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Application News

No.A482

Spectrophotometric Analysis

Simple Quantitative Measurement of Nannochloropsis Micro Algae in Water Using Shimadzu UV Micro Algae Analysis System

Global warming due to the burning of fossil fuels is now widely recognized as a problem, and among the alternatives to fossil fuels, the extraction of fuel from micro algae has been actively investigated in recent years. While the yield per unit area of micro algae is high, and harvesting is possible year round, these advantages are offset by many challenges in the development process that must be overcome for micro algae to become a viable source of alternative fuel. Currently, various studies at each stage in the production process, including breeding, cultivation, harvesting, and oil extraction are being undertaken.
In studying micro algae, measurement of the daily growth (concentration) is important. Currently, this measurement is conducted by the dry weight method, in which the sample is filtered using filter paper, and then dried prior to weighing. However, a simpler, faster method is being sought to replace this time-consuming dry weight method. Here, we introduce Shimadzu's newly developed UV micro algae analysis system which permits simple measurement of the micro algae concentration using a spectrophotometer.
Measurement of a single sample can be accomplished very quickly in about one minute using a disposable screw-cap vial (or disposable cell). This Application News introduces an example of measurement of Nannochloropsis micro algae using the Shimadzu micro algae analysis system.

Sample – Nannochloropsis Micro Algae –

The Nannochloropsis micro algae sample (Higashimaru Co., Ltd., 10 billion cells/mL) was diluted to prepare measurement samples consisting of twelve sequential concentrations from 0 to 100 %. Fig. 1 shows a photograph of the prepared samples. These samples were divided into two groups, one to serve as standard samples for generating a calibration model, and the other to serve as calibration model validation samples. These are listed in Table 1 and Table 2, respectively.

Fig. 1 Measurement Samples of Nannochloropsis Micro Algae

Table 1 Standard Samples

Standard Sample	Concentration (%)
①	100
②	90
③	80
⑤	60
⑥	50
⑦	40
⑨	20
⑩	10
⑫	0 (Pure water)

Table 2 Validation Samples

Validation Sample	Concentration (%)
④	70
⑧	30
⑪	5

Measurement Instrument

Analysis was conducted using the Shimadzu UV micro algae analysis system consisting of the UV-2600 UV-VIS spectrophotometer combined with the integrating sphere attachment for algae analysis. Reflectance measurement of the samples was conducted using this system. The disposable screw-cap vials containing the samples were set in the integrating sphere, and the total reflectance of each sample was measured. The UV-2600 with mounted integrating sphere attachment is shown in Fig. 2. The use of disposable screw-cap vials for these measurements eliminated the need for manual operations such as the washing of vials, which also eliminated any concerns regarding contamination and carry-over due to insufficient washing.

Fig. 2 UV-2600 with Mounted Integrating Sphere Attachment

Measurement Results

Reflectance measurement of the 12 samples listed in Table 1 and Table 2 was conducted. Replacing the screw-cap vial for each sample, each sample of the same concentration was measured twice (12 samples × 2 = total of 24 data sets). Those results are shown in Fig. 3, and the analytical conditions are shown in Table 3. The results indicated that the higher the concentration, the greater the reflectance, and the lower the concentration, the lower the reflectance, demonstrating a correlation between the reflection spectrum and the concentration. This can be attributed to the fact that the greater the number of micro algae cells, the greater the degree to which light is reflected from the vicinity of liquid surface.

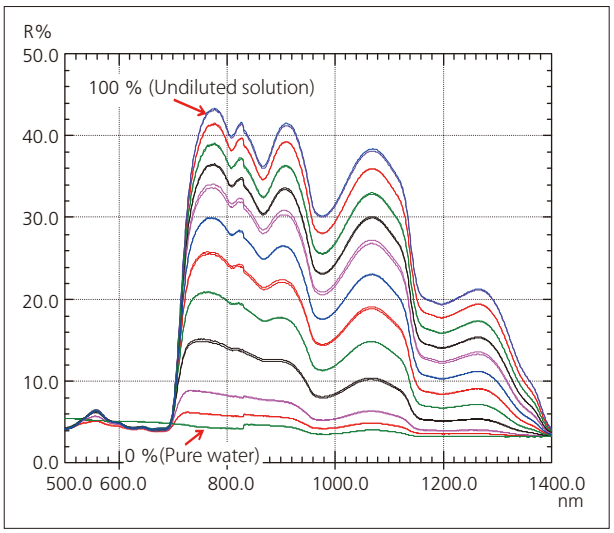

Fig. 3 Reflection Spectra of Micro Algae in Disposable Vials

Table 3 Analytical Conditions

Instrument	: UV-2600 UV-VIS spectrophotometer Integrating sphere attachment for algae analysis
Measurement Wavelength Range	: 500 nm – 1400 nm
Scan Speed	: Medium
Sampling Pitch	: 1.0 nm
Photometric Value	: Reflectance
Slit Width	: 5 nm

