Enhanced Growth Performance Of Microalgal Biomass For Lipid Production

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Abstract

The supply of petroleum-based fuel decreases, and the need to produce viable renewable fuel increases. In the wake of the uprising global energy crisis, microalgae serve as a promising feedstock for oil production. The researcher gained interest due to its hitting-two birds-with-one-stone approach as a potential solution for the continuously depleting source of oil reservoir worldwide and to reduce carbon dioxide emission into the atmosphere using microalgae.

The potential of the strains (*Chlorella vulgaris*, *Monoraphidium* sp., *Scenedesmus obliquus*, *Desmodesmus denticulatus*, and *Chlorophyta* sp.) in the production of lipid was assessed in this study. Furthermore, optimization of the growth conditions of each microalgae strain were identified to enhance biomass production and lipid accumulation. Two microalgae strains namely *Monoraphidium* sp. and *Desmodesmus denticulatus* exhibited higher lipid productivity compared to other microalgae strains on biomass and lipid content. The following optimized growth conditions that support algal growth are as follows: pH 5, 0.5 moles in salinity level, outside temperature, and sunlight. The production of lipids is dependent on the microalgae strain, including the optimum condition that supports its biomass production.

Introduction

Sources of petroleum-derived fuel have become limited due to the rapid industrialization and population growth which threatens governments and organizations across the world. Approximately, 21.3 billion tons per year and 2.4 million pounds per second of carbon dioxide (CO_2) is being emitted into the atmosphere resulting from the burning of fossil fuels causing adverse effects for humans and earth as a whole (National Academies Report and Earth Institute, 2017).

The Philippines is one of the major producers of first-generation feedstock namely food crops like corn, soybean, rapeseed, sunflower, and palm oil for biofuel production. Globally, second-generation biofuels are derived from non-edible feedstock like *Jatropha*, *Miscanthus*, switchgrass, and other organic wastes. However, the increasing demand for edible feedstock as food sources and their necessity posted another concern because these demands bigger areas of arable land for its propagation and be developed as both first and second-generation biofuel sources (Chen, 1994).

These prompted researchers to find ways in converting the lignocellulosic material to produce oil from cellulosic feedstock. This is expensive because of lignin and hemicellulose contents which reduce the surface area for enzyme access thus production cost is very high. Its removal needs high and complex technology which makes it impractical and unfeasible, while in the case of microalgae, the absences of lignin and hemicellulose contents make it the best cellulosic feedstock for oil production (Gao & Rehman, 2016).

As a response, this study enhanced the growth performance and evaluated the capability of microalgae strains namely, *Chlorella vulgaris, Monoraphidium* sp., *Scenedesmus obliquus, Desmodesmus*

denticulatus, and *Chlorophyta* sp. in producing lipids which could potentially provide an economically-feasible solution for the continuously depleting source of oil reservoir worldwide. Microalgae derived biofuels are expected to produce less CO_2 emission as it can utilize carbon dioxide during its photosynthetic phase.

Results And Discussion

To determine the optimal conditions that support maximum growth, the microalgae strains were exposed to different ranges of optimal parameters such as pH level, salinity level, light intensity, and temperature. Harvesting and weighing of microalgae biomass were also evaluated to determine its relationship with lipid production.

Microalgae Strains

Chlorella vulgaris, Monoraphidium sp., *Scenedesmus obliquus, Desmodesmus denticulatus, Chlorophyta* sp. are the microalgae strains isolated and collected from different body of water and selected for further evaluation based on consistent growth (Thao et al., 2017) (see Fig. 1). The different microalgae were from the germplasm collection of the Biotechnology and Analytical Laboratory of the Central Luzon State University.

Optimal pH level for growth

Different pH levels of the growth medium of microalgae strains were assessed to determine the best condition that supports microalgae growth based on the absorbance value of the samples (see Fig. 2).

Different pH levels of the growth medium of the microalgae strains were assessed to determine the best condition that supports microalgae growth based on the absorbance value of the samples. According to Hirooka et al., 2014, microalgae are reported to grow in highly acidic environments. Contamination of lethal fungus is inhibited in acidic range of pH in growth media (Hwang et al., 2019). Thus, it can be seen in Fig. 2 that pH level 5 supports higher algal growth of the following strains: *Monoraphidium* sp., *Scenedesmus obliquus*, and *Chlorophyta* sp. similar to the previous works of Hirooka et al., 2014 and Bogen et al., 2013. However, there are microalgae species which have their optimal pH and salinity wherein cells are harmed when it is severely above or below the optimal level. *Chlorella vulgaris* has shown enhanced growth in a neutral, pH 7 level while *Desmodesmus denticulatus* preferred the unadjusted pH of control media. According to Tham et al., (2019), *Chlorella vulgaris* can adapt in a wide range of pH, from 4 to 10 with the highest biomass productivity obtained at pH 9 to 10.

