Chlorella* sp. exceeding 10^6 cells/mL was successfully enhanced for mass cultivation in wastewater. Baldisserotto et al. (2023a) reported that an initial density of *Chlorella* sp. 1.3×10^6 cells/mL in distillery wastewater reached maximum density of 6×10^6 cells/mL after 7 days. Meanwhile, *Chlorella* sp. cultured in soybean

sauce factory wastewater and under photoautotrophic conditions in community wastewater reached peak cell densities of 6.48×10^6 and $11.9\text{--}13.6 \times 10^6$ cells/mL within 7 days, respectively (Canedo-López et al., 2016; Mohammadi & Arabian, 2023). This evidence shows that wastewater from a grease trap with a satisfactory initial algal density has the potential to be an effective and sustainable substrate for microalgal biomass production, with performance comparable to or superior to that of other industrial and agricultural wastewaters.

Comparison of the phytoplankton growth rate in the same medium (formula A) with the same initial cell density (5×10^6 cells/L) showed that the specific growth rate in the experiment with the suitable nutrient formula (Figure 1) was much higher than that determined in the experiment with the optimal inoculum density (Figure 2). This may be due to differences in cell integrity. In this study, the experiment on the suitable nutrient formula was implemented first. Then, the second experiment (the optimal inoculum density) was run afterward. The *Chlorella vulgaris* cell used in the first experiment was a pure culture directly obtained from TISTR. The integrity of algal cells was checked under the compound light microscope. Meanwhile, the *C. vulgaris* cell employed in the second experiment was subcultured many times. During the subculture, cells may lose their integrity. Under the compound light microscope, cell color faded, and cell size decreased. Therefore, the algal cells used in the first experiment may have produced more efficiently than those used in the second experiment. These results suggest that to achieve the most efficient cell production, algal cell integrity should be checked before use.

3.4 Water quality assessment before and after the experiment

The preliminary water characteristics before and after the experiments are summarized in Tables 2 and 3. Throughout the experimental period, the water temperature was consistently maintained at 25°C, which is within the optimal temperature range for green algae growth (Becker, 2007). Green algae exhibit optimal growth between 25–30°C. Temperatures above this range can reduce photosynthetic rates and growth, ultimately leading to cell death, while lower temperatures similarly limit biomass production.

The dissolved oxygen (DO) levels before the experiment ranged from 5.82–6.02 mg/L, increasing to an average range of 7.14–9.82 mg/L after the experiment (Table 3). These DO concentrations are within or above the optimal range for *Chlorella vulgaris* cultivation, typically 4–8 mg/L (Wang et al., 2010). The increase in DO after the experiment was attributed to continuous aeration. More oxygen can enhance phytoplankton growth because it is required for their respiration (Wang et al., 2010). During cell development, phytoplankton uses organic matter to produce cell organelles such as the nucleus, mitochondria, and ribosomes. Therefore, oxygen enrichment supports the growth of phytoplankton and other microorganisms involved in wastewater treatment, subsequently enhancing the overall remediation process. The oxygen level in water is not directly affected by pH because there is no physical-chemical connection between the two parameters. However, the pH of a water column can be influenced by carbon dioxide, a byproduct of respiration or dissolved from the atmosphere (Emerson & Hedges, 2008).

The statistical analysis, a paired t-test, showed that the pH values differed significantly across treatments (P value < 0.01). As shown in Table 3, the pH values of the water ranged from 5.49–6.82 prior to the experiment and increased to 6.19–8.82 afterward. This pH range was consistent with Williams et al., (2012), who noted that grease trap wastewater typically exhibits acidic conditions (pH values between 5.5 and 7.5) depending on the wastewater source and grease filtration processes. The post-experiment pH, which

exceeded 6 in all treatments, indicated favorable conditions for *C. vulgaris* growth, as green algae thrive in neutral to slightly alkaline environments (pH 8.0-10.0) (Al-Hammadi & Güngörmüşler, 2024). As shown in Table 2, the pH values increased only in medium A. This was because the medium contained calcium hydroxide, which allowed more alkaline. Adding medium to the experiment may increase pH level in A1-A3 (Table 3). Meanwhile, the pH values increased in Controls A1-A3. This may be due to the addition of pure culture during the algal cell adjustment. The leftover medium in the pure culture may alter the pH level in the experiment. Moreover, the pH increase was likely due to CO₂ uptake during photosynthesis, which reduced the concentration of carbonic acid in the water, thereby stabilizing the pH and enhancing microbial degradation efficiency (Wang et al., 2010).