Results of Quantitative Analysis

In this experiment, we attempted to quantify micro algae using reflection spectra. Quantitation was conducted using the single regression method and the multivariate analysis multiple linear regression method, and the respective quantitative accuracies were then compared. A regression expression of the calibration curve model was obtained for each standard sample based on the samples of Table 1. For the multiple linear regression method, the regression equation is entered in the photometric measurement method window of the standard UVProbe software as shown in Fig. 4. With this setting, the predicted value calculated via the regression equation for each sample measured is output by the software.

Table 4 shows the calculated concentration results based on the measurements of the validation samples of Table 2. Comparing the results, it is clear that better results are obtained using the multiple linear regression method than by the single regression method. In this way, measurement of unknown samples can easily be conducted by simply entering the regression equation in the standard UVProbe software.

In addition, the regression equation using the multiple linear regression method was determined using the "regression analysis" function in the Excel®1) spreadsheet software of Microsoft Corporation. RMSEP of Table 4 refers to the value defined in Fig. 5, and is an index that expresses the average difference between the predicted and true value. Thus, the smaller the RMSEP value, the better the prediction accuracy. Regarding the single regression method, quantitation was conducted using the standard calibration function of the UVProbe software.

Note: Using the single regression method, calculation was conducted based on the single wavelength data obtained at 910 nm. As for the multiple linear regression method, calculation was conducted using four wavelength data sets, 870 nm, 910 nm, 980 nm, and 1070 nm.

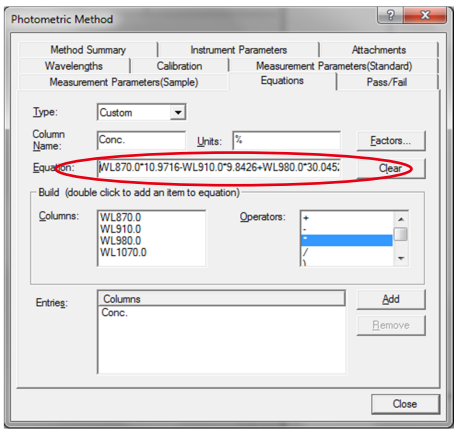

Fig. 4 Photometric Method in UVProbe Software

Table 4 Prediction Results Based on Concentration Calculated by Each Calibration Model for Validation Samples

Validation Sample	Actual Concentration (%)	Predicted Results by Single Regression Method (%)	Predicted Results by Multiple Linear Regression Method (%)
④ -1	70	72.9	69.2
④ -2	70	73.4	69.1
⑧ -1	30	32.4	28.3
⑧ -2	30	32.3	30.0
⑪ -1	5	1.4	5.3
⑪ -2	5	1.5	5.4
RMSEP		3.1	0.87

* RMSEP: Root Mean Sqaure Error of Prediction

$$RMSEP = \sqrt{\frac{\sum_{i=1}^{n} (y'_i - y_i)^2}{N}}$$

y_i' is the predicted value, y_i is the actual value, and N is the number of validation samples.

Fig. 5 Defined Expression for RMSEP

Measurement of Absorption Spectra (Transmission Measurement)

When the concentration of micro algae is low, accurate quantitation becomes difficult due to low reflectance. To address this, we investigated the use of transmission measurement using an integrating sphere, making it possible to measure the absorption spectrum of the total transmitted light including both the linear transmitted light and diffused transmitted light.

The measurement samples, including those listed in Table 1 and Table 2 were uniformly diluted 20-fold. Disposable cells were used for the measurements. Fig. 6 shows a sample-filled disposable cell (10 mm optical path length) mounted in the integrating sphere. Measurements were conducted twice for each sample (12 samples × 2 = total of 24 data sets).

