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Haematococcus pluvialis Cell-Mass Sensing Using Ultraviolet Fluorescence Spectroscopy

LABABPOUR, ABDOLMAJID, SEONG-JOO HONG, AND CHOUL-GYUN LEE*

Institute of Industrial Biotechnology, Department of Biotechnology, Inha University, Incheon 402-751, Korea

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Abstract A simple whole-cell-based sensing system is proposed for determining the cell mass of *H. pluvialis* using ultraviolet fluorescence spectroscopy. An emission signal at 368 nm was used to detect the various kinds of green, green-brown, brown-red, and red *H. pluvialis* cells. The fluorescence emission intensities of the cells were highest at 368 nm with an excitation wavelength of 227 nm. An excitation wavelength of 227 nm was then selected for cell-mass sensing, as the emission fluorescence intensities of the cell suspensions were highest at this wavelength after subtracting the background interference. The emission fluorescence intensities of HPLC-grade water, filtered water, and HPLC-grade water containing a modified Bold's basal medium (MBBM) were measured and the difference was less than 1.6 for the selected wavelengths. Moreover, there was no difference in the emission intensity at 368 nm among suspensions of the various morphological states of the cells. A calibration curve of the fluorescence emission intensities and cell mass was obtained with a high correlation (R^2=0.9938) for the various morphological forms of *H. pluvialis*. Accordingly, the proposed method showed no significant dependency on the various morphological cell forms, making it applicable for cell-mass measurement. A high correlation was found between the fluorescence emission intensities and the dry cell weight with a mixture of green, green-brown, brown-red, and red cells. In conclusion, the proposed model can be directly used for cell-mass sensing without any pretreatment and has potential use as a noninvasive method for the online determination of algal biomass.

Keywords: Algal cell mass, astaxanthin production, spectrofluorophotomety, *H. pluvialis*, noninvasive, whole-cell assay

The photosynthetic unicellular microalga *H. pluvialis* is a rich source of natural astaxanthin and already used for the large-scale production of astaxanthin [10, 18]. Astaxanthin (3,3'-dihydroxy-β,β-carotene-4,4'-dione), a red ketocarotenoid with high antioxidant activity, is used in aquaculture as a feed pigment additive and has been tested in clinical studies for treating heart and neurodegenerative diseases [6]. Genomics and proteomics studies [4, 11] as well as a novel bioreactor design [21] have been reported extensively in order to overproduce astaxanthin [10]. However, monitoring of the astaxanthin production process by *H. pluvialis* requires accurate information on variations in the cell-mass during cultivation [13]. In addition, the cell-mass determination system needs to be fast, accurate, easy to handle, and inexpensive for use in the laboratory and on an industrial scale.

Determining the cell mass is a daily routine analysis in any production process using microorganisms, including the *H. pluvialis* cultivation process. Existing methods reported in the literature for measuring the *H. pluvialis* cell-mass generally quantify the cell mass using the dry cell weight (DCW) or fresh cell weight (FCW). The DCW can be directly determined by drying a known amount of cells after washing off extra salts from the cells [2, 5, 12], whereas the FCW can be directly measured by weighing the cells after centrifugation and washing, or indirectly by electronic particle counting, preferably by multiplying the cell concentration by the average cell volume [2]. Although the electronic counting technique is a precise and sensitive method, a Coulter Counter is a costly instrument and inappropriate for *in situ* and continuous cell monitoring.

Another well-established technique is spectrophotometry, whereby the chlorophyll pigments in cells can be measured using the absorbance peaks [16]. This method has also been adapted to measure chlorophyll and carotenoids in *H. pluvialis* based on the chlorophyll and carotenoid absorption peaks [2]. Moreover, changes in the chlorophyll absorption peaks and baseline of the *H. pluvialis* cell suspension have been adopted for cell-mass determination in *H. pluvialis* [12]. Nonetheless, although this chlorophyll-based method is inexpensive and easy for routine analyses, it is less accurate and sensitive than electronic particle counting.

