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Fourier Transform Infrared Spectrometry Detection of *Phaeodactylum tricornutum* Biomacromolecules in Response to Environmental Changes

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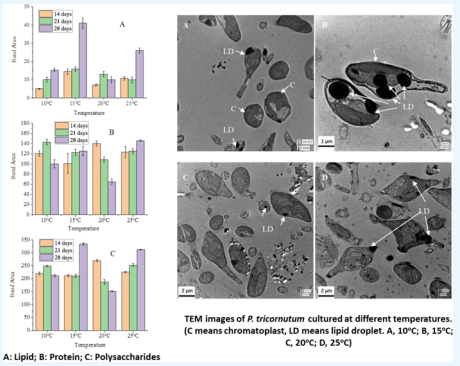
ABSTRACT: Environmental factors play an important role in the lipid, protein, and carbohydrate compositions of microalgae, wherein temperature and light are key influencing factors. Fourier transform infrared (FTIR) spectrometry was used in this study to detect biomacromolecules in *Phaeodactylum tricornutum* cells under different temperatures (10, 15, 20, and 25 °C) and different illumination conditions (1000, 2000, 3000, and 4000 lx) to study the corresponding changes in lipid, protein, and carbohydrate contents. Results indicate that the biomacromolecule content at different temperatures has different patterns. Specifically, the patterns at 15 and 25 °C are similar to each other and the contents accumulate with extended culture time. However, the pattern at 20 °C is different. The carbohydrate and protein contents peaked during the early stage of the exponential phase, whereas lipid accumulation lagged behind the former two, peaking during the middle of the culture stage and then decreasing. Lipid content was analyzed by transmission electron microscopy (TEM), which revealed that the highest lipid content was observed at 15 °C. Results also show that all of the lipid, protein, and carbohydrate contents in cells were the highest when the illumination was at 2000 lx and that the contents decreased with increasing illumination. By using FTIR, less samples were needed as compared to the traditional chemical quantitative detection methods. Moreover, the relative content changes of various biomacromolecules during the growth of *P. tricornutum* could be accurately determined by a single detection, thereby providing a new technique for the further study of metabolic mechanisms.



1. INTRODUCTION

Microalgae has the potential to produce high-value products, such as carbohydrates, proteins, unsaturated fatty acids, and pigments (i.e., chlorophyll and carotenoid). Furthermore, in the last two decades, it has been recognized as an important and promising biofuel feedstock.[1,2] *Phaeodactylum tricornutum* is a kind of diatom that has been widely studied and reported as a model diatom with the potential to produce large amounts of biomass.[3,4] It is rich in proteins, polysaccharides, polyunsaturated fatty acids, and other active substances and is often used in aquaculture as an important food. It is also an important source of neutral lipids and eicosapentaenoic acid.[5,6] External environmental conditions can cause changes in the biochemical composition of algal cells, which may result in the variable production of high-value biomacromolecules; thus, studying changes in the *P. tricornutum* biomacromolecule composition under different temperatures and illumination conditions could provide basic data for large-scale culture techniques of *P. tricornutum* in regions at different latitudes, as well as lending insight into the production and utilization of its biomacromolecules.

Microalgal polysaccharide is a potential medicinal resource, which has biological effects of antitumor, antivirus, lowering blood lipid, and regulating the immune response of the body.[7,8] Microalgae lipids are rich in nutrients necessary for the growth of aquatic economic animals and are widely used in aquaculture; they are also a kind of bioactive substances with a high nutritional value and health-care functions for human beings. At the same time, lipids in microalgae are of high value; it has been studied as a promising feedstock for biodiesel's production.[9−11] The protein in microalgae can be used in food formulation to make products with nutritional value and therapeutic function, such as fortified food, nutritional milkshakes, and sports drinks.[12,13]

At present, chemical quantitative detection methods are mainly applied in the analysis of biomacromolecules in microalgae cells. For instance, carbohydrates are determined by the anthrone method,[14] lipids by the chloroform-methanol extraction method,[15] and proteins by the Coomassie brilliant blue method.[16] All of these aforementioned methods require large sample quantities, and each indicator needs to be tested

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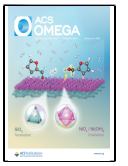

