EFFECT OF LOCALLY ISOLATED *SCENEDESMUS* SP. TN1 ON BIOMASS PRODUCTION AND NUTRIENT REMOVAL FROM COOKING COCOON WASTEWATER: EFFECTS OF INITIAL ALGAL CELL DENSITY AND TEMPERATURE

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Abstract

Scenedesmus sp. TN1, a local freshwater microalga, was cultivated in cooking cocoon wastewater. The effects of initial algal cell density and temperature on microalgae growth and the removal of total nitrogen (T-N), total phosphorus (T-P), and chemical oxygen demand (COD) were studied. The initial algal cell densities were 5, 10, 15, and 20 mg/l. The experimental temperatures varied from 20 °C to 30 °C. The growth rate increased with the increase in initial algal cell density up to 15 mg/l and was statistically stable thereafter. A similar trend of total nitrogen, total phosphorus, and COD removal efficiency was observed. The optimum temperature for microalgae growth and nutrient removal was 30 °C. After 9 days of cultivation, Scenedesmus sp. TN1 exhibited nutrient removal efficiencies of 88.28%, 82.91%, 92.01%, and 89.16% for T-N, T-P, biological oxygen demand (BOD_5), and COD, respectively. The maximum biomass production and nutrient removal efficiencies of Scenedesmus sp. TN1 were observed at cultivation conditions of 15 mg/l initial algal cell density at 30 °C. This study compiled information on the cultivation conditions for cooking cocoon wastewater treatment practices.

Keywords: BOD_5; COD; Microalgae; Total nitrogen; Total phosphorus.

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1. INTRODUCTION

Microalgae-based wastewater treatment processes are among the most ideal and sustainable approaches for eliminating pollutants in wastewater (Kong et al., 2010; Morillas-España et al., 2022; Srimongkol et al., 2022). Using microalgae to treat wastewater has attracted much attention because of their potential to reduce nutrients, heavy metals, toxic compounds, and other compounds while the biomass can be used to produce fuel and nonfuel products (Morillas-España et al., 2022; Plöhn et al., 2021; Srimongkol et al., 2022). One of the major requirements of wastewater treatment is to remove nitrogen and phosphorus that can cause eutrophication of aquatic environments (Yamashita & Yamamoto-Ikemoto, 2014). Microalgae are able to assimilate nitrogen and phosphorus for their metabolism (Yamashita & Yamamoto-Ikemoto, 2014). Therefore, many algal genera such as *Chlorella*, *Scenedesmus*, and *Spirulina* have been proposed for widespread use in household, agricultural, industrial, and hospital wastewater treatment (Bashir et al., 2020).

Algal cells can assimilate nutrients and other compounds in wastewater. Many studies in Vietnam have been conducted using microalgae for wastewater treatment. *Chlorella vulgaris* and *Scenedesmus obliquus* achieved high removal rates in reducing nutrients and organic contaminants in wastewater from a pig farm (Ji et al., 2013; Nguyen et al., 2022). *Spirulina platensis* SP4 has the ability to remove ammonia, nitrate, and phosphate from household wastewater (Tran et al., 2019). Various microalgae have been applied to treat wastewater from shrimp cultivation ponds, fertilizer plants, and other sources (Luu et al., 2020; Pham & Bui, 2020; Tran et al., 2022; Tran et al., 2019; Trinh et al., 2022). Various domestic algal strains have been isolated that are suitable for wastewater treatment (Luu et al., 2020; Tran et al., 2020). Among wastewater types, cooking cocoons during silk production generates large amounts of wastewater that contain high concentrations of organic matter (Capar et al., 2008; Fabiani et al., 1996). Thus, this wastewater has the potential for microalgae cultivation (Capar et al., 2008; Deng et al., 2020; Gao et al., 2021, 2022; Li et al., 2019; Mondal et al., 2007; Xue et al., 2021).

Cooking cocoon wastewater is rich in nutrients such as nitrogen, phosphorus, and organic matter (Xue et al., 2021). It was reported that 800–1000 tons of wastewater are discharged in producing 1 ton of raw silk (Zhang, 2002). Silkworm cocoons consist of 20% to 25% sericin, a globular protein and macromolecule, and 75% to 80% fibroin, a fibrous protein (Mondal et al., 2007). The silk reeling process removes the sericin that coats the outside of the silk fiber to obtain raw silk or completely removes it during the refining and scrubbing process of raw silk to produce silk fiber and biomedical materials (Li et al., 2019). Thus, reeling wastewater often contains large amounts of sericin protein and pupal oil. It has a high pH without the toxic compounds that can inhibit the growth of microorganisms (Gupta et al., 2014). Therefore, cooking cocoon wastewater would be a possible culture to cultivate algae.