A fluorometric technique called *in vivo* fluorometry has been used for about 40 years by oceanographers and

*Corresponding author
Phone: 82-32-860-7518; Fax: 82-32-872-4046;
E-mail: leecg@inha.ac.kr

limonologists to measure algae by directly measuring the fluorescence of chlorophyll in living algal cells [15]. The determination of chlorophyll and secondary carotenoids in *Haematococcus* using fluorometry has also been reported [9]. The fluorometric determination of chlorophyll both by extraction and *in vivo* measurement uses excitation in the blue region of the spectrum (about 450 nm) and emission at the near infrared (about 670 nm) [8]; however, it would appear that no analytical techniques employing fluorescence spectroscopy have yet been reported for determining the cell mass of *H. pluvialis*.

A common difficulty with using spectrofluorometric and spectrophotometeric methods to determine the cell mass of *H. pluvialis* is that the patterns of change for chlorophyll, carotenoids, and other pigments do not correspond with changes in the cell mass. In addition, the absorbance of *H. pluvialis* cells does not correspond to its cell mass, as the small motile green cells with a diameter of around 15 μm expand during cultivation into red haematocysts with a diameter of up to 150 μm because of the accumulation of astaxanthin in the lipid bodies [17]. However, these morphological changes are functions of the environmental conditions of the cells, such as nutrient availability and light intensity, making it difficult to find a relationship among these variables [5].

Marine bacteria and phytoplankton have intrinsic fluorophores attributed to proteins, nicotinamide adenine dinucleotide (NADPH), chlorophyll, carotenoids, and other unknown compounds, such as cell-wall complex compounds [3]. It has already been established that in most cases the emission fluorescence intensity of phytoplankton at around 368 nm is proportional to the cell concentration, plus there is a linear relationship between the fluorescence intensity and the cell concentration [3], thereby allowing the cell mass to be evaluated using a coefficient factor between the fluorescence intensity and the cell mass. Therefore, this sensing method could potentially facilitate measurement of the *H. pluvialis* cell mass using a spectrofluorophotometer.

Fluorescence spectroscopy is a sensitive technique for the recognition and detection of fluorophores, and as much as 1,000 times more sensitive than absorption spectroscopy, since the emission signal is measured above a low background level [1]. This method is also widely used because of its relative simplicity and high sensitivity, plus it only requires a small aqueous sample with a low concentration (usually <20 mg/l) [1].

Fluorescence exists in conjugated molecules; *i.e.*, molecules with delocalized electronic structures. The practical limit of fluorescence sensitivity is set by varying the background, which largely consists of two things: the fluorescence of extraneous materials in the water, and an inescapable emission from the water itself (called the Raman shift). Raman scattering is the inelastic scattering of photons by water molecules, and converts a small fraction of incident photons into photons with a lower energy and therefore longer wavelength.

Accordingly, this study presents a simple *in vivo* fluorescence-based sensing system for determining the total cell mass of *H. pluvialis*. Although several other studies have already investigated the spectrofluorometric determination of the cell mass of phytoplankton [7, 9, 14, 19, 22], the most important advantage of the proposed method is its independence from the chlorophyll and astaxanthin pigment contents, making it a rapid and accurate method for measuring the cell mass of *H. pluvialis*. Therefore, this paper describes the application of fluorescence spectroscopy for the indirect determination of the *H. pluvialis* cell mass in a culture broth, thereby eliminating the need for pretreatment when determining the cell mass.