Table 3. Water characteristics before and after cultivation, temperature (°C), dissolved oxygen (DO, mg/L), pH, and chemical oxygen demand (COD, mg/L) in grease trap wastewater samples

Before Experiment				
	Temp (°C)	DO (mg/L)	pH	COD (mg/L)
WW	25.00±0.00	6.02±0.00	5.49±0.00	228.80±0.10
Control A1	25.00±0.00	5.88±0.00	6.40±0.00	276.80±0.14
Control A2	25.00± 0.00	5.90±0.00	6.00±0.00	244.80±0.17
Control A3	25.00±0.00	5.90±0.00	6.01±0.00	224.00±0.12
A1	25.00±0.00	5.98±0.01	6.67±0.01*	225.33±0.10*
A2	25.00±0.00	5.88±0.01	6.69±0.02*	232.00±0.12*
A3	25.00±0.00	5.82±0.02	6.82±0.01*	228.00±0.11*
After Experiment				
	Temp (°C)	DO (mg/L)	pH	COD (mg/L)
WW	25.00±0.00	8.29±0.00	6.19±0.00	212.80±0.25
Control A1	25.00±0.00	8.83±0.00	7.99±0.00	158.40±0.06
Control A2	25.00±0.00	7.14±0.00	7.96±0.00	132.80±0.12
Control A3	25.00±0.00	9.82±0.00	8.52±0.00	126.40±0.06
A1	25.00±0.00	7.86±0.20	8.60±0.04*	125.33±0.05*
A2	25.00±0.00	7.67±0.22	8.63±0.03*	74.67±0.13*
A3	25.00±0.00	7.57±0.83	8.82±0.11*	66.67±0.04*

Note: WW is grease trap wastewater without nutrient supplementation; Controls A1, A2, A3 are grease trap wastewater without nutrient supplementation, inoculated with *Chlorella vulgaris* at 5×10^4 , 5×10^5 , and 5×10^6 cells/mL, respectively; A1, A2, A3 are supplemented with nutrient formula A, inoculated at 5×10^4 , 5×10^5 , and 5×10^6 cells/mL, respectively. *Significance level of Paired t-test at $P < 0.01$

Chemical oxygen demand (COD) values, indicating the amount of oxygen required to chemically oxidize organic compounds, decreased from an initial range of 228.00-276.80 mg/L to 66.67-212.80 mg/L after the cultivation period (Table 3). Statistical analysis also revealed that COD values were significantly different before and after the experiment (P value < 0.01). The highest removal efficiency, 70.76% was achieved at an initial cell concentration of 5×10^6 cells/mL, followed by 67.81% and 44.38% at initial cell concentrations of 5×10^5 and 5×10^4 cells/mL, respectively. The observed reduction in COD post-cultivation suggested that organic substances were consumed and degraded by *C. vulgaris* and associated microorganisms, consistent with findings that microalgae could reduce COD by absorbing nutrients and organic matter (Wang et al., 2010). The COD removal efficiency observed in this study (70.76%) was comparable to that reported by Li et al. (2021) in distillery wastewater (72.24%), higher than *Scenedesmus* sp. in municipal wastewater (45-50%) (Rawat et al., 2011), but lower than that achieved by a mixed microalgal consortium in swine wastewater (72.1%) (Chen et al., 2022) and *Chlorella* sp. in hospital wastewater (80.8%) (Le & Nguyen, 2024). These results indicate that COD removal is influenced not only by the algal species but also by wastewater composition, organic matter concentration, and cultivation conditions.