The green microalgae *Scenedesmus* has shown the potential to grow rapidly in wastewater and is highly tolerant of variations in environmental conditions (Jebali et al., 2015; Juan et al., 2012; Kabir et al., 2017; Latiffi et al., 2017). *Scenedesmus* is a genus with high photosynthetic capability and lipid content. Excellent nutrient removal efficiency and lipid biosynthesis ability of *Scenedesmus* species in a variety of wastewater types have been reported in multiple studies (Korozi et al., 2023; Moreno Osorio et al., 2019; Russel et al., 2020; Sacristán de Alva et al., 2013; Ye et al., 2020). As a photosynthetic microorganism, *Scenedesmus* can fix carbon dioxide by converting energy from sunlight into biomass (Li et al., 2022). The growth of microalgae in wastewater is influenced by various factors such as pH, temperature, and initial algal cell density (Benedetti et al., 2018; Habibi et al., 2019; G. Li et al., 2023; Morillas-España et al., 2022; Saranya & Shanthakumar, 2020). Previous studies showed that the nutrient removal efficiency of *Scenedesmus* is affected by wastewater type and cultivation conditions (Morillas-España et al., 2021; Saranya & Shanthakumar, 2020). Biomass yield and lipid production of this alga correlated with cultivation conditions as well (Sacristán de Alva et al., 2013; Wong et al., 2015; Ye et al., 2020). Even though a number of research studies on wastewater treatment using *Scenedesmus* have been reported, studies on cooking cocoon wastewater treatment are limited and focused on using *Chlorella sorokiniana* (Deng et al., 2020; Gao et al., 2021, 2022; Li et al., 2019; L. Li et al., 2023; Lin et al., 2022; Xue et al., 2021). Moreover, there is no research on using locally isolated *Scenedesmus* to remove nutrients from cooking cocoon wastewater in Vietnam. Therefore, the aim of this study is to assess the potential of locally isolated *Scenedesmus* sp. TN1 to remove total nitrogen, total phosphorus, and chemical oxygen demand (COD) from cocoon wastewater by varying the initial algal cell density and temperature.

2. MATERIALS AND METHODS

2.1. Materials

Chemicals and reagents were obtained from Fisher Scientific (Pittsburgh, PA) and Sigma-Aldrich Co. (St. Louis, MO). *Scenedesmus* sp. TN1 is locally isolated from lakes that have received cooking cocoon wastewater from a local silk processing plant in Ta Nung, Lam Dong Province, Vietnam. The strain is available from the resource unit of Dalat University, Lam Dong Province, Vietnam. The wastewater was filtered to remove the solids for the experiments.

2.2. Microalgae cultivation

Pure *Scenedesmus* sp. TN1 was isolated from cooking cocoon wastewater that has been used in this study. *Scenedesmus* sp. TN1 typically occurs in 4-cell colonies of length 10–15.5 µm and width 5.5–6.5 µm, with long, spiny projections at the cell ends (Figure 1). The cells were cultivated in Bold's Basal Medium (Bischoff & Bold, 1963). The culture was incubated in a closed chamber at 25 °C ± 2 °C and shaken at 90 rpm with a light intensity of 3200 lux. The microalgae were collected by centrifugation and washed twice with distilled water for the experiments.

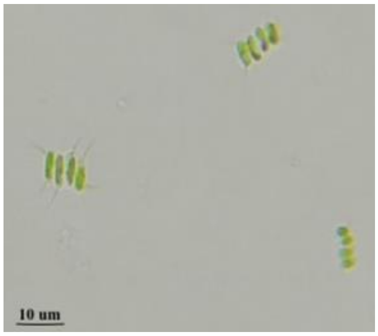

Figure 1. Morphology of *Scenedesmus* sp. TN1

2.3. Experimental set-up

The effect of cultivation conditions on the biomass production and nutrient removal of *Scenedesmus* sp. TN1 was investigated in sterilized cooking cocoon wastewater for different initial algal densities and temperatures. *Scenedesmus* sp. TN1 was tested in initial algal cell densities of 5, 10, 15, and 20 mg/l to investigate the effect of initial algal cell density on cell growth and nutrient removal, including total nitrogen (T-N), total phosphorus (T-P), and COD. The effect of temperature on microalgae was determined at 20 °C, 25 °C, and 30 °C. Microalgae growth was measured in terms of algal dry weight every 2 days after the first day, and the nutrient removal efficiency of total nitrogen, total phosphorus, and COD was determined over 9 days. BOD_5 was measured at the beginning of the experiment and at the end of the incubation on the ninth day.

2.4. Analytical methods

The algae biomass was determined by measuring algal dry weight. The pH was measured with a pH meter. Total nitrogen and total phosphorus were investigated using a water analysis (Pawlowski, 1994). BOD_5 and COD were measured according to the standard method (Walter, 1961).

3. STATISTICAL ANALYSIS

A one-way analysis of variance (ANOVA) and *t*-test were performed using Excel 2011 statistical tools. A p value < 0.05 was used as the criterion for significance level. ANOVA was used to determine whether growth and nutrient removal of *Scenedesmus* sp. TN1 at different initial algal cell densities and temperatures are statistically different.

4. RESULTS

Cooking cocoon wastewater was sterilized before the optimization test. Table 1 shows the characteristics of the wastewater before and after autoclaving. There was no statistical difference between T-N, T-P, and COD of the wastewater before and after autoclaving, while BOD_5 was higher. Therefore, the characteristics of T-N, T-P, and COD were measured to investigate the nutrient removal efficiency of *Scenedesmus* sp. TN1 for cocoon wastewater. The N/P ratio of the effluent was 22.75, much higher than the optimal ratio. Thus, this wastewater was a phosphorus limitation medium.