MATERIALS AND METHODS

Microorganism and Cultivation Conditions

The photosynthetic microalga *Haematococcus pluvialis* (UTEX 16, Culture Collection of Algae, University of Texas, Austin, TX, U.S.A.) was precultivated in a 200-ml Erlenmeyer flask under a continuous light intensity of 40 $\mu E\ m^{-2}\ s^{-1}$ using a fluorescent lamp (FL 20 SSEX_D/18; Osram, Ansan, Korea) in the photoautotrophic mode of operation. A modified Bold's basal medium (MBBM), containing 246.5 mg/l $NaNO_3$, 24.99 mg/l $CaCl_2 \cdot 2H_2O$, 73.95 mg/l $MgSO_4 \cdot 7H_2O$, 74.9 mg/l K_2HPO_4, 175 mg/l KH_2PO_4, 25.13 mg/l NaCl, 49.68 mg/l $C_{10}H_{16}N_{20}Na$, 30.86 mg/l KOH, 4.98 mg/l $FeSO_4 \cdot 7H_2O$, 1.0 mg/l H_2SO_4, 11.13 mg/l H_3BO_3, 8.83 mg/l $ZnSO_4 \cdot 7H_2O$, 1 mg/l $MnCl_2 \cdot 4H_2O$, 6.06 mg/l MoO_3, 2 mg/l $CuSO_4 \cdot 5H_2O$, 0.49 mg/l Co $(NO_3)_2 \cdot 6H_2O$, and 1.19 mg/l $Na_2MoO_4 \cdot 2H_2O$ (pH=6.3±0.5) was used for the cultivation [2]. The preculture was then used to prepare the seed culture in a 500-ml column photobioreactor (45 cm high and 2.8 cm diameter). The aeration (0.2 vvm) and mixing were carried out by bubbling 95% air and 5% CO_2, while light was supplied by two white fluorescent lamps from opposite sides with 40 $\mu E\ m^{-2}\ s^{-1}$ at the inner surface of the column. The exponentially growing cells of the seed organism were inoculated into 500-ml column photobioreactors at a working volume of 400 ml. The pH was kept constant at 6.3±0.5 by introducing CO_2.

Analytical Instruments

The fluorescence excitation and emission spectra were recorded using a spectrofluorophotometer (Model RF 5301; Shimadzu Co., Kyoto, Japan) equipped with a 1.0 cm path length quartz cell. The fluorescence spectra were recorded within the 220–550 nm region at room temperature with a 1.0 nm resolution based on an integration time of 0.5 s. The excitation and emission slits of the spectrofluorophotometer were 5.0 nm wide. A 3.0 ml sample, contained in a 1.0 cm

path length quartz cuvette, was utilized for the fluorometry after mixing to maintain a uniform suspension during the measurements. The excitation spectra were generated at an emission set at 368 nm using water and the cell suspensions. The fluorescence intensities were then averaged from a minimum of three replicates. The fluorescent intensity of the cell suspensions and water background peak were subtracted.

A Coulter Counter Multisizer II equipped with a Channelyzer Z2 256 (Coulter Electronics, Hialeah, FL, U.S.A.) with a 200-μm aperture was also used to acquire the mean cell number, cell size, and fresh weight of *H. pluvialis* cells during the cultivation. The data were analyzed using AccuComp Software, Ver. 2.01. A microscope (CSB-HP3, Samwon Scientific Ind., Co., Seoul, Korea) equipped with 40× and 100× plan apochromatic objectives was used to determine the color, viability, and morphology of the cells.

All the spectrophotometric measurements were made using a UV-visible spectrophotometer (HP8453B, Hewlett Packard, Waldbronn, Germany) equipped with 1-cm path length quartz cells. In addition, all the spectra were recorded from 200 to 800 nm based on a 1.0 nm bandwidth.

Preparation of Samples

The *H. pluvialis* samples included various morphological cell forms, divided into 4 types based on the color: green, green-brown, brown-red, and red. The samples were centrifuged at 3,000 ×*g* for 10 min and the spectrofluorophotometric spectra taken after washing the cells three times and resuspending them in HPLC-grade water (Mallinckrodt Baker, Phillipsburg, U.S.A.) to eliminate the effects of the MBBM culture medium components. Suspensions of the various morphological cell forms were prepared in HPLC-grade water to enable a 1 to 20 measurement range.