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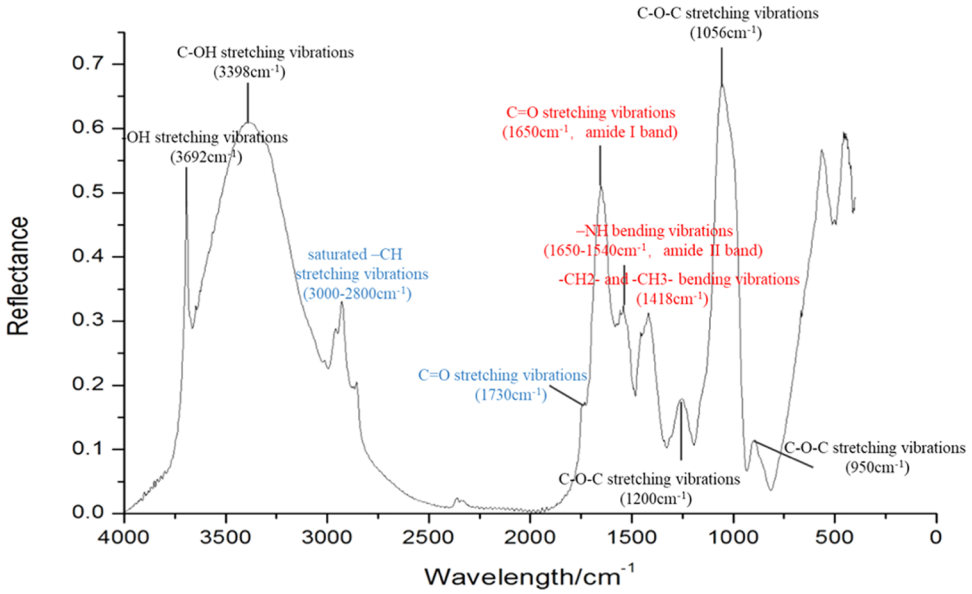

Figure 1. Mean infrared spectra of *P. tricornutum* (the different font colors indicate different molecular groups: black, polysaccharide; blue, lipid; red, protein).

separately. Fourier transform infrared (FTIR) spectrometry is an analytical technology based on the vibrations of polar bonds and functional groups within compounds and is mainly applied to the analysis of the molecular structures of organic compounds.[17] Biomacromolecules, such as proteins, carbohydrates, nucleic acids, and lipids, have their own unique molecular vibration patterns and characteristic functional groups known as vibrational fingerprints.[18] Based on differences in the characteristic infrared absorptions and intensities in organic molecules, such as C−H, N−H, O−H, and other hydrogen-containing groups, different macromolecular components in biological cells can be qualitatively and quantitatively analyzed.[19,20] Some studies have used FTIR technology to identify compositional changes in plant cells under low-phosphorus,[21,22] low-nitrogen,[23] and other nutritional stress conditions. Dean et al.[24] used FTIR to show that under nitrogen-deficient conditions, the carbohydrate and lipid contents in freshwater algae underwent significant changes as treatment time progressed. In this study, FTIR and transmission electron microscopy (TEM) were used to explore the dynamic changes in the biomacromolecular content of *P. tricornutum* under different temperature and light conditions that were investigated. Results show that biomacromolecules in microalgae can be determined quickly through FTIR, thereby rapidly elucidating their physiological condition, which provides a basis for further research into the application and development of microalgae technologies.

2. MATERIALS AND METHODS

2.1. Algal Species and Culture Conditions. The algal strain used in this experiment, *P. tricornutum* (CCMM2004), was obtained from the algal species group at the Institute of Oceanology, Chinese Academy of Sciences. To test the effects of temperature on the biomacromolecule content, cultures were incubated at 10, 15, 20, and 25 °C. The illumination intensity was fixed at 4000 lx, and the light−dark cycle was 12 h:12 h. Cultures were prepared by adding L1 culture medium (2 L), with an initial inoculation density of 9×10^7 cells/L, into 3 L conical flasks.[25] Samples were collected on days 14, 21, and 28 for FTIR determination; samples collected on day 28 were also used for TEM analysis.