As shown in Figure 3, during the cultivation of *Chlorella vulgaris* in grease trap wastewater, ammonia-nitrogen ($\text{NH}_3\text{-N}$) concentration tended to increase over time within the range of 0.50-3.14 $\mu\text{g/L}$. The results revealed that nutrient formula A combined with an inoculum density of *C. vulgaris* at 5×10^4 (A1) cells/mL in grease trap wastewater resulted in higher concentrations of ammonia-nitrogen ($\text{NH}_3\text{-N}$) compared to samples inoculated at 5×10^6 (A3) and 5×10^5 (A2) cells/mL, respectively. This outcome can be attributed to the high urea content in the nutrient formula and the biodegradation of organic matter by other microorganisms contaminated in the water, resulting in increased ammonia levels. In addition, fewer algal cells resulted in less ammonia use for cell growth. Therefore, higher ammonia accumulation in water was observed at lower inoculum densities. Ammonia-nitrogen levels in grease trap wastewater are a common pollutant resulting from the degradation of protein and organic matter in food processing effluents (Tchobanoglous et al., 2014). Elevated $\text{NH}_3\text{-N}$ towards the experiment's end, particularly in nutrient-amended samples, may be attributed to urea content in the added media and metabolic waste released by algal cells. Nevertheless, $\text{NH}_3\text{-N}$ concentrations remained within the optimal growth range for *C. vulgaris* (0.5-5 $\mu\text{g/L}$) (Mata et al., 2010). These $\text{NH}_3\text{-N}$ levels were lower than those reported by Rawat et al. (2011), who observed ammonia concentrations of 50-300 mg/L in grease trap wastewater, reflecting differences in effluent source and treatment. The efficient uptake of $\text{NH}_3\text{-N}$ by *C. vulgaris* as a nitrogen source for growth highlights its suitability for nitrogen removal in wastewater treatment (Wang et al., 2010).

Conversely, concentrations of nitrate-nitrogen ($\text{NO}_3\text{-N}$) significantly decreased over time during the experiment. As shown in Figure 3, samples inoculated at the highest density 5×10^6 cells/mL (A3) showed greater $\text{NO}_3\text{-N}$ reduction than those at 5×10^4 (A1) and 5×10^5 (A2) cells/mL resulting in the removal efficiency at 91.03%, 18.09%, and 4.55%, respectively, within 3 days of the cultivation. This was because nitrates were actively utilized by the algal cells at higher densities. Phytoplankton, like other primary producers, requires nitrogen as a key macronutrient to support their growth and metabolism. Nitrates (NO_3^-) are the most common and bioavailable form of dissolved inorganic nitrogen in aquatic ecosystems. Phytoplankton assimilate nitrates through specific transport systems and reduce them enzymatically (via nitrate reductase and nitrite reductase) to ammonium (NH_4^+), which is then incorporated into amino acids,