Correction of Fluorescence Spectra

The effects of the background interference (*i.e.*, Raman scattering and water impurities) were eliminated by subtracting the background interference from the mean emission fluorescence intensity of the cell spectra. To clarify the effect of the fluorescent impurities in the filtered water and MBBM culture medium components on the cell suspension spectra, the fluorescence spectra of the HPLC-grade water, filtered water obtained from a Pentair water purification system (Pentair, Golden Valley, MN, U.S.A.), and HPLC-grade water containing the MBBM culture medium components were taken at a selected excitation wavelength of 227 nm and emission of 368 nm (EX227/EM368). Further tests were also performed using solutions of the different MBBM culture medium components based on the original concentrations, and the spectra of the solutions taken to investigate any possible cross-interference caused by the MBBM culture medium components. The fluorescence spectra of the algal suspensions in HPLC-grade water were then corrected by subtracting the spectra for the water impurities and MBBM culture medium (background spectra) from the obtained peaks.

Procedure for Cell-Mass Sensing

The first step in developing the cell-mass sensing system was to characterize the fluorescence spectra of the *H. pluvialis* cell suspensions, including the peak maxima, and optimum excitation and emission wavelengths. To obtain primary information about the excitation and emission wavelengths of the cells, the absorption spectra of the cell suspensions were taken within a range of 200–800 nm and the emission fluorescence spectra taken within a range of 220–550 nm with the excitation set at 220 nm. To identify the optimum fluorescence excitation wavelength for determining the cell mass, emission spectra were generated using an emission with various excitation wavelength ranges from 220 to 350 nm based on 5 nm intervals using HPLC-grade water and the cell suspensions. Additionally, further excitation wavelengths were scanned within the range of 220–235 nm based on 1 bandwidth intervals. In addition, to identify the fluorescence interference from the water Ramen, impurities, and MBBM culture medium components, various excitation wavelengths were also determined from 220 to 230 nm based on a 1 bandwidth interval. To determine the impartiality of the system to green, green-brown, brown-red, and red *Haematococcus* cells, the fluorescence spectra of the various morphological cell forms were compared in HPLC-grade water based on the same concentrations. Thus, samples of green (GN), green-brown (GB), brown-red (BR), red (RD), green - green-brown (GG; GN:GB=1:1), green - brown-red (GB; GN:BR=1:1), green - red (GR; GN:RD=1:1), and a mixture of all (MX; GN:GB:BR:RD=1:1:1:1) cell suspensions were prepared based on the same concentration within a range of 0–140 mg/ml and the excitation and emission wavelengths tested at 227 nm and 368 nm, respectively. Each sample was analyzed 10 times to determine the average peak intensities and standard deviation of the results.

The calibration curve for *H. pluvialis* cell-mass sensing was then made based on the cell mixture (MX; GN:GB:BR:RD=1:1:1:1). Five standard samples with concentrations of 20, 50, 80, 110, and 140 μg/ml were prepared and their fluorescence emission intensity at 368 nm plotted against the cell mass, as determined by the DCW. Each standard was analyzed ten times and the average peak intensities were detected to obtain calibration data, and the regression was obtained from the individual standard points of the cell suspensions. The slope of the fluorescence intensity versus the cell mass was then used to measure the cell mass of unknown samples. The dry cell weight of 50.0 ml of the same samples was also analyzed in parallel to the fluorescence measurements after drying at 90°C for 48 h to

investigate the correlation of the calibration factor of the fluorescence intensity. The sensitivity of the proposed cell-mass sensing system was also evaluated against the DCW measured by drying the cells. No correction was made for possible cell fragility in the samples.