Optimal light intensity for Growth

The growth of microalgae and lipid production was assessed by exposing the samples to different light intensities such as 100 flux, 200 flux, 300 flux, 400 flux, and 500 flux (Fig. 3). The light conditions affect directly the growth and photosynthesis of microalgae through the duration of exposure with different

intensities. It is showed that the light intensity of 400 flux is the optimal light intensity that supports the growth of the microalgae strain *Chlorella vulgaris* and the control variable for microalgae strains *Monoraphidium* sp., *Scenedesmus obliquus*, *Desmodesmus denticulatus*, *Chlorophyta* sp., as it recorded the highest absorbance value. Figure 3 showed that having sunlight as the light source exhibited high biomass production in comparison to artificial light. Moreover, there were recommendations based on findings that alternation of light and dark periods is essential for photosynthesis in microalgae as the adenosine-5'-triphosphate (ATP) synthesis and nicotinamide adenine dinucleotide phosphate (NADPH) requires light to activate the dark reaction process to produce carbon skeletons (Cheirsilp & Torpee, 2012).

Optimal Temperature Condition for Growth

Assessment of the temperature condition favors microalgae growth and lipid accumulation (Fig. 4).

It is presented that room temperature (20–25ºC) is the optimal condition that supports the growth of the microalgae strains *Scenedesmus obliquus*, *Desmodesmus denticulatus*, and Chlorophyta sp. related to the study of Wang et al.,(2012); Vanags et al., (2015). Meanwhile, outside temperature (30–35ºC) is the optimal temperature that supports the growth of the microalgae strain *Chlorella vulgaris* and *Monoraphidium* sp. similar to the previous works stated by Converti et al., (2009) and Powell et al., (2008).

Optimal Salinity level for Growth

As presented in Fig. 5, 1.1 molars (M) is the optimal salinity level that supports the growth of the microalgae strain *Chlorophyta* sp., but as well as in 0.5 molar, similar to *Chlorella vulgaris*, *Monoraphidium* sp., *Scenedesmus obliquus* and *Desmodesmus denticulatus*. In a study of Pancha et al., (2015) on the effect of salinity to *Scenedesmus* sp., it was stated that the physiological and biochemical composition of microalgae is affected by salinity stress. It emphasized the importance of oxidative stress for neutral lipid accumulation and a two stage salinity stress is suggested as best approach for biomass and lipid production.

Harvested Biomass and Lipids Extracted

Optimized conditions for growth of each strain was consolidated in 3 liters of growth media (see Table 1). The microalgae biomass was harvested through centrifugation which is used in separating microalgae from their growth medium. Table 2 shows that after cultivating the microalgae strains in their optimized growth conditions, *Chlorophyta* sp. has the highest produced wet biomass of 601.54 grams per 3 liters of the optimized growth media and *Chlorella vulgaris* being the lowest with 456.97 grams per 3 liters. Biomass production and lipid quantification vary widely in the strains. Furthermore, *Monoraphidium* sp. produced 56. 965 milliliters of lipid and *Desmodesmus denticulatus* produced 77.624 mL as shown in Table 2. The weight of the extracted lipid was measured through the gravimetric method which consists of the lipid extraction using solvents, and lipid quantification done by recording the weight of extracted lipids after evaporating the extracting solvents. In a study of Massimi and

Kirkwood (2016), *Scenedesmus* sp. and *Desmodesmus* sp. can take over 13 hours to double up, faster than other microalgae that generally doubled every 24 hours.

Table 1
Summary of Optimum Environmental Growth Conditions and Cell Count (at 680 nm absorbance value) of Microalgae Strains

Microalgae	Salinity	pH level	Light intensity	Temperature
Chlorella vulgaris	0.5 mole (M)	7	400	Outside Temperature
Monoraphidium sp.	0.5 mole (M)	5	Control	Outside Temperature
Scenedesmus obliquus	0.5 mole (M)	5	Control	Room Temperature
Desmodesmus denticulatus	0.5 mole (M)	Control	Control	Room Temperature
Chlorophyta sp.	1.1 mole (M)	5	Control	Room Temperature

Table 2
Summary of the amount of produced biomass and lipid extracted from the microalgae strains.

Microalgae	Biomass (g)	Lipids (ml)
Chlorella vulgaris	456.97	0.00
Monoraphidium sp.	513.33	56.97
Scenedesmus obliquus	544.96	0.00
Desmodesmus denticulatus	533.96	77.62
Chlorophyta sp.	601.54	0.00

Validation of the presence of lipids

In this study, the emulsion test was done to determine the presence of lipids in the sample. Lipids are insoluble in water and soluble in ethanol. After lipids have been dissolved in ethanol and then added to H2O, they formed tiny dispersed droplets in the water, which is called an emulsion. These droplets scatter light as it passes through the water so, it appears white and cloudy. Microalgae strains *Monoraphidium* sp. (56.965 mL of extracted lipids per 513.33 grams of microalgae biomass) and *Desmodesmus denticulatus* (77.624 mL of extracted lipids per 533.96 grams of microalgae biomass) gained positive results after performing the emulsification test. Two (2) among the five isolated microalgae strains exhibited positive results regarding lipid accumulation. *Monoraphidium* sp. produced 56.965 mL of lipid from the harvested 513.33 grams and *Desmodesmus denticulatus* produced 77.624 mL of lipid from the harvested 533.96 grams of wet algae biomass.