To test the effects of illumination intensity on the biomacromolecule content, cultures were exposed to 1000, 2000, 3000, and 4000 lx. The incubation temperature was fixed at 20 °C, and the light−dark cycle was 12 h:12 h. Cultures were prepared by adding L1 culture medium (600 mL), with an initial inoculation density of 9×10^7 cells/L, into 1 L conical flasks.[25] Samples were collected on day 28 for FTIR determination. Cultures were performed in the light incubator (GXZ-280C-CO_2-LED), and all experiments were done in triplicate.

2.2. Sample Preparation and Fourier Transform Infrared Spectrometry. Algal cultures (600 mL) were subjected to centrifugation at 3500 rpm for 10 min; the supernatant was discarded and algae were collected. After lyophilization, algae were ground to a powder, breaking the cell wall, in liquid nitrogen. The broken cells (1 mg) were placed in an agate mortar with potassium bromide (150 mg) and further ground for 3−5 min to fully mix the samples. The grindate was transferred to a tablet mold and spread out using a small flat stainless steel spade. A tablet press was used to apply pressure (10 MPa) on the mold for a strictly controlled time of 2 min. The pressed tablet was removed and placed into a Nicolet iS10 FTIR instrument for analysis. The scan was conducted 36 times, with a scanning wave scope of 4000−500 cm^{-1} and a spectral resolution of 6 cm^{-1}.

2.3. Relative Content Analysis. The relative absorption peak areas of biological macromolecules under different culture conditions were dissimilar. The contents of three bioactive substances were quantified by the peak area during their wavelength range. The absorption peak area of lipid at a wavenumber of 1730 cm^{-1} represents its relative content. The protein relative content could be observed from the peak area at a wavelength of 1418 cm^{-1} (C═N) and 1650 cm^{-1} (C═O). The polysaccharide relative content in *P. tricornutum* was mainly determined by combining the peak heights of the C−O−C absorption (1200−950 cm^{-1}) and the stretching vibrations of the O−H groups (near 3398 cm^{-1}). Omnic 8.0

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software was used to process spectral data and integrate the peak area.

2.4. Transmission Electron Microscopy Sample Preparation. *P. tricornutum* cells from the algal solution (10 mL) were collected after centrifugation at 3500 rpm, fixed for 2 h with 2.5% glutaraldehyde in phosphate buffer solution (PBS), rinsed three times in PBS (0.1 M), fixed for 2 h in 1% osmic acid, and then rinsed three times with PBS (0.1 M). Gradient dehydration was performed at 4 °C by successively applying 50% ethanol, 70% ethanol, 90% ethanol, a 1:1 ratio of 90% ethanol and 90% acetone, and then 90% acetone. Pure acetone was added for dehydration at room temperature. For the embedding process, a 2:1 ratio of pure acetone and embedding liquid was applied at room temperature for 3−4 h. Finally, the sample was solidified at 37 °C overnight. The samples were sectioned into 50−60 nm thick segments using an ultramicrotome and double-stained with uranyl acetate (3%) and lead citrate. Sections were observed and photographed by TEM (Hitachi H-7650).

3. RESULTS AND DISCUSSION

3.1. Fourier Transform Infrared Spectrometry Peak Affiliation Analysis of *P. tricornutum*. The mean infrared spectrum of *P. tricornutum* was obtained by averaging 12 FTIR spectra measured at different temperatures and different growth stages (Figure 1). Different peaks in the spectrum represent different molecular groups within the macromolecules, and the absorption intensity reflects the content of the molecular group (Figure 1). Different molecular groups in the algal cells have different absorption frequencies. Peak wavelengths representing the stretching vibrations of carbohydrate functional groups were seen near 3692 cm^{-1}, identified as −OH groups, such as sugar; at 3398 cm^{-1}, identified as C−OH groups; and near 1056 cm^{-1}, identified as C−O−C groups.[21,26] The peaks near 3000−2800 and 1730 cm^{-1} are the stretching vibrations of the saturated −CH groups (Figure 1B) and the C═O groups[26] in lipids, respectively. The peaks near 1650 cm^{-1} are mainly the stretching vibrations of the C═O groups in amino acid and protein, also known as the amide I band. The peaks between 1650 and 1540 cm^{-1} mainly represent the bending vibrations of the −NH groups, also known as the amide II band. The peaks between 1418 cm^{-1} are the bending vibrations of the $-CH_2-$ and $-CH_3-$ groups, mainly from proteins.[26] The peaks between 1200 and 950 cm^{-1} represent the stretching vibrations of the C−O−C groups in carbohydrates.[21] The above results demonstrate that the characteristic peaks of the biomacromolecules in *P. tricornutum* cells can be obtained by FTIR analysis and that they can be applied in the following comparative analysis of the corresponding substance contents.