The measurement results are shown in Fig. 7, and the analytical conditions are shown in Table 5. Fig. 8 shows an enlarged view of the wavelength region in the absorbance range of 0 – 2 of Fig. 7. The results indicated an absorption spectrum that correlated with the concentration, such that the higher the concentration, the greater the absorbance, and the lower the concentration, the lower the absorbance. It should be noted that when conducting measurement by the transmission method, the integrating sphere attachment designed for algae measurement by the reflection method is unnecessary, permitting use of the standard type integrating sphere attachment.

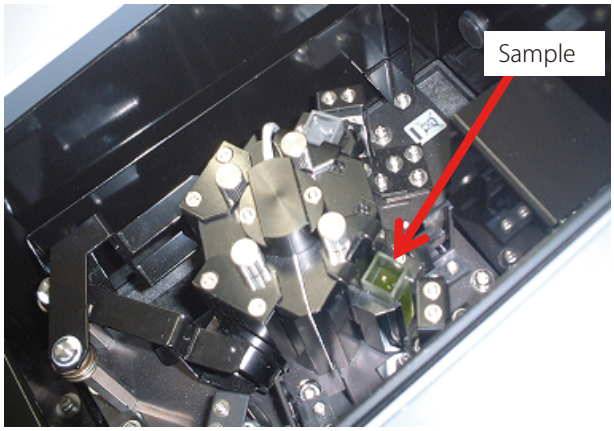

Fig. 6 Disposable Cell Set in Integrating Sphere Attachment

Table 5 Analytical Conditions

Instrument	: UV-2600 UV-VIS spectrophotometer ISR-2600Plus integrating sphere attachment
Measurement Wavelength Range	: 300 nm – 800 nm
Scan Speed	: Medium
Sampling Pitch	: 1.0 nm
Photometric Value	: Transmittance
Slit Width	: 5 nm

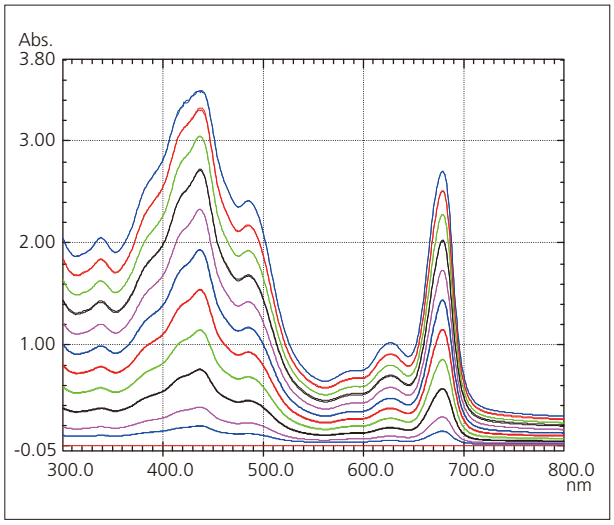

Fig. 7 Absorption Spectra

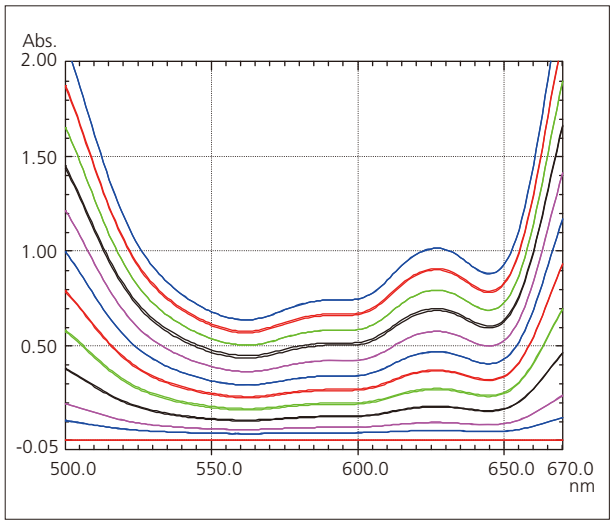

Fig. 8 Enlarged Spectra of Fig. 7

Results of Quantitative Analysis (Transmission Measurement)

We tried quantifying the micro algae using the absorption spectrum. As when using the reflection method, we conducted quantitation by both the single and multiple linear regression methods, and compared the quantitative accuracy. The results are shown in Table 6. Once again, better results were obtained using the multiple linear regression method. This time, since all of the samples measured by the reflection method were uniformly diluted 20-fold, the "Actual Concentration (%)" in Table 6 is 1/20 that of the values shown for the Validation Samples of Table 2.