Table 1. Properties of cooking cocoon wastewater (CCW) before and after autoclaving

Characteristic	Raw CCW	Autoclaved CCW
T-N (mg/l)	112 ± 2.34	117 ± 4.65
T-P (mg/l)	4.95 ± 0.52	5.13 ± 0.83
BOD_5 (mg/l)	319 ± 5.95	342 ± 8.47
COD (mg/l)	499.1 ± 8.39	502.17 ± 7.61
pH	8.14 ± 0.16	8.15 ± 0.29

4.1. Effect of initial algal cell density on growth of *Scenedesmus* sp. TN1

The purpose of algae-based wastewater treatment is to reduce the nutrient content and increase the biomass of algae. *Scenedesmus* sp. TN1 was incubated in cooking cocoon wastewater with different initial algal cell densities to evaluate biomass production (Figure 2). Growth was estimated from dry cell weight over 9 days. An increase in the initial algal cell density led to an increase in biomass production. The maximum biomass yields obtained after 9 days were 370.16 ± 25.32, 398.45 ± 24.12, 571.12 ± 36.51, and 599.98 ± 12.87 mg/l for initial algal cell densities of 5, 10, 15, and 20 mg/l, respectively. The optimal biomass production was achieved on the ninth day with the initial algal cell densities of 15 and 20 mg/l ($p < 0.05$). There was no statistical difference between the biomass yields from initial algal cell densities of 15 and 20 mg/l after the fifth day.

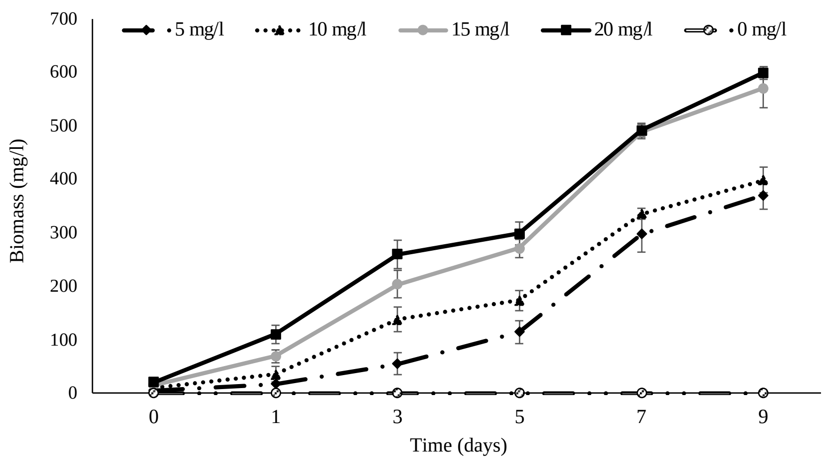

Figure 2. Growth of *Scenedesmus* sp. TN1 in cooking cocoon wastewater with different initial agal cell densities

Nitrogen removal over 9 days by the different initial cell densities of *Scenedesmus* sp. TN1 is shown in Figure 3. In the first 5 days, the reduction of nitrogen depended on initial cell density. Higher initial densities led to faster removal rates (Figure 3). A dramatic reduction in total nitrogen for all initial algal cell densities was observed over the first 3 days. The highest total nitrogen removal efficiencies achieved were 66.46% ± 2.23%, 65.19% ± 1.79%, 71.49% ± 2.51%, and 71.25% ± 2.59% after 9 days for initial cell densities of 5, 10, 15, and 20 mg/l, respectively. A similar trend was observed in total phosphorus removal efficiency. The phosphorus reduction by microalgae over 9 days was influenced by the initial cell density (Figure 4). Higher initial algal cell density increased the phosphorus removal rate. After the fifth day, there was no statistically significant difference in phosphorous removal between the initial cell densities of 5 and 10 mg/l. A similar pattern was observed for the initial algal cell densities of 15 and 20 mg/l. After 9 days, phosphorous removal efficiency of the initial algal cell densities of 15 and 20 mg/l showed no statistical difference.

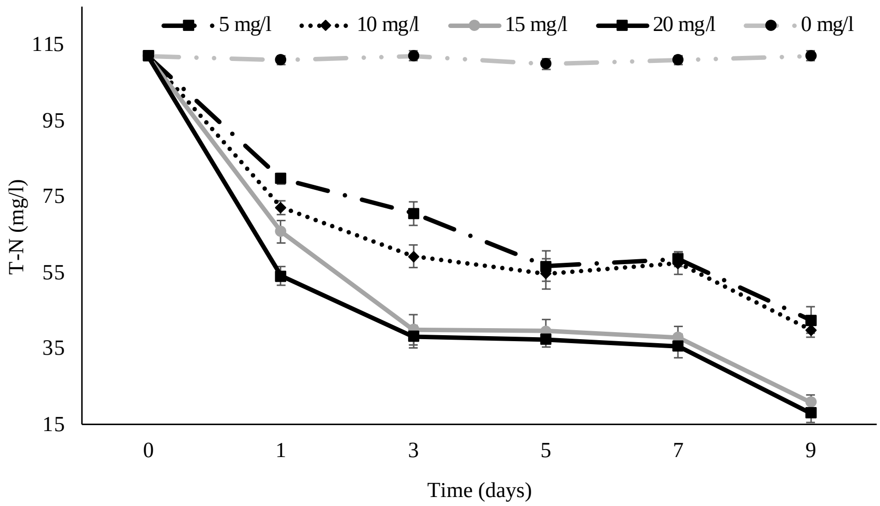

Figure 3. Removal of total nitrogen from cooking cocoon wastewater by *Scenedesmus* sp. TN1 for different initial algal cell densities