RESULTS AND DISCUSSION

Characterization of Cell Fluorescence Spectra

In certain cases, a spectrophotometric absorption peak spectrum corresponds to a transition, in which case there is typically a corresponding peak in the fluorescence emission spectrum, yet with a smaller photon energy. Thus, to investigate this situation and the relative sensitivity of the absorption and fluorescence intensity, the absorption spectra of various morphological cell suspensions were obtained. Fig. 1 shows the typical spectrophotometric spectra of the green, green-brown, brown-red, and red cells, along with the spectrofluophotometric emission spectrum of a cell mixture, for the same concentration of 100 mg/ml in HPLC-grade water, after three washes. The difference altitude of the fluorescence emission intensity was ~167 at 368 nm with the excitation at 227 nm within a range of 300–450 nm, whereas the difference altitude of the absorption spectra was ~0.02 within a range of 200–800 nm. However, since the absorption peaks for the cell suspensions were very low and showed no similarities with the fluorescence spectra, the UV-visible data were of no value to find the optimum fluorescence excitation wavelength of the *H. pluvialis* cells with fluorescence spectroscopy (Fig. 1). In contrast, the fluorometric measurements significantly increased the cell-detection sensitivity in comparison with the absorption spectroscopy, indicating their potential use in accurately determining the *H. pluvialis* cell mass.

Fig. 2A shows the emission fluorescence intensities of the cells in HPLC-grade water with excitation wavelength ranges from 220 to 270 nm based on 5 bandwidth intervals after subtracting the background interference. A high peak overlap of the cells and water Raman was seen in the range above 270 nm (data not shown). According to Fig. 2A, the highest emission fluorescence intensity of 86.67±0.99% was obtained with an excitation wavelength of 230 nm. Further analysis of the optimum excitation wavelength, with 1 nm bandwidth intervals within a range of 220–235 nm, is shown in Fig. 2B. Consequently, after subtracting the background impurities and water Raman scattering from the fluorescence intensities within an excitation range of 220–235 nm, the maximum emission fluorescence

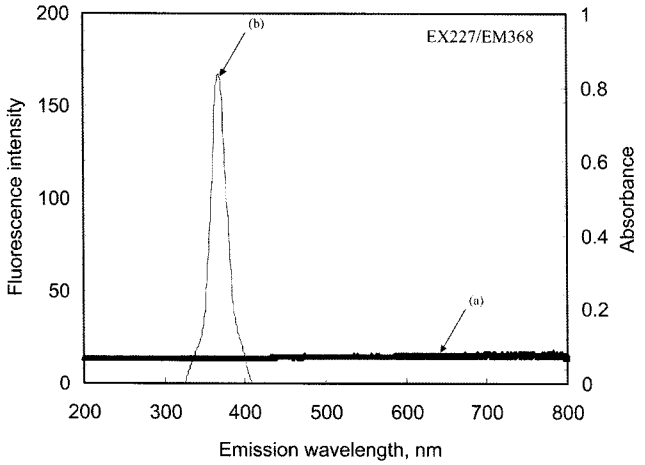

Fig. 1. Typical fluorescence emission spectrum and absorption spectra of *H. pluvialis* cells in HPLC-grade water: (a) spectrofluorophotometric spectrum and (b) spectrophotometric spectra for green, green-brown, brown-red, and red cells that totally overlapped.