3.2. Fourier Transform Infrared Spectrometry of *P. tricornutum* Cultured under Different Temperature Conditions. Temperature can affect enzyme activity, nutrient absorption, and cell division during the growth and metabolism process of microalgae, thus affecting the composition of intracellular macromolecules.[27] On the 14th day of culture, no significant lipid-associated absorption peak (1730 cm^{-1}) was detected under the four temperature conditions (Figure 2A). Furthermore, there was little intracellular lipid accumulation under these four temperature conditions. On the 21st day of culture, small absorption peaks were observed at 1730 m^{-1}, and based on the peak height comparison, the intracellular lipid content was the highest at 15 °C (Figure 2B). On the

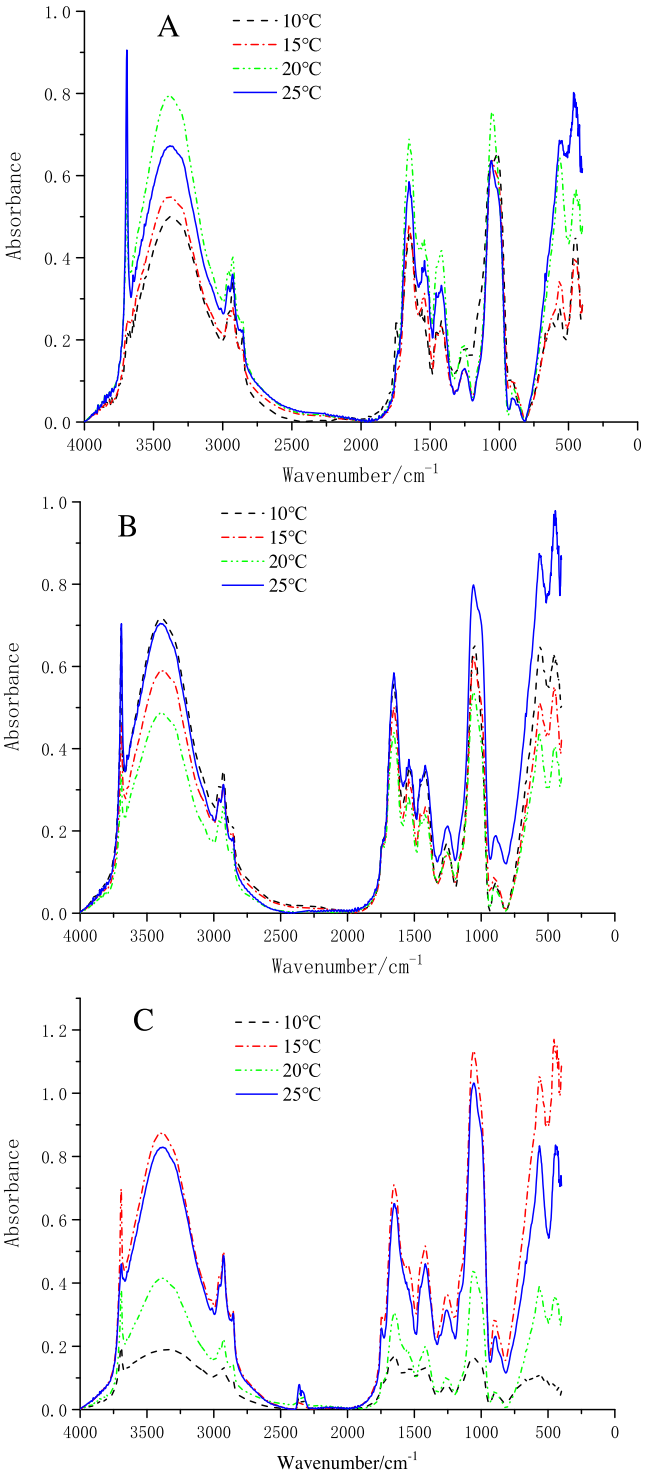

Figure 2. Infrared spectra of *P. tricornutum* at different growth phases cultured under different temperature conditions. (A) Day 14; (B) day 21; and (C) day 28.