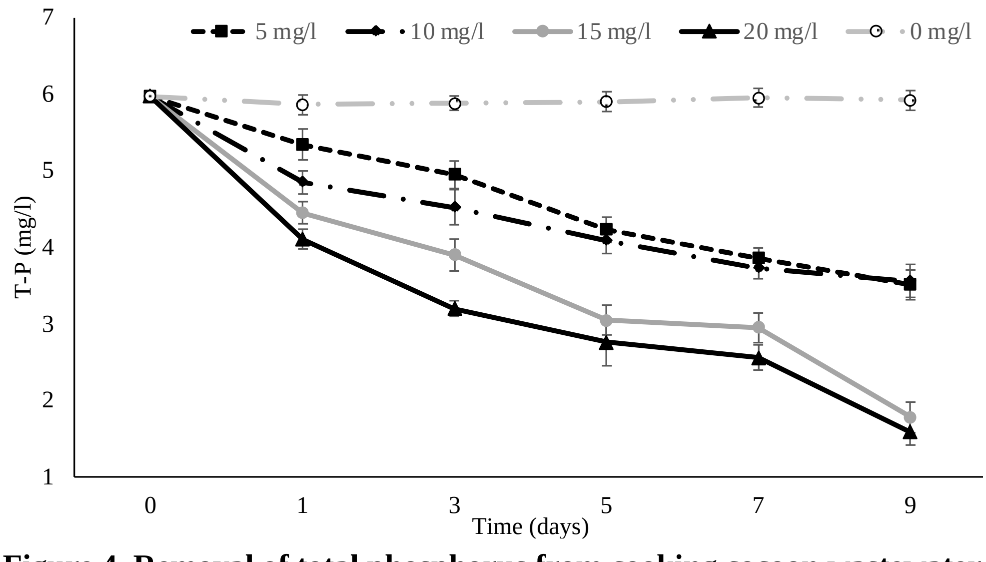

Figure 4. Removal of total phosphorus from cooking cocoon wastewater by *Scenedesmus* sp. TN1 for different initial algal cell densities

The reduction in COD for the four initial algal cell densities is shown in Figure 5. The COD of the culture dropped over the first 5 days. Increasing the initial cell concentration led to an increase in the removal efficiency. At the end of cultivation, COD values were 137.19 ± 17, 128.12 ± 14, 92.01 ± 12, and 71.99 ± 16 mg/l with removal efficiencies of 72.68% ± 3.39%, 74.49% ± 2.77%, 81.68% ± 2.39%, and 85.66% ± 3.19% for initial cell densities of 5, 10, 15, and 20 mg/l, respectively. Among the four initial algal cell densities, biomass production and nutrient removal, including total nitrogen, total phosphorus, and COD, were higher for initial algal densities of 15 and 20 mg/l than for the others. There was no statistical difference between the initial algal cell density of 15 and 20 mg/l ($p < 0.05$). Therefore, the initial algal cell density of 15 mg/l was chosen for testing the temperature effect on biomass production and nutrient removal.

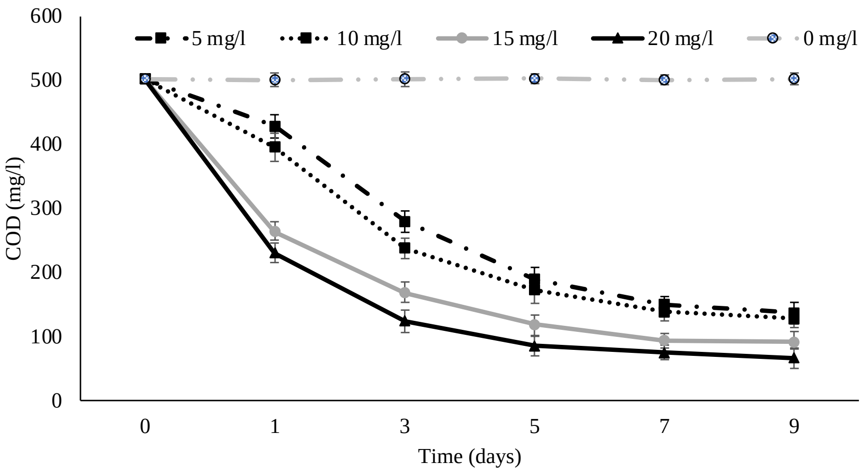

Figure 5. Reduction of COD in cooking cocoon wastewater by *Scenedesmus* sp. TN1 for different initial algal cell densities