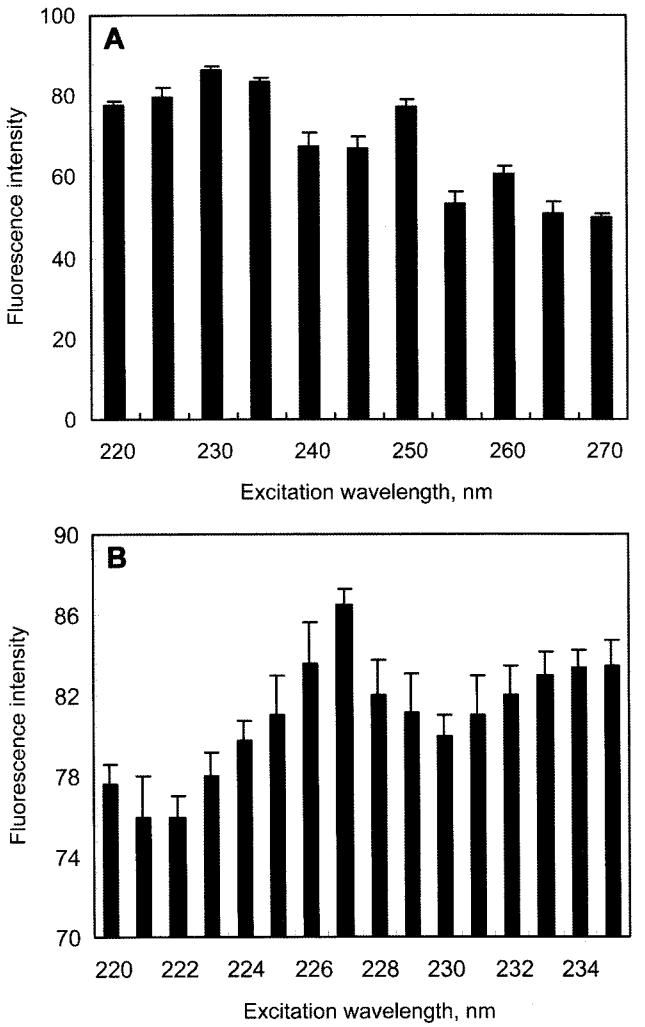

Fig. 2. Emission fluorescence intensities at 368 nm for cells (**A**) within excitation range of 220–270 nm and 5 bandwidth intervals and (**B**) within the range from 220 to 235 nm and 1 bandwidth interval, in HPLC-grade water after subtraction of background interference.

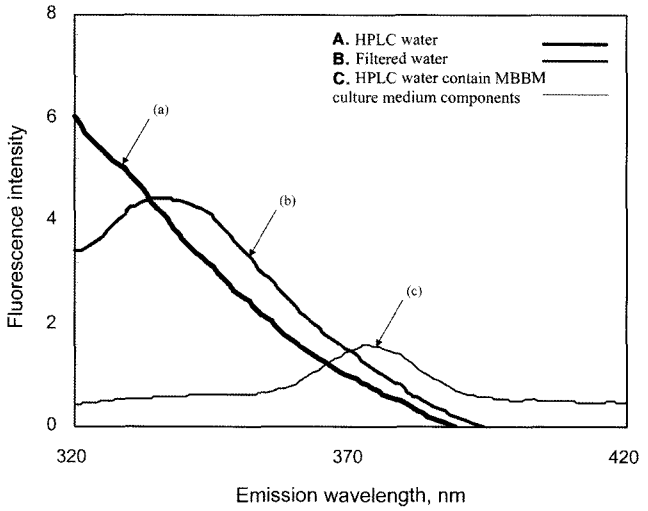

Fig. 3. Fluorescence spectra of (**A**) HPLC-grade water (n=10, RSD=0.86%), (**B**) filtered water (n=10, RSD=0.99%), and (**C**) HPLC-grade water plus all the components of MBBM culture medium (n=10, RSD=1.24%), with excitation wavelength set at 227 nm and emission set at 368 nm.

intensity of the cells at 87.56±0.81% obtained with the excitation set at 227 nm was identified as the optimum for cell-mass sensing. Therefore, the excitation and emission wavelengths were set at 227 nm and 368 nm, respectively, for the cell-mass sensing.

Fig. 3 shows the fluorescence emission spectra for the HPLC-grade water, filtered water, and HPLC-grade water containing all the MBBM culture medium components. The fluorescence spectrum for the water containing all the MBBM culture medium components agreed well with the spectra obtained for the HPLC-grade water