Conclusions

Variation of pH level affects microalgae growth including its photosynthetic ability. Also, salinity and temperature are the two major factors vital for microalgae growth. Different salinity levels affect biochemical and physiological mechanisms such as lipid production and growth of microalgae. It also uses light to respond to their environment. In outdoor cultures, sunlight provides energy for these organisms. Controlled growing systems using artificial light with varying intensities have been taken into consideration in this study for the optimization of growth conditions. Therefore, enhancement of the growth performance of the collected microalgae strains namely: *Chlorella vulgaris*, *Monoraphidium* sp., *Scenedesmus obliquus*, *Desmodesmus denticulatus*, and *Chlorophyta* sp. was done by cultivating the strains in different conditions of pH level, salinity level, light intensity, the temperature necessary for microalgae growth to identify which among these best supports biomass production.

The assessment of the relationship between the amounts of the harvested wet microalgae biomass (in grams) and the amount of extracted lipids (in mL) was also performed in this study. Biomass production and lipid accumulation vary widely in the strains.

The study proved that microalgae are one of the most promising feedstocks for the production of lipids. Two microalgae strains namely *Monoraphidium* sp. and *Desmodesmus denticulatus* were determined to contain a high amount of lipid among the five. The production of lipids is dependent on the microalgae strain, including the optimum condition that supports its growth.

Methdology

Preparation of Microalgae strains

Microalgae strains used in this study were obtained from the germplasm collection of the Biotechnology and Analytical Laboratory, Department of Biological Sciences, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines. The purified samples of the microalgae were morphologically confirmed using a Compound Light Microscope under High Power Objective at 100x magnification. The identification of the sample was done using characteristic features in reference to its previous identity confirmed using molecular identification through DNA sequencing. An aliquot of the samples was mounted into microscope slides and viewed under the microscope. Pure culture method was done by isolating microalgae with specific morphologies using pipette tips and inoculated into a new bottle of sterile nutritious water.

Preparation of Materials and Culture Media

Three hundred fifteen (315) empty plastic bottles of 500 milliliters (mL) capacity was collected and sterilized with commercial dishwashing liquid and power sprayed. The Nitrogen-Phosphorus-Potassium (NPK) media were prepared using the ratio of 21:21:21 having N = 0.07 g/L, P = 0.70 g/L, K = 1.33 g/L. One hundred milliliters (10 mL) of the microalgae strain was transferred to a 500 ml plastic bottle containing 90 mL of NPK media.

Optimization of Environmental Growth Conditions

To enhance the biomass production of the microalgae strains, assessment of the optimal condition that affects maximum growth and lipid accumulation of the algae strains was done, considering the different factors such as pH level, light intensity, salinity level, and temperature. Each treatment for the following experiments were conducted in triplicates. To minimize the effect of environmental heterogenicity of natural conditions, such as daylight, the bottles for each factor (e.g. light intensity) were randomly distributed in the experimental plot. The schematic diagram for the optimization of the microalgae environmental growth condition to enhance its lipid production was provided in Fig. 6.

pH level

The algal cultures were grown in N-P-K growth media with varying pH levels of 5, 6, 7, 8, and 9 (Nigam *et al.*, 2015). The pH value of each sample was adjusted by adding 1M Sodium hydroxide (NaOH) and 1M Hydrochloric acid (HCl). A variation on pH level affects algae growth including its photosynthetic ability, therefore, determination of the optimal pH level that supports the microalgae growth was conducted.

Light intensity

The microalgae samples were grown under different conditions of light intensity ranging from 100 to 500 fluxes to determine the optimal light amount that enhances algal growth. A 9 watts LED light tube was used as an artificial light source. This was covered by different layers of cellophane paper to adjust the light intensity with the aid of the Lux Meter Application for accurate flux lighting measurement.

Temperature

Temperature is another important factor in the growth of microalgae affecting biochemical processes such as photosynthesis. Each species has its optimal growth temperature. Room temperature, outside temperature, and air-conditioned room temperature was considered as ranges in the study to assess which temperature certainly affects the growth of the algal strains (Khan et al., 2018).

Salinity

The varying concentrations of Sodium chloride (NaCl) and distilled water was prepared to determine different salinity levels (0M, 0.2M, 0.5M, 0.8M, 1.1M) based on procedures of Nigam *et al.*, (2015).

Determination of Algal Growth

One hundred microliters (100 µL) of each replicate of the microalgae strains exposed at different pH levels, light intensity, salinity level, and temperature was transferred into a 96-well plate. For each analyzed microalgae species, the maximum absorbance values were examined by scanning sample cultures at the same wavelength, 680 nanometers (nm) using Ultraviolet-Visible Spectroscopy (UV-VIS) (Havlik et al., 2013).