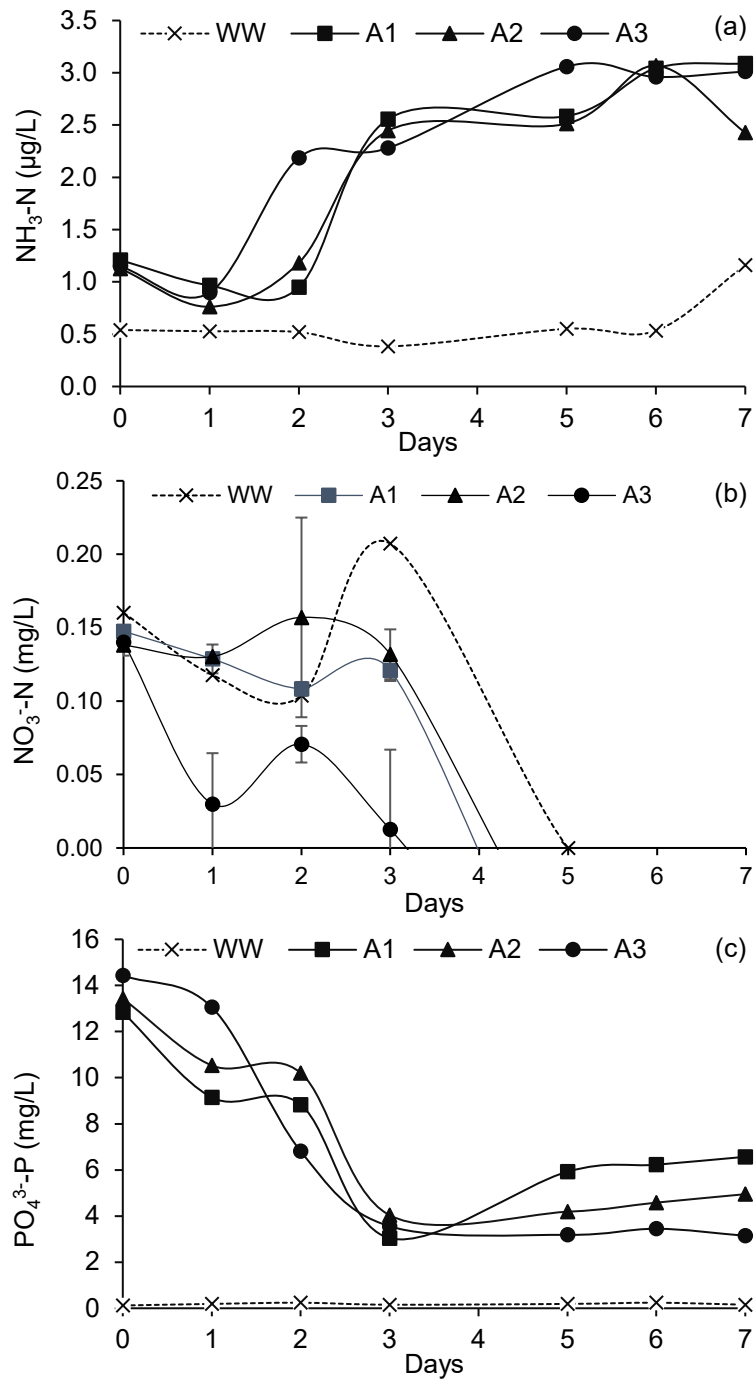


Figure 3. Concentration of ammonia-nitrogen ($\text{NH}_3\text{-N}$) (a), nitrate-nitrogen ($\text{NO}_3\text{-N}$) (b), and phosphate-phosphorus ($\text{PO}_4^{3-}\text{-P}$) (c) in grease trap wastewater inoculation at initial *Chlorella vulgaris* of 5×10^4 (A1), 5×10^5 (A2), and 5×10^6 (A3) cells/mL

nucleotides, and chlorophyll molecules. These nitrogen-containing compounds are essential for protein synthesis, photosynthetic activity, and cell division. Therefore, nitrate availability strongly regulates primary productivity and biomass accumulation in both marine and freshwater systems. Limited nitrate supply can restrict phytoplankton growth, while elevated concentrations can promote algal blooms (Falkowski & Raven, 2007). The results from this study demonstrate that the reduced nitrate in algal treatments suggested effective assimilation by *C. vulgaris* during photosynthesis, indicating its capacity to mitigate nitrate pollution.

Similar to $\text{NO}_3\text{-N}$, the concentration of phosphate-phosphorus ($\text{PO}_4^{3-}\text{-P}$) in all treatments decreased rapidly within the first 3 days of the cultivation period (Figure 3). Consequently, the $\text{PO}_4^{3-}\text{-P}$ removal efficiencies were found at 76.31%, 69.94%, and 75.36% in the inoculum cell densities of 5×10^4 (A1), 5×10^5 (A2), and 5×10^6 (A3) cells/mL, respectively. These results demonstrate that the green algae *Chlorella vulgaris* effectively took up phosphate for new cell synthesis. Phytoplankton requires phosphorus for growth and cell enlargement. Our hypothesis was that a higher density of algal cells could perform more phosphorus utilization from the water column. However, this study found that the initial cell inoculum did not affect phosphorus removal. This may be due to the high efficiency of phosphorus accumulation by the phytoplankton cells. When phosphorus is enriched in the water column, phytoplankton attempts to accumulate phosphorus as much as they can in their cells so that they can later assimilate it into the cell tissues (Tantanasarit et al., 2013). Therefore, the results imply that phytoplankton can effectively remove phosphorus from the water column, even at low density. Phytoplankton primarily assimilate phosphorus in the form of inorganic phosphate (PO_4^{3-}) to sustain growth and metabolic activity. Phosphate is a critical constituent of nucleic acids (DNA and RNA), adenosine triphosphate (ATP), phospholipids of cellular membranes, and various coenzymes, which collectively support energy transfer, biosynthesis, and cell division. The availability of phosphate in aquatic environments directly influences phytoplankton productivity, photosynthetic efficiency, and biomass accumulation. Limitation of phosphate can substantially constrain growth, whereas sufficient phosphate concentration promotes cellular proliferation and ecosystem-level productivity (Sharoni & Halevy, 2022). Hence, our results indicate that *C. vulgaris* effectively absorbed phosphate for growth, contributing to a significant reduction in phosphorus in wastewater.