4.2. Effect of temperature on microalgae production

Figure 6 shows the growth curves of *Scenedesmus* sp. TN1 in raw cooking cocoon wastewater at 20 °C, 25 °C, and 30 °C for the initial cell density of 15 mg/l for all experiments over 9 days. The biomass yield obtained at the different temperatures varied in a statistically significant way. Temperature had a strong influence on the biomass production of *Scenedesmus* sp. TN1. Increasing temperature enhanced biomass production over 9 days. The maximum biomass production was recorded for *Scenedesmus* sp. TN1 cultivated at 30 °C, while the lowest production was observed at 20 °C. The difference in biomass production varied significantly among the three incubation temperatures after the third day ($p < 0.05$). The highest biomass productions obtained after 9 days were 355.67 ± 17, 570.61 ± 19, and 623.14 ± 20 mg/l at 20 °C, 25 °C, and 30 °C, respectively. The growth phase slowed down after the seventh day for the test temperature of 20 °C, while algae cultivated at 25 °C and 30 °C kept growing.

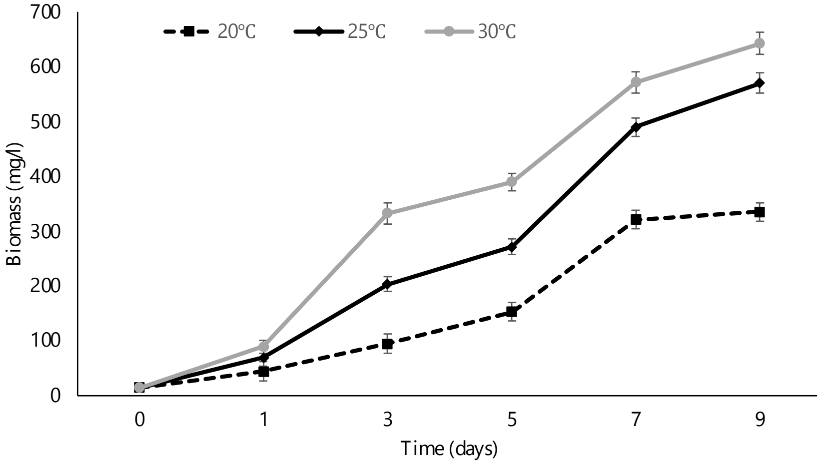

Figure 6. Growth curves of *Scenedesmus* sp. TN1 at different temperatures

The variation of total nitrogen and total phosphorus removal rates with temperature indicated that temperature is a sensitive parameter for metabolic activities involved in nutrient removal of *Scenedesmus* sp. TN1 (Figures 7 and 8). Biomass production is correlated with the T-N removal. Nitrogen removal efficiency was positively affected by temperature. After 9 days at the maximum growth rate, the maximum nitrogen removal rates achieved were 71.28% ± 2.68%, 81.31% ± 2.54%, and 88.29% ± 1.78% at 20 °C, 25 °C, and 30 °C, respectively. The removal efficiency of T-N was achieved below 35 mg/l for all incubations that meet the permissible discharge limit of Vietnam for many types of wastewater (Bộ Tài nguyên và Môi trường, 2011, Table 1, C values, column B). Phosphorus removal efficiency showed a similar pattern. Increasing biomass yield led to a rise in the total nitrogen and phosphorus removal rates. Lower temperatures had a negative impact on phosphorus removal. There were statistical differences ($p < 0.05$) among incubations in terms of both nitrogen and phosphorus removal efficiency.

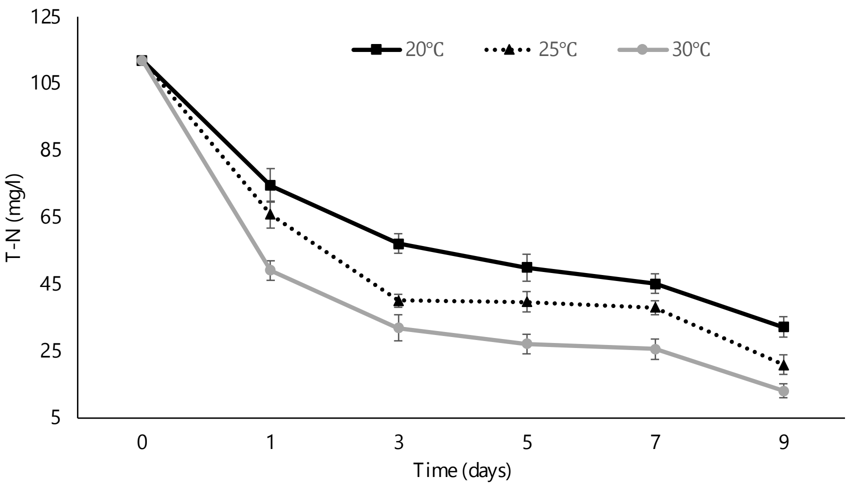

Figure 7. Removal of total nitrogen from cooking cocoon wastewater by *Scenedesmus* sp. TN1 for different cultivation temperatures