28th day, the intracellular lipid content was also the highest at 15 °C and lowest at 20 °C (Figure 2C).

Combination analysis of the lipid absorption peak areas indicates that the intracellular lipid contents experienced relatively large changes under different temperature conditions (Figure 3A). The relative lipid content on the 28th day was the highest at 15 °C, followed by 25 °C, and showed a significant increasing trend as cultural time progressed. The maximum

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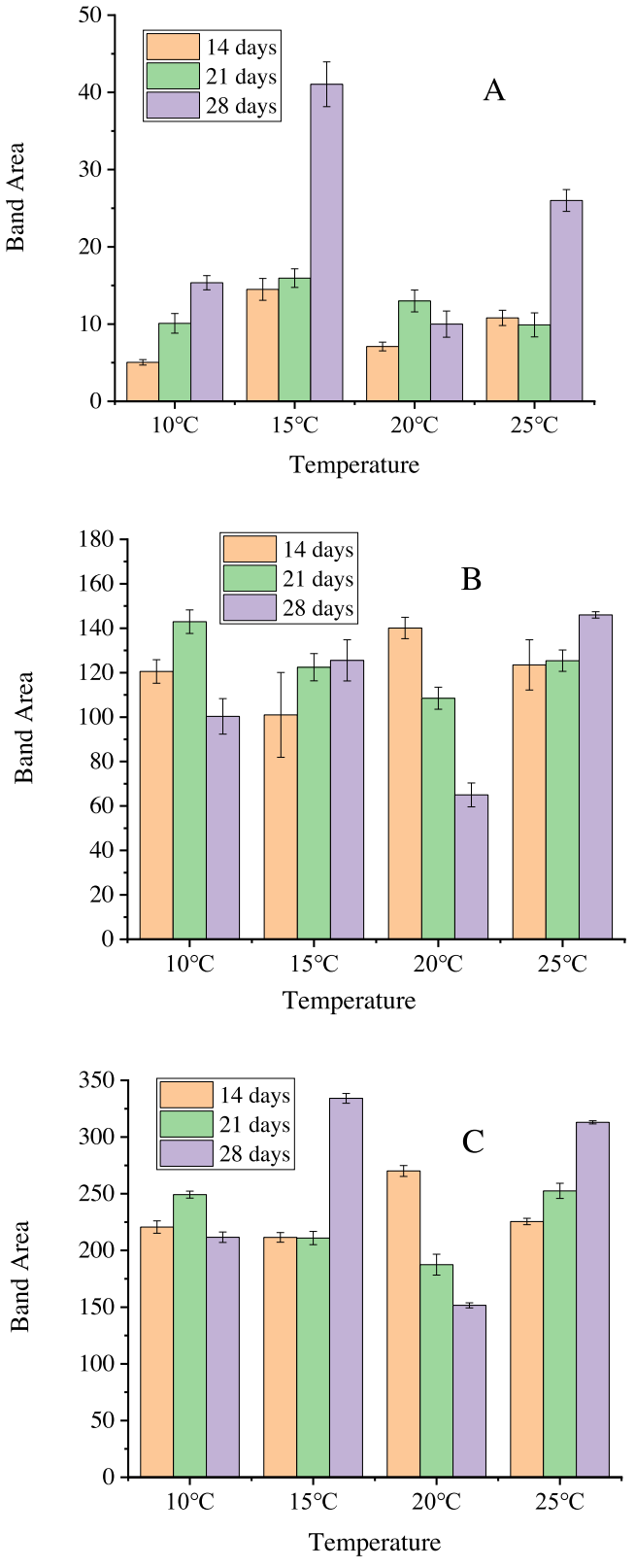

Figure 3. Content variations in lipids, proteins, and polysaccharides of *P. tricornutum* under different temperatures. (A) Lipid; (B) protein; and (C) polysaccharides.

lipid content in algae cultured at 10 and 20 °C was measured at half, or less, than that seen at 15 °C. It could be speculated that the relatively low temperature of 15 °C and the relatively high temperature of 25 °C contribute to the accumulation of intracellular lipid in *P. tricornutum*.