3.5 Capability of *Chlorella vulgaris* cultivation in grease trap wastewater

The efficiency of *Chlorella vulgaris* cultivation in wastewater is influenced by several factors, most notably the nutrient formulation and the initial inoculum density. These parameters have a significant impact on algal growth rates, nutrient uptake efficiency, and system stability. Excessively high inoculum densities may lead to overcrowding, nutrient depletion, or cell mortality due to self-shading and oxygen limitation. Conversely, low inoculum densities may result in delayed growth and unstable treatment performance.

The results from this study showed that grease trap wastewater contained available essential nutrients for phytoplankton growth. Phytoplankton requires carbon, nitrogen, and phosphorus for cell development-related processes such as DNA replication, respiration, and cell division (Thingstad, 2000; Seymour et al., 2017). In this study, organic matter, the carbon source in grease trap wastewater, was expressed as COD. The results demonstrated that the wastewater contained COD 228.80 mg/L (Table 3). This value fell within the range of algal cultivation conditions (Wang et al., 2010). The concentrations of ammonia-nitrogen and nitrate-nitrogen in the wastewater were 0.54

µg/L and 0.16 mg/L, respectively, which were within the suitable conditions for algal cell enhancement (Mata et al., 2010). However, phosphate-phosphorus ($\text{PO}_4^{3-}\text{-P}$) may not be sufficient for algal mass culture. The concentration of $\text{PO}_4^{3-}\text{-P}$ in the grease trap wastewater was only 0.12 mg/L, which was relatively low level compared to other wastewater effluents (Wang et al., 2010; Rawat et al. 2011). Phosphorus is an essential nutrient for phytoplankton due to the importance of biochemical substances such as ATP, cell membranes, and lipids. Lack of this nutrient results in a limitation of algal growth. Hence, nutrient supplementation, especially $\text{PO}_4^{3-}\text{-P}$ is required for enhancing mass culture of *C. vulgaris* in grease trap wastewater.

Additionally, our results showed that pH level had a significant influence on the *C. vulgaris* mass culture. The medium A used in this study was the only nutrient formula that contained calcium hydroxide. This allowed the adjustment of the pH of wastewater from acid (3.87 ± 0.06) to base (8.48 ± 0.14) (Table 1), which was suitable for *C. vulgaris* growth. Acidic wastewater can inhibit algal growth, and mass mortality can be observed. Acidic conditions inhibit algae because low pH can disrupt enzyme activity, damage cell membranes, and reduce nutrient uptake, all of which impair photosynthesis and cell division (Falkowski & Raven, 2007). *Chlorella vulgaris*, like many green microalgae, thrives in slightly basic conditions (pH 7-8) where photosynthetic efficiency, nutrient assimilation, and growth rates are optimized (Wang et al., 2010; Rawat et al., 2011). Therefore, prior adjustment of the acid-base balance of the culture medium is essential for successful mass cultivation of *C. vulgaris*.

This study is initial research on the cultivation of *Chlorella vulgaris* in grease trap wastewater. Although there is limited information on *C. vulgaris* cultivation in this wastewater, our results are comparable to those in other types of wastewaters. Compared to other researchers, the *C. vulgaris* cultivation in this study had low mass production. The present study revealed that cultivating *C. vulgaris* in grease trap wastewater yielded a final biomass concentration of 0.76 g/L. This estimate was derived from a preliminary experiment that determined the dry weight of *C. vulgaris* to be 15.74 pg/cell. When compared to the findings of Sasiboonyarat (2014), the biomass yield from *C. vulgaris* cultured in grease trap wastewater was lower than that obtained from swine farm wastewater, seafood processing wastewater, and concentrated latex factory effluent, for which biomass concentrations of 0.96, 0.82, and 0.76 g/L, respectively, were reported. The lower cell biomass and density in this study may be due to cultivation conditions that were suffocating, including water temperature, sterile conditions, pH, nutrient levels, and other contaminants in the wastewater. However, our results provide unique information for the mass culture of this algal species using recycled water, especially wastewater from the grease trap. When supplemented with nutrients and adjusted to a suitable pH, grease trap wastewater can support *C. vulgaris* cultivation. This is relevant to Sustainable Development Goal 12: responsible consumption and production, particularly in minimizing water use, nutrient removal, and commercial algal mass production.