Harvesting of Microalgae biomass and Quantification of Lipid Content

Each microalgae strains were grown in 3 liters of growth media treated with the optimized conditions for maximum growth for 3 weeks. Forty milliliters (40 mL) from each microalgae strain container was transferred in 50 ml conical tubes. Tubes were centrifuged at 4000 rpm for 15 minutes, the microalgae cells were combined in one tube (for each strain) and then re-suspended in 1 mL distilled water. The sample was mixed with 2.5 mL chloroform and 1.25 mL methanol (2:1, v/ v) and separation of the chloroform and aqueous methanol layers occurred. A 1.25 mL of water was added, and the chloroform layer at the bottom part of the conical tube was removed using a pipettor. A second extraction was performed by adding 2.5 mL chloroform and mixed using a vortex. The chloroform portion was collected and washed with 5 mL of 5% NaCl solution and was evaporated on a hot plate at 50 °C. The weight of the lipid that was obtained from each sample was measured using an analytical balance (Bligh and Dyer, 1959).

Presence of Lipids Verification

Emulsion test was applied wherein ethanol (C_2H_6O) was added to the microalgae sample allowing lipids to dissolve. Two droplets of extracted oil were put into a conical tube and 2 mL of 96% ethanol was added. The solution was shaken thoroughly for about a minute then 2 ml of distilled water was added. A layer of cloudy white suspension was seen at the top of the solution as the lipids became more noticeable that appeared after being added by distilled water (Kumar et al., 2006).

Declarations

Ethics Approval and Consent to Participate-Not applicable

Consent for Publication-Not applicable

Availability of data and materials

The datasets generated and/or analyzed during the current study are not publicly available due to the memorandum of agreement signed by each of the author's institutions but are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

Funding

There was no funding received for the conduct of this study.

Authors' contributions

YFNP, CAPSM, and their group performed the collection and preparation of materials, optimization of environmental growth conditions and the harvesting, extraction, quantification and verification of lipids

from microalgae. The morphological and molecular identification of the strains of microalgae used in this research was conducted by RMMP, JDCA and JRU. The latter also served as the supervisors of the group of YFNP and CAPSM in conducting each experiment.

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Figures

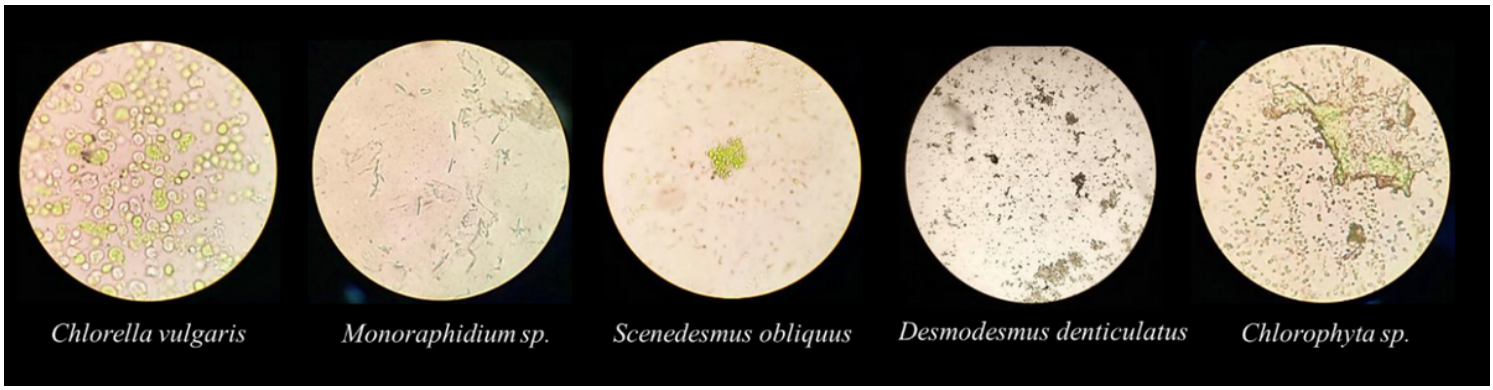

Figure 1

Microalgae strains from the water samples viewed in microscope under HPO at 100x magnification