By combining the relative absorption peak area of the proteins in Figure 3B, the intracellular protein content on the 14th day was observed to be the highest at 20 °C, followed by 25 and 10 °C, and was the lowest at 15 °C. On the 21st day, the protein content in *P. tricornutum* cultured at 20 °C was the lowest than the other three temperature conditions. On the 28th day, the relative intracellular protein content in algae cultured at 20 °C continued to decrease; however, the content in algae cultured at 15 and 25 °C continued to increase, while the protein content in algae cultured at 10 °C began to decrease.

Extended culture time resulted in the intracellular polysaccharides content of *P. tricornutum* to increase at 15 and 25 °C, while a gradual decrease was seen at 20 °C (Figure 3C). These results are similar to the changes observed in the intracellular protein and lipid contents. Additionally, the intracellular polysaccharide contents at 10 °C appeared to first increase and then decrease. The sharp absorption peaks showed some regularities. As culture time progressed, the peak heights at 15 °C gradually increased (Figure 2, red color); at 20 °C, they gradually decreased, and at 10 and 25 °C the peak heights first decreased and then increased.

Analysis of the lipid, protein, and polysaccharide absorption peak areas on the 28th day of culture (Figure 3) shows that at 15 °C, the lipid and polysaccharide contents in *P. tricornutum* reached the maximum observed value, while the protein content was relatively lower. At 25 °C, protein and polysaccharide contents reached the maximum observed value on day 28, while the lipid content was lower as compared with the other two macromolecules. On the 28th day of culture, the lipid contents were less than the protein and polysaccharide contents at 10 and 20 °C, and they were also less than the lipid contents observed at 15 and 25 °C. Therefore, a culture temperature of 15 °C is most conducive to the formation of lipids and polysaccharides in *P. tricornutum*.

Significant differences in FTIR spectra were observed at different temperatures and different growth stages of *P. tricornutum*. Both the peak heights and areas could reflect the varying patterns of lipid, protein, and polysaccharide contents, thereby supporting FTIR as an effective technique for identifying the varying biomacromolecular composition patterns in *P. tricornutum* cells at different physiological states.

3.3. Ultrastructure of *P. tricornutum* under Different Temperature Conditions. Ultrastructure of microalgae could be used to directly characterize their intracellular lipid content and quantity and further verify FTIR results. TEM images of *P. tricornutum* show the organelles and the relative content and size of the lipid particles (Figure 4). After glutaraldehyde and osmic acid staining and fixation, lipids appeared as round black homogeneous droplets of different sizes. At 10 °C, the chromatoplasts in *P. tricornutum* were well developed and occupied half, or more, of the cell, with the storage material appearing in the form of starch (Figure 4A). At 15 °C, only one chloroplast was observed, and lipid droplets were larger and detected in a higher quantity, with the main storage material in the form of lipid droplets. However, at 25 °C, the size and amount of lipid droplets were less than that seen at 15 °C, while the number of lipid droplets was the lowest at 20 °C.

Lipid droplets are the major storage site for intracellular neutral lipids and they exist extensively in the cells of bacteria,