4. Conclusions

The findings of this research highlight the potential of *Chlorella vulgaris* as an efficient microalgal species for biomass production and bioremediation applications in grease trap wastewater environments. The results demonstrated that nutrient formula A, which included calcium hydroxide, provided the most suitable nutrient environment for promoting the growth of *C. vulgaris* in grease trap wastewater. This was because the medium adjusted the pH from acidic to slightly alkaline, which was appropriate for *C. vulgaris* cell

division. The acidity of water can inhibit the photosynthesis process and algal growth. Our results also revealed that the maximum growth rate was found in the algal cell culture initiated at 5×10^6 cells/mL. This indicated that a sufficiently high initial algal density may enable rapid cell proliferation. Water observation during the experiment period successfully demonstrated that *C. vulgaris* had strong potential for the sustainable bioremediation of grease trap wastewater, effectively reducing nitrate-nitrogen and phosphate-phosphorus concentrations while improving the dissolved oxygen and pH conditions that were conducive to microbial degradation and algal proliferation. To achieve algal mass production and bioremediation in grease trap wastewater, the suitable nutrient concentration at N/P ratio 27.411 with an optimal acid-base balance at pH 8-10 must be considered, and the initial cell density must be adequate (at least 5×10^6 cells/mL). Further studies should focus on the details of algal mass culture, the cost of the operation, and the overall worthiness, which refers to the benefits in both environmental remediation and financial assets.

5. Acknowledgements

The authors are thankful to the Environmental Science and Technology Programme, Faculty of Science and Technology, Bansomdejchaopraya Rajabhat University for providing laboratory space, and the Thailand Institute of Scientific and Technological Research (TISTR) for providing the green algae *Chlorella vulgaris* TISTR 8580 for the experiment design.

6. Authors' Contributions

Suwat Chunharat wrote the paper, revised the manuscript, and discussed the data; Prepawarin Wongkeetee, Sumalee Saipan, Panlop Pommee performed research; Chayarat Srisunont designed the research, analyzed the data, and coordinated the research.

7. Conflicts of Interest

No conflicts of interest.

References

- Al-Hammadi, M., & Güngörmüşler, M. (2024). New insights into *Chlorella vulgaris* applications. *Biotechnology and Bioengineering*, 121(5), 1486-1502. <https://doi.org/10.1002/bit.28666>
- APHA. (2012). *Standard methods for the examination of water and wastewater* (22nd ed.). American Public Health Association, American Water Works Association and Water Environment Federation.
- Amin, S. A., Parker, M. S., & Armbrust, E. V. (2012). Interactions between diatoms and bacteria. *Microbiology and Molecular Biology Reviews*, 76(3), 667-684. <https://doi.org/10.1128/MMBR.00007-12>
- Baldisserotto, C., Demaria, S., Arcidiacono, M., Benà, E., Giacobbe, P., Marchesini, R., Ferroni, L., Benetti, L., Zanella, M., Benini, A., & Pancaldi, S. (2023a). Enhancing urban wastewater treatment through an isolated *Chlorella* strain-based phytoremediation in