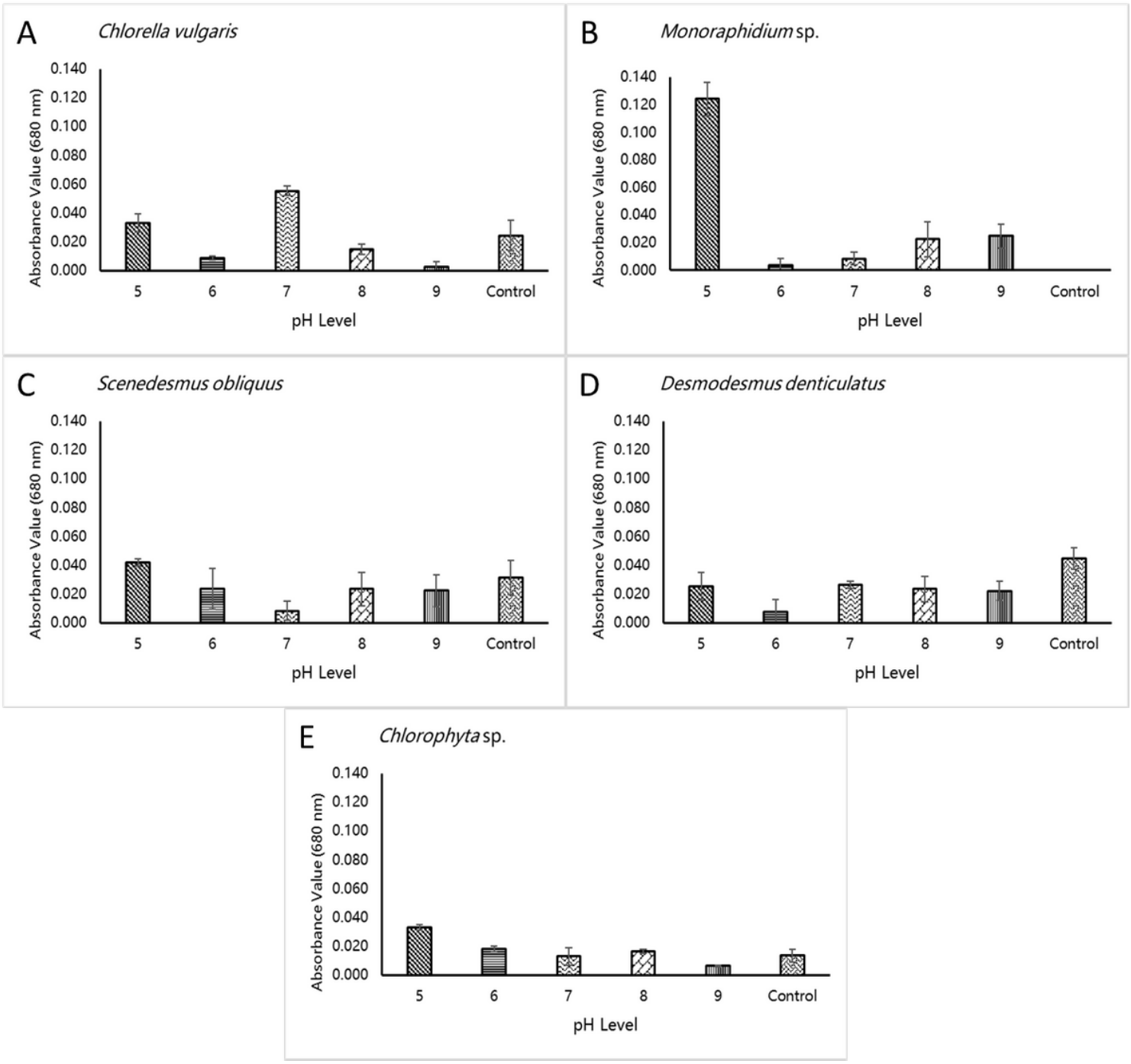

Figure 2

Absorbance values (680 nm) of Chlorella vulgaris (A), Monoraphidium sp. (B), Scenedesmus obliquus (C), Desmodesmus denticulatus (D) and Chlorophyta sp. (E) in the optimization of pH Level of culture media.