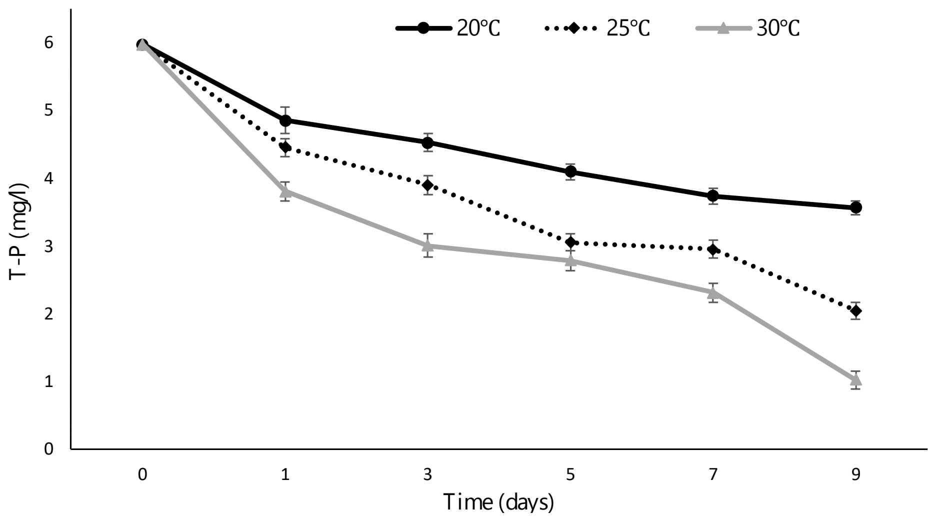

Figure 8. Removal of total phosphorous from cooking cocoon wastewater by *Scenedesmus* sp. TN1 for different cultivation temperatures

The effect of temperature on the removal of carbon by *Scenedesmus* sp. TN1 is shown in Figure 9. In the first 5 days, COD decreased dramatically. Temperature influenced COD reduction. The lowest COD values were obtained after 9 days; these were 141.34 ± 2.39, 92.01 ± 2.79, and 54.45 ± 1.83 mg/l for temperatures of 20 °C, 25 °C, and 30 °C, respectively. A slight increase in COD was observed at 30 °C on the seventh day but was not statistically different ($p < 0.05$). This observation was not detected for incubations at 20 °C and 25 °C.

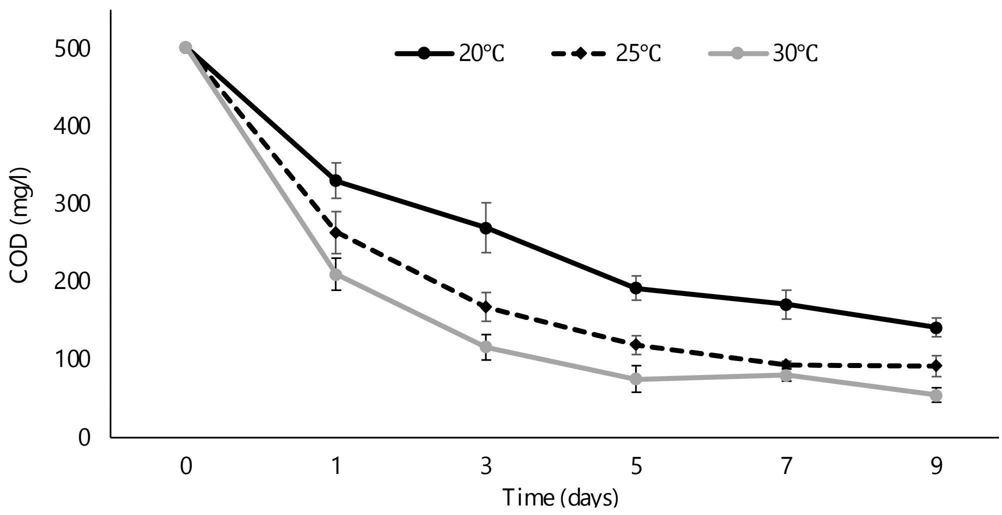

Figure 9. Reduction of COD in cooking cocoon wastewater by *Scenedesmus* sp. TN1 for different cultivation temperatures

Characteristics of wastewater before and after being treated with *Scenedesmus* sp. TN1 are presented in Table 2 for algae cultivated at 30 °C and an initial algal cell density of 15 mg/l after 9 days. Cooking cocoon wastewater was rich in nitrogen, phosphorus, and carbon sources. High levels of BOD_5 and COD were observed. The BOD_5:COD ratio was 0.64 (second column, Table 2). The percentage of BOD_5 removal was 92.01% after 9 days, and the percentage of COD removal was 89.16%. The removal efficiencies of T-N and T-P were 88.28% and 82.91%, respectively. The pH of the effluent after treatment was 8.19.

Table 2. Removal efficiency by *Scenedesmus* sp. TN1 before and after cultivation with *Scenedesmus* sp. TN1 after 9 days

Characteristic	Raw CCW	Autoclaved CCW	Treated CCW
T-N (mg/l)	112 ± 2.34	117 ± 4.65	13.12 ± 0.93
T-P (mg/l)	4.95 ± 0.52	5.13 ± 0.83	1.02 ± 0.01
BOD_5 (mg/l)	319 ± 5.95	342 ± 8.47	25.49 ± 2.72
COD (mg/l)	499.1 ± 8.39	502.17 ± 7.61	54.45 ± 9.21
pH	8.14 ± 0.16	8.15 ± 0.29	8.19 ± 0.61

Note: (CCW) cooking cocoon wastewater.