- centrate stream: An analysis of algae morpho-physiology and nutrient removal efficiency. *Plants*, 12(5), Article 1027. <https://doi.org/10.3390/plants12051027>
- Becker, E. W. (2007). Micro-algae as a source of protein. *Biotechnology Advances*, 25(2), 207-210. <https://doi.org/10.1016/j.biotechadv.2006.11.002>
- Canedo-López, Y., Ruiz-Marín, A., & Zavala-Loría, J. D. C. (2016). A two-stage culture process using *Chlorella vulgaris* for urban wastewater nutrient removal and enhanced algal lipid accumulation under photoautotrophic and mixotrophic conditions. *Journal of Renewable and Sustainable Energy*, 8(3), Article 033102. <https://doi.org/10.1063/1.4954078>
- Chen, C.-Y., Kuan, S.-P., Nagarajan, D., Chen, J.-H., Ariyadasa, T. U., & Chang, J.-S. (2022). A novel two-stage process for the effective treatment of swine wastewater using *Chlorella sorokiniana* AK-1 based algal-bacterial consortium under semi-continuous operation. *Bioresource Technology*, 365, Article 128119. <https://doi.org/10.1016/j.biortech.2022.128119>
- Chisti, Y. (2007). Biodiesel from microalgae. *Biotechnology Advances*, 25(3), 294-306. <https://doi.org/10.1016/j.biotechadv.2007.02.001>
- Dang, H., & Lovell, C. R. (2016). Microbial surface colonization and biofilm development in marine environments. *Microbiology and Molecular Biology Reviews*, 80(1), 91-138. <https://doi.org/10.1128/MMBR.00037-15>
- Emerson, S. R., & Hedges, J. I. (2008). *Chemical oceanography and the marine carbon cycle*. Cambridge University Press.
- Falkowski, P. G., & Raven, J. A. (2007). *Aquatic photosynthesis* (2nd ed.). Princeton University Press.
- Farooq, W., Naqvi, S. R., Sajid, M., Shrivastav, A., & Kumar, K. (2022). Monitoring lipids profile, CO₂ fixation, and water recyclability for the economic viability of microalgae *Chlorella vulgaris* cultivation at different initial nitrogen. *Journal of Biotechnology*, 345, 30-39. <https://doi.org/10.1016/j.jbiotec.2021.12.014>
- Hinga, K. R. (2002). Effects of pH on coastal marine phytoplankton. *Marine Ecology Progress Series*, 238, 281-300. <https://doi.org/10.3354/meps238281>
- Jefri, U. H. N. M., Khan, A., Lim, Y. C., Lee, K. S., Liew, K. B., Kassab, Y. W., Choo, C.-Y., Al-Worafi, Y. M., Ming, L. C., & Kalusalingam, A. (2022). A systematic review on chlorine dioxide as a disinfectant. *Journal of Medicine and Life*, 15(3), 313-318. <https://doi.org/10.25122/jml-2021-0180>
- Le, T. T. A., & Nguyen, T. (2024). Potential of hospital wastewater treatment using locally isolated *Chlorella* sp. LH₂ from cocoon wastewater. *Bioresources and Bioprocessing*, 11, Article 35. <https://doi.org/10.1186/s40643-024-00748-6>
- Li, F., Amenorfenyo, D. K., Zhang, Y., Zhang, N., Li, C., & Huang, X. (2021). Cultivation of *Chlorella vulgaris* in membrane-treated industrial distillery wastewater: Growth and wastewater treatment. *Frontiers in Environmental Science*, 9, Article 770633. <https://doi.org/10.3389/fenvs.2021.770633>
- Lohalaksanadech, D., Chuanprachum, T., & Kaewsut, E. (2011). Comparison of culture media for *Chlorella ellipsoidea* culture. *Fisheries Technology Research Journal*, 5(2), 48-54.
- Mata, T. M., Martins, A. A., & Caetano, N. S. (2010). Microalgae for biodiesel production and other applications: A review. *Renewable and Sustainable Energy Reviews*, 14(1), 217-232. <https://doi.org/10.1016/j.rser.2009.07.020>
- Mayali, X., & Azam, F. (2004). Algicidal bacteria in the sea and their impact on algal blooms. *Journal of Eukaryotic Microbiology*, 51(2), 139-144. <https://doi.org/10.1111/j.1550-7408.2004.tb00538.x>
- Mehrpour, P., Mirbagheri, S. A., Kavianimalayeri, M., Sayyahzadeh, A. H., & Ehteshami, M. (2023). Experimental pH adjustment for different concentrations of industrial