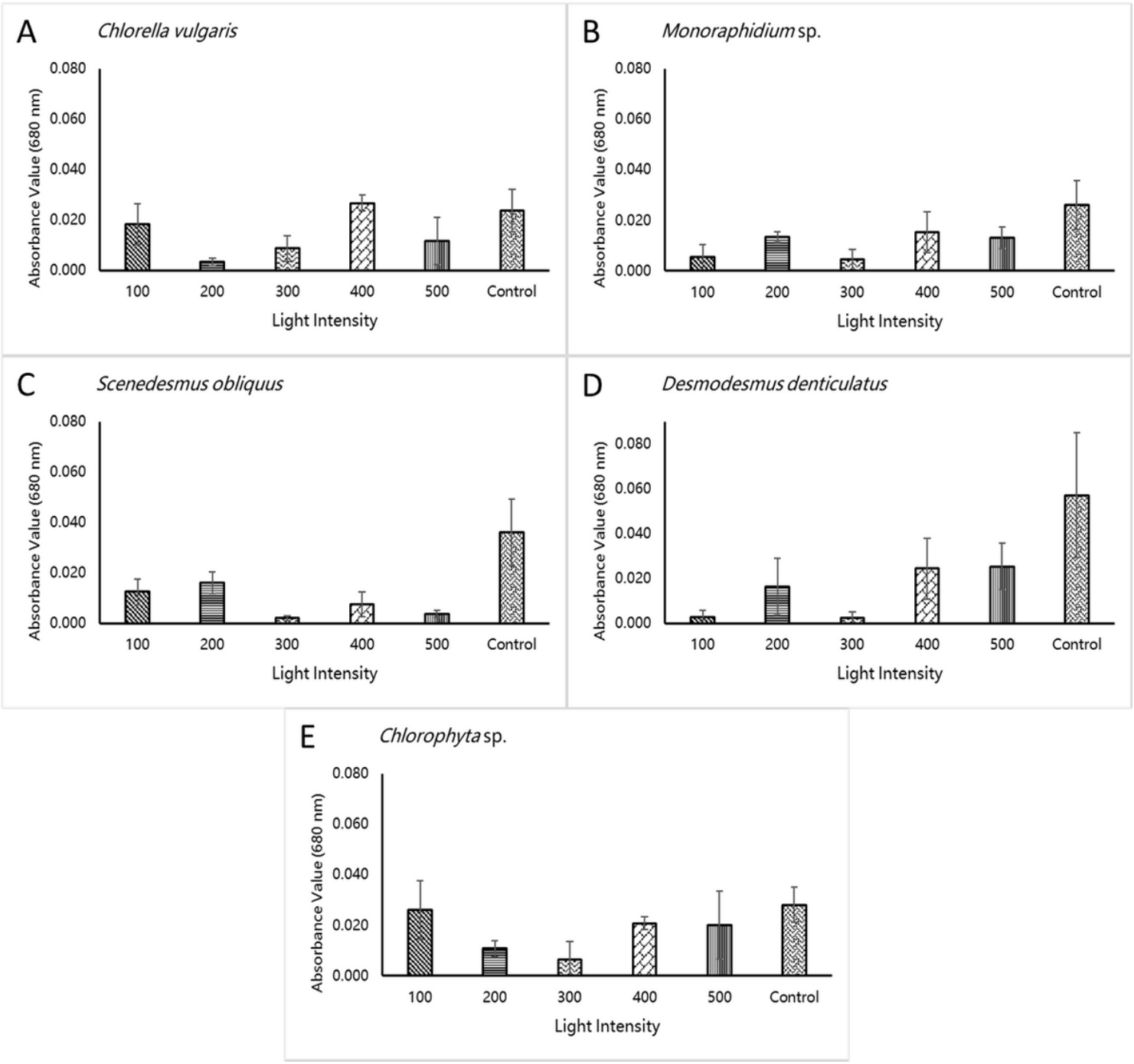

Figure 3

Absorbance values (680 nm) of Chlorella vulgaris (A), Monoraphidium sp. (B), Scenedesmus obliquus (C), Desmodesmus denticulatus (D) and Chlorophyta sp. (E) in the optimization of light intensity.