Note: Using the single regression method, calculation was conducted based on the single wavelength data obtained at 625 nm. As for the multiple linear regression method, calculation was conducting using three wavelength data sets: 580 nm, 625 nm and 660 nm.

Table 6 Predicted Results for Concentrations of Validation Samples Calculated Using Each Calibration Model

Validation Sample	Actual Concentration (%)	Predicted Results by Single Regression Method (%)	Predicted Results by Multiple Linear Regression Method (%)
④ -1	3.5	3.52	3.52
④ -2	3.5	3.47	3.50
⑧ -1	1.5	1.42	1.50
⑧ -2	1.5	1.45	1.51
⑪ -1	0.25	0.35	0.25
⑪ -2	0.25	0.36	0.25
RMSEP		0.073	0.009

Investigation of the Use of Other Analytical Instruments for the Study of Micro Algae

A variety of analytical instruments other than ultraviolet-visible (near infrared) spectrophotometers (UV) are used in the study of micro algae. Here we summarize these according to the following respective research objectives.

(1) Culture Process Management

Currently, the monitoring of micro algae cell mass (concentration) is primarily conducted using the dry weight method, but the required filtration and drying operations are cumbersome and time-consuming. For this reason, UV and total organic carbon analysis (TOC) are now used as simple methods. In addition, TOC is also used to study the physiological state of the culture and micro algae.

Qualitative analysis of the fats and oils, and other organic substances produced by the micro algae typically require the use of a gas chromatograph mass spectrometer (GCMS) or liquid chromatograph mass spectrometer (LCMS). For quantitation, instruments such as a liquid chromatograph (LC) or gas chromatograph (GC) are used. In addition, simple qualitative and quantitative analyses are conducted using such instruments as a UV (including near-infrared) spectrophotometer, Fourier transform infrared spectrophotometer (FTIR), and fluorescence spectrophotometer (RF).

Objective	Instrument
Monitoring of micro algae, generated organic matter	TOC, UV
Research of physiological state of micro algae	TOC
Qualification of generated organic matter	GCMS, LCMS
Quantitation of generated organic matter	GC, LC, GCMS, LCMS
Simple quantitation, simple qualification of generated organic matter	UV, FTIR, RF

(2) Purification and Modification

Organic matter produced by micro algae is not suitable as a fuel until it is first subjected to such processing as purification and reforming to a low molecular weight substance. Following such impurity removal and reforming processes, LCMS and GCMS are useful for characterization of the resulting products.

Objective	Instrument
Purification, and qualification and quantitation of organic matter following reforming	GCMS, LCMS

(3) Metabolomics

Metabolic flux analysis and profile analysis are being promoted to elucidate the mechanisms by which micro algae produce useful organic matter, and to increase production volume. GCMS and LCMS are required at this time to conduct such studies.

Objective	Instrument
Metabolomics	GCMS, LCMS

(4) Harvesting of Organic Matter

The size and hardness of the algae cells affect the ease with which micro algae cells can be harvested. Particle size analyzers (SALD) and scanning probe microscopes (SPM) are presently being considered for such evaluation.

Objective	Instrument
Cell surface hardness	SPM
Cell particle size distribution	SALD

Conclusion

We developed a method which permits easy measurement of micro algae concentrations.

With this method, reflectance measurement is conducted when the sample concentration is high, and transmittance measurement is used if the concentration is low. A screw-cap vial is used with the reflection method, and a disposable cell is used with the transmission method.

When conducting quantitative experiments using Nannochloropsis micro algae, good results were obtained using both the reflection method and the transmission method, as demonstrated by the RMSEP values. Using this technique, it is possible to measure micro algae concentrations quickly and easily.

Further, in micro algae research, in addition to the monitoring of the cell mass, it is necessary to perform a variety of measurement and research functions, including culture process management, purification and reforming, metabolomics research, and evaluation of harvesting methods. Various instruments are effective for these activities, including UV, TOC, FTIR, GCMS, LCMS, etc.

1) Excel is a registered trademark or trademark of Microsoft Corporation.

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