- wastewater and modeling by Artificial Neural Network. *Environmental Technology and Innovation*, 31, Article 103212. <https://doi.org/10.1016/j.eti.2023.103212>
- Mohammadi, F. S., & Arabian, D. (2023). Optimization of *Chlorella vulgaris* cultivation grown in waste molasses syrup using mixture design. *Journal of the American Oil Chemists' Society*, 100(1), 45-56. <https://doi.org/10.1002/aocs.12656>
- Petchmanee, T. (1999). *Plankton cultivation manual*. Coastal Aquaculture Institute, Songkhla Province.
- Peter, A. P., Chew, K. W., Pandey, A., Lau, S. Y., Rajendran, S., Ting, H. Y., Munawaroh, H. S. H., Phuong, N. V., & Show, P. L. (2023). Artificial intelligence model for monitoring biomass growth in semi-batch *Chlorella vulgaris* cultivation. *Fuel*, 333(Part 2), Article 126438. <https://doi.org/10.1016/j.fuel.2022.126438>
- Rawat, I., Kumar, R. R., Mutanda, T., & Bux, F. (2011). Dual role of microalgae: Phycoremediation of domestic wastewater and biomass production for sustainable biofuels production. *Applied Energy*, 88(10), 3411-3424. <https://doi.org/10.1016/j.apenergy.2010.11.025>
- Sasiboonyarat, T. (2014). *A study on the cultivation of Chlorella algae in wastewater from the food industry*. [unpublished Master thesis]. Mahidol University.
- Seymour, J. R., Amin, S. A., Raina, J.-B., & Stocker, R. (2017). Zooming in on the phycosphere: the ecological interface for phytoplankton–bacteria relationships. *Nature Microbiology*, 2, Article 17065. <https://doi.org/10.1038/nmicrobiol.2017.65>
- Sharoni, S., & Halevy, I. (2022). Geologic controls on phytoplankton elemental composition. *Proceedings of the National Academy of Sciences of the United States of America*, 119(1), Article e2113263118. <https://doi.org/10.1073/pnas.2113263118>
- Stevenson, R. J., Bothwell, M. L., Lowe, R. L. & Thorp, J. H. (1996). *Algal ecology: Freshwater benthic ecosystems*. Academic Press.
- Tang, L. Y., Wong, N. H., Chieng, T. A., Kiu, A. K. J., Choo, C. S., Li, Y., Tan, C. P., Yaser, A. Z., Khaerudini, D. S., Chen, G. H., & Sunarso, J. (2024). Physicochemical characteristics of grease-trap wastewater with different potential mechanisms of FOG solid formation, separation, and accumulation inside grease traps. *Water Research*, 256, Article 121607. <https://doi.org/10.1016/j.watres.2024.121607>
- Tantanasarit, C., Englande, A. J., & Babel, S. (2013). Nitrogen, phosphorus and silicon uptake kinetics by marine diatom *Chaetoceros calcitrans* under high nutrient concentrations. *Journal of Experimental Marine Biology and Ecology*, 446, 67-75. <https://doi.org/10.1016/j.jembe.2013.05.004>.
- Tchobanoglous, G., Stensel, H. D., Tsuchihashi, R., & Burton, F. L. (2014). *Wastewater engineering: Treatment and resource recovery* (5th ed.). McGraw-Hill Education.
- Thakwang, N. (2007). *Cultivation of Spirulina (Spirulina platensis) in dairy farm wastewater for use as dairy cow feed*. [unpublished Master thesis]. Maejo University.
- Thewaratmaneekul, P., Setkit, S., & Watcharakornyothin, T. (2006). *Red worm cultivation*. Cooperative Community Printing of Agricultural Cooperatives of Thailand Ltd.
- Thingstad, T. F. (2000). Elements of a theory for the mechanisms controlling abundance, diversity, and biogeochemical role of lytic bacterial viruses in aquatic systems. *Limnology and Oceanography*, 45(6), 1320-1328. <https://doi.org/10.4319/lo.2000.45.6.1320>
- Vovk, L., Matsiyevska, O., & Zhdanov, O. (2020). *Chlorella vulgaris* in wastewater treatment processes – practical experience. *Theory and Building Practice*, 2(2), 21-27. <https://doi.org/10.23939/jtbp2020.02.021>
- Wang, L., Min, M., Li, Y., Chen, P., Chen, Y., Liu, Y., Wang, Y., & Ruan, R. (2010). Cultivation of green algae *Chlorella* sp. in different wastewaters from municipal wastewater treatment plant. *Applied Biochemistry and Biotechnology*, 162(4), 1174-1186. <https://doi.org/10.1007/s12010-009-8866-7>

- Williams, J. B., Clarkson, C., Mant, C., Drinkwater, A., & May, E. (2012). Fat, oil and grease deposits in sewers: Characterisation of deposits and formation mechanisms. *Water Research*, 46(19), 6319-6328. <https://doi.org/10.1016/j.watres.2012.09.002>
- Zhu, L., Wang, Z., Shu, Q., Takala, J., Hiltunen, E., Feng, P. & Yuan, Z. (2013). Nutrient removal and biodiesel production by integration of freshwater algae cultivation with piggery wastewater treatment. *Water Research*, 47(13), 4294-4302. <https://doi.org/10.1016/j.watres.2013.05.004>