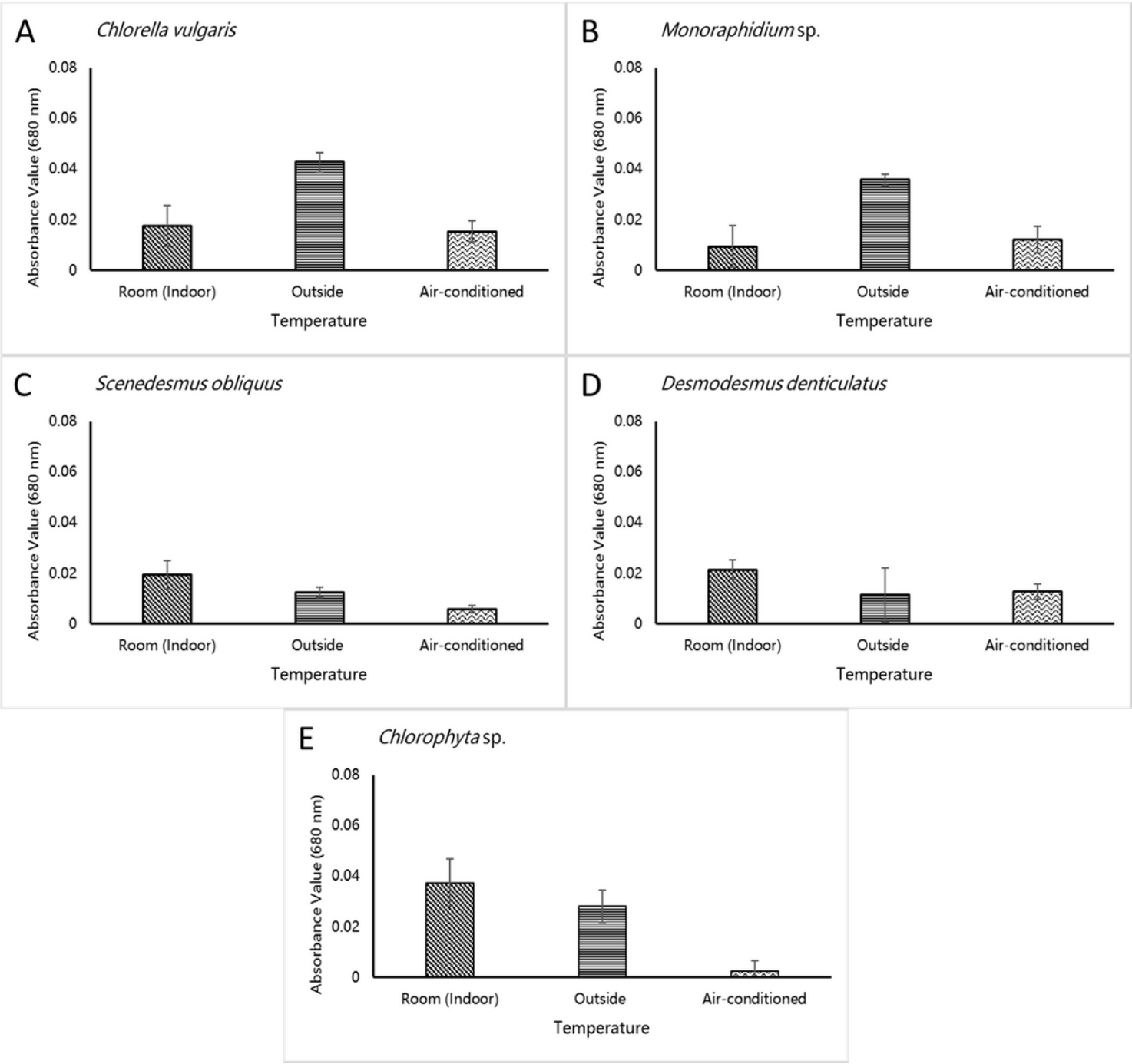

Figure 4

Absorbance values (680 nm) of Chlorella vulgaris (A), Monoraphidium sp. (B), Scenedesmus obliquus (C), Desmodesmus denticulatus (D) and Chlorophyta sp. (E) in the optimization of temperature.