5. DISCUSSION

Microalgae are one of the potential candidates for wastewater treatment due to their ability to convert solar energy and assimilate nutrients from wastewater. *Scenedesmus* sp. TN1 was isolated in water bodies that have received wastewater from a cooking cocoon silk processing plant in Ta Nung, Lam Dong Province. The assimilation of nutrients from wastewater by microalgae to reduce the nutrient content in wastewater and then promote their growth is recorded in many previous studies (Dunn & Manoylov, 2016; Hodaifa et al., 2010; Korozi et al., 2023; Latiffi et al., 2017; Yaakob et al., 2021; Ye et al., 2020). Biomass production was also achieved during this process. The effect of the initial cell density and temperature on wastewater treatment ability has been examined for different microalgae (Abdelfattah et al., 2023; Bashir et al., 2020; Bischoff & Bold, 1963; Capar et al., 2008). The growth rate of *Scenedesmus* sp. TN1 in terms of biomass production under the influence of the initial algal cell density was recorded. Higher initial algal cell densities promoted faster growth. Less growth and nutrient removal were observed at the lower initial cell densities of 5 and 10 mg/l compared with 15 and 20 mg/l. Pham and Bui (2020) reported that *Scenedesmus* grew well in fertilizer plant wastewater, but no statistical difference in growth rate was observed among initial cell densities (10, 20, 30, 40, and 60 mg/l). Different microalgae strains and/or types of wastewater can lead to a variety of growth and nutrient removal (Pham & Bui, 2020). Excess initial cell density may enhance the rapid growth rate, while a small amount of initial cell density extends the cultivation time (Bumbak et al., 2011; Dunn & Manoylov, 2016). The length of the fermentation cycle affects the treatment efficiency of the process. Using waste mineral water from the RO column to culture *Chlorella vulgaris* algae for biomass treatment and production is ideally feasible for harvesting a high biomass yield in a short time (Benedetti et al., 2018). Additionally, there was no statistical difference between the initial algal cell density of 15 and 20 mg/l on the ninth day. This can be caused by the limitation of nutrients in the effluent.

Nitrogen and phosphorus are essential nutrients for microalgae cultivation and biomass production. Algae are able to assimilate nitrogen nutrients in various forms to synthesize proteins. Higher biomass production leads to higher nitrogen removal efficiency. The high algal cell density of *Scenedesmus* sp. TN1 assimilates more nutrients during growth, and thus the depletion rate is enhanced (Dunn & Manoylov, 2016; Xue et al., 2021). The T-N removal efficiency by *Scenedesmus* sp. TN1 achieved more than 80% after 9 days. The highest T-N removal efficiency was 95.95% after 18 days in brewery wastewater by one strain of *Scenedesmus* sp. with higher initial cell density (Benedetti et al., 2018). Another study reported 100% total nitrogen removal after 9 days with an initial total nitrogen concentration of 100 mg/l (Benedetti et al., 2018). The differences can be explained by the variety of algal strains, wastewater, and cultivation conditions. A similar pattern was observed in phosphorus removal efficiency. Phosphorus uptake efficiency of *Scenedesmus* sp. TN1 was more than 70% after 9 days for initial cell densities of 15 and 20 mg/l. The results are consistent with Pham and Bui (2020) in that higher initial cell densities led to significantly increased T-P removal rates. Carbon is an element found in microalgae biomass (Bolognesi et al., 2021; Jebali et al., 2015; Nguyen et al., 2022). Microalgae also uptake carbon sources in wastewater during their growth. A linear correlation between biomass production and nutrient removal efficiency of *Scenedesmus* sp. TN1 was observed. The COD of cooking cocoon wastewater was reduced with the higher accumulation of microalgae biomass. The selection of microalgae with high biomass production that can be used for other purposes and high nutrient removal efficiency is a crucial factor for further practical applications.

Acclimation of microalgae can enhance their efficiency in nutrient removal (Lau et al., 1996; Osundeko et al., 2014). However, every single algal strain possessed an optimum range for each cultivation condition for different purposes (Okcu, 2019; Silambarasan et al., 2023; Voltolina et al., 2005). Temperature is a critical parameter that affects algal growth, nutrient removal, lipid production, and pigment production (Delgadillo-Mirquez et al., 2016; El-Sheekh et al., 2017; Ruiz-Martínez et al., 2015). Our results indicated that an increase in temperature had a favorable effect on biomass production and nutrient removal. The optimal temperature for cultivation of *Scenedesmus* sp. TN1 is 30 °C. The optimal temperature for cultivation of *Scenedesmus acuminatus* is 27.5 °C, and the optimal cultivation temperature of *Scenedesmus maximus* is 35 °C (Bouterfas et al., 2002; Lürling, 2003). The optimal cultivation temperature for *Scenedesmus obliquus* ranges between 27.5 °C and 29.5 °C (Hodaifa et al., 2010; Lürling, 2003). Cell division and compound accumulation are influenced by temperature as well (Brown, 1951). Raising temperature promotes cell division and then increases biomass production (Isiramen et al., 2022). Metabolic processes in the cell are also affected by temperature (Yahya et al., 2020).