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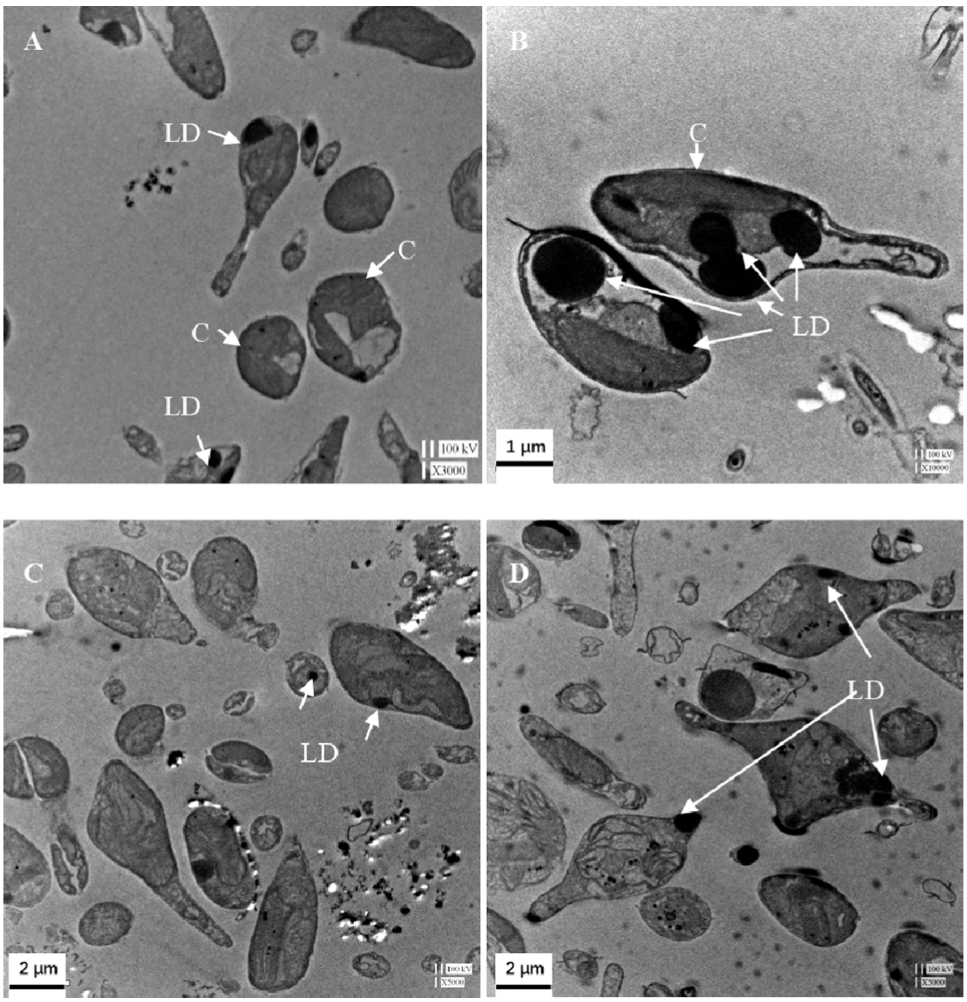

Figure 4. TEM images of *P. tricornutum*. (C means chromatoplast, LD means lipid droplet. (A) 10 °C; (B) 15 °C; (C) 20 °C; (D) 25 °C).

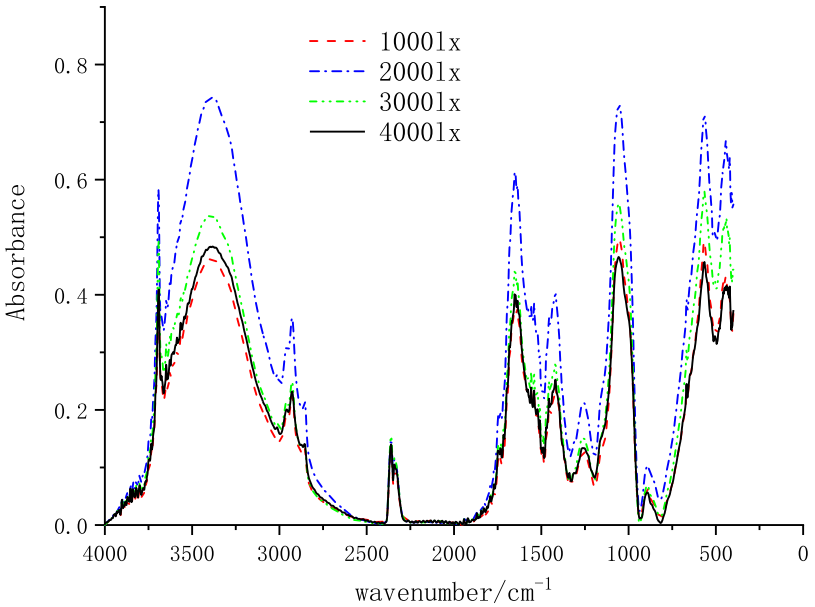

Figure 5. Infrared spectra of *P. tricornutum* under different illumination conditions.