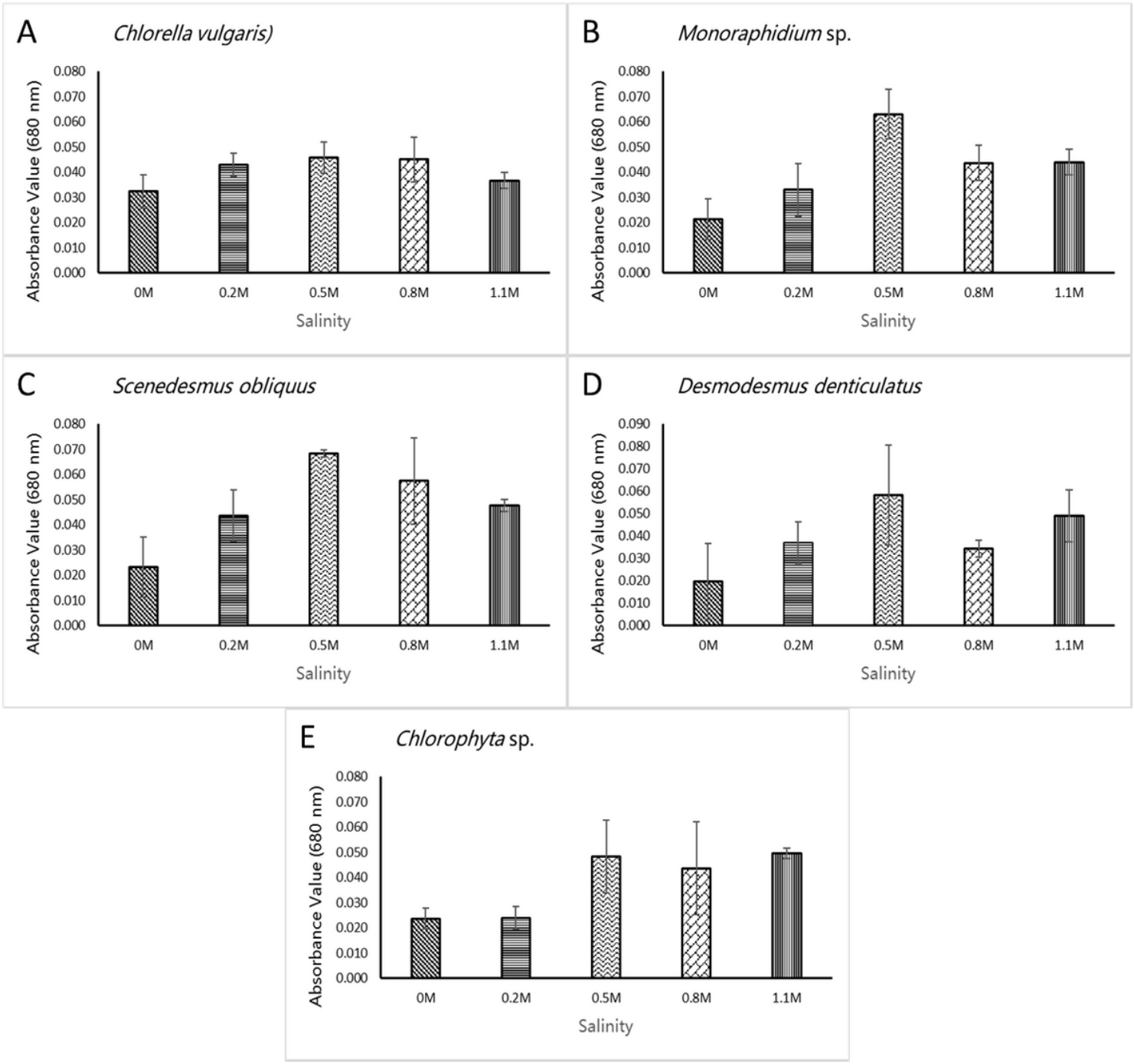

Figure 5

Absorbance values (680 nm) of Chlorella vulgaris (A), Monoraphidium sp. (B), Scenedesmus obliquus (C), Desmodesmus denticulatus (D) and Chlorophyta sp. (E) in the optimization of salinity.

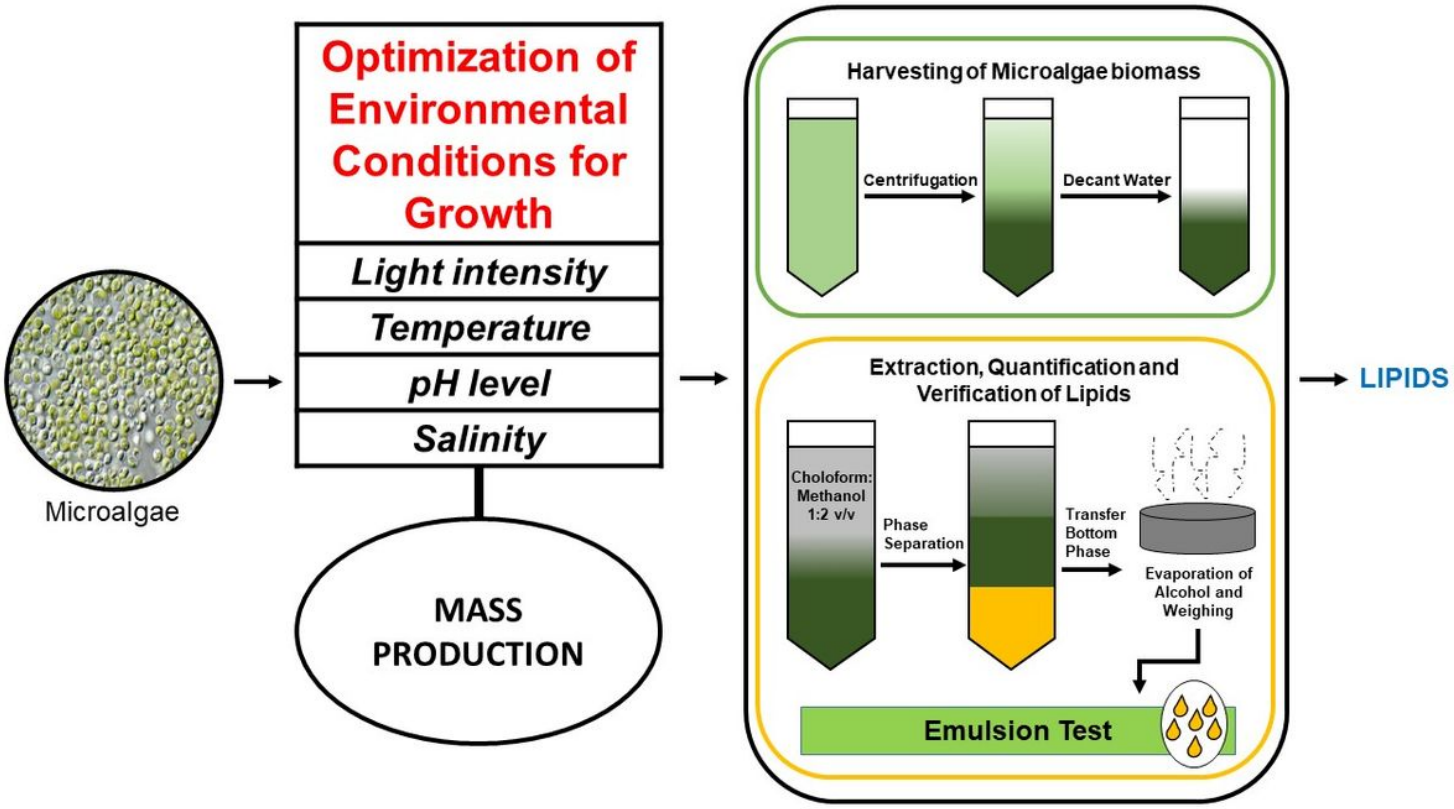

Figure 6

Schematic diagram used in this study to optimize the microalgae environmental growth condition such as light intensity, temperature, pH level and salinity in relation to lipid production.

Supplementary Files

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