The nutrient removal by *Scenedesmus* sp. TN1 was observed at a low temperature of 20 °C. Nutrient removal rates at 20 °C were 71.26%, 65.82%, and 71.85% for nitrogen, phosphorus, and COD, respectively. The removal of nitrogen, phosphorus, and COD achieved the highest rate at a cultivation temperature of 30 °C (Figures 7, 8, and 9). This can be explained in that the microenvironment for the enzymes involved in photosynthesis and metabolism is accelerated when temperature increases (Bolognesi et al., 2021; Wang et al., 2018). Additionally, the pH of the cultivation medium falls with lower temperature due to the solubility of carbon dioxide (< 20 °C) and inhibits microalgal growth (G. Li et al., 2023). An increase in COD was observed at the cultivation temperature of 30 °C on the seventh day. However, this phenomenon was not recorded at 20 °C or 25 °C. Algae can release organic compounds under stress conditions to transfer stress information to other cells. This process can help algal cells prepare for stresses such as light, temperature, nutrition conditions, and abiotic stresses (Bonsang et al., 2010; Bouterfas et al., 2002; Xu et al., 2017; Ye et al., 2018; Zuo et al., 2012). A temperature of 30 °C could be the upper temperature limit of *Scenedesmus* sp. TN1.

Nitrogen, phosphorus, and carbon sources in wastewater are essential nutrients (Lima et al., 2019). Therefore, microalgae can be cultivated in the wastewater as tertiary treatments to enhance nutrient removal (Cuellar-Bermudez et al., 2017). The T-N, T-P, BOD_5, and COD in cooking cocoon wastewater decreased after cultivating *Scenedesmus* sp. TN1 for 9 days (Table 2). Cooking cocoon wastewater can be treated by biological approaches (BOD_5:COD of 0.66) (Cossu et al., 2017). The relationship between BOD_5 and COD after 9 days of cultivation indicated that *Scenedesmus* sp. TN1 can grow and assimilate nutrients in this wastewater, but some compounds could not be consumed by algae in 9 days. Therefore, an amount of these nutrients still remained. These results agree with previous studies on the removal of nutrients by *Scenedesmus* strains (Wong et al., 2015; Yirgu et al., 2020). COD reduction rates of 71.85%, 81.68%, and 89.16% were obtained after 9 days for the initial algal density of 15 mg/l at 20 °C, 25 °C, and 30 °C, respectively. A COD reduction rate of 78.4% after 10 days for an initial cell density of 20 mg/l at 27 °C ± 1 °C was reported for other *Scenedesmus* sp. in fertilizer plant wastewater (Pham & Bui, 2020). While the initial COD of cooking wastewater is 499.1 mg/l compared to the COD of 180 mg/l in fertilizer plant wastewater, *Scenedesmus* sp. TN1 may be considered to have a high capacity of COD uptake of algal strains in cooking cocoon wastewater. The BOD_5 reduction rate achieved after 9 days was 92%. This result indicated that a large amount of biodegraded carbon sources in cooking cocoon wastewater was taken up by *Scenedesmus* sp. TN1. The pH range of 6–8.5 is indicated as the optimum pH for *Scenedesmu*s strain growth (Bakuei et al., 2015; Zhang et al., 2019). The pH value of 8.19 after 9 days of cultivation did not differ statistically compared with the pH of the original effluent. *Scenedesmus* sp. TN1 did not alter the pH of cooking cocoon wastewater during growth.

6. CONCLUSIONS

The ability of cultivation and nutrient removal of a local algal isolate, *Scenedesmus* sp. TN1, in cooking cocoon wastewater has been examined. Different initial algal cell densities of 5, 10, 15, and 20 mg/l were tested to determine the optimum initial cell density of *Scenedesmus* sp. TN1 for biomass production and nutrient removal. An increase in the initial algal cell density resulted in an increase in biomass production. Nutrient removal efficiency was also improved along with increased biomass production.

However, there was no statistical difference between the 15 and 20 mg/l cell densities; therefore, 15 mg/l was used to study the effect of temperature. The effects of temperature on microalgae growth in terms of biomass production, total nitrogen, total phosphorus, and COD removal in the effluent were investigated from 20 °C to 30 °C. The maximum biomass was obtained at a temperature of 30 °C, even though this alga could be cultivated from 20 °C to 25 °C. *Scenedesmus* sp. TN1 assimilated nutrients from the cooking cocoon wastewater and produced a high biomass yield after 9 days. This alga exhibited nutrient removal efficiency at 30 °C of 88.28%, 82.91%, 92.01%, and 89.16% for T-N, T-P, BOD_5, and COD, respectively. However, at 30 °C, an increase in COD was observed on the seventh day. High nutrient and COD removal efficiency indicated that *Scenedesmus* sp. TN1 is a promising candidate for nutrient removal and biomass production in cooking cocoon wastewater treatment.

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