plants, and animals. The size of lipid droplets varies greatly, ranging in diameter from 40 nm to 100 μm.[28] The biological function of lipid droplets mainly involves development and stress resistance.[29] The optimum growth temperature for *P. tricornutum* is between 10 and 20 °C, with growth inhibition occurring above 25 °C.[30] In *P. tricornutum*, low-temperature

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environments promote the synthesis of fatty acid desaturase to maintain membrane mobility and normal physiological function, thereby promoting polyunsaturated fatty acid accumulation.[31] The TEM results show that the maximum number of intracellular lipid droplets appears at 15 °C, followed by 25 °C, which is consistent with the FTIR analysis of the lipid content. Both analyses demonstrate that the highest contents are seen at 15 °C, followed by 25 °C, and that the lowest content is seen at 10 and 20 °C. These results further demonstrate that FTIR can rapidly detect lipid content variation in *P. tricornutum*.

3.4. Fourier Transform Infrared Spectrometry of *P. tricornutum* Cultured under Different Illumination Conditions. The infrared spectra of *P. tricornutum* when exposed to different illumination conditions are shown in Figure 5. The lipid content indicated by the absorption peak at a wavelength of 1730 cm^{-1} reached the maximum value under 2000 lx. The intracellular protein content, indicated by the absorption peaks at 1418−1455 cm^{-1} (C═N) and 1650 cm^{-1} (C═O), were also the highest under 2000 lx, followed by those under 3000 lx, while they were the smallest under the 1000 and 4000 lx illumination conditions. The intracellular polysaccharide contents indicated by the absorption peak heights at wavelengths 1200−950 and 3398 cm^{-1} showed a similar pattern. The suitable illumination intensity conditions for *P. tricornutum* are between 1000 and 8000 lx, with the optimum range between 3000 and 5000 lx.[32] Nymark et al.[33] subjected a *P. tricornutum* culture, which was adapted to a low-light environment to high-light conditions, and found that light-protective metabolites increased significantly. It is believed that under high-light conditions, *P. tricornutum* can rapidly and effectively initiate a protective response mechanism. The light-harvesting system on the thylakoid membrane of *P. tricornutum* can adjust to the variation of the external light condition correspondingly, thus changing its photosynthetic efficiency and thereby adapting to changes in the natural environment. In this study, the contents of the macromolecular substances under the illumination intensity of 2000 lx were relatively high. The reason for this might be that under low illumination conditions, *P. tricornutum* can adjust the intracellular biomacromolecular substances to adapt to changes in external illumination intensity. Furthermore, significant differences in the FTIR spectra under different illumination conditions were observed, and the characteristic peaks were specific, indicating that the FTIR spectra can be used to rapidly detect varying patterns in intracellular biomacromolecules.

4. CONCLUSIONS

FTIR can be used as a simple and convenient way to detect changes in the intracellular components of *P. tricornutum*. This study demonstrates that, when cultured for the same amount of time, the intracellular biomacromolecular contents of *P. tricornutum* have similar patterns under the low-temperature (15 °C) and high-temperature (25 °C) conditions. Moreover, under both conditions, the intracellular biomacromolecular contents accumulate as culture time progresses. However, at 20 °C, the protein and carbohydrate contents were the highest at the early stage of the exponential phase and then decreased continuously; however, lipid accumulation lagged behind that of the carbohydrates and proteins, with its content reaching the highest level during the middle of the cultural stage and then decreasing as culture time progressed. Under different illumination conditions, lower illumination was more conducive to biomacromolecule accumulation. To adapt to different temperatures, *P. tricornutum* is able to regulate the contents of carbohydrates, lipids, and proteins, thus negating the effects of temperature and illumination. Therefore, the increase in algal biomacromolecular content is a positive self-protection method against temperature and illumination stress. In conclusion, FTIR analysis is a powerful technique to detect intracellular biomacromolecular compositions rapidly and conveniently and provides an effective method for analyzing the physiological state of microalgae.

■ ASSOCIATED CONTENT

Data Availability Statement

The data sets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c05933.

Datasets used and analyzed (XLSX)

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Notes

The authors declare no competing financial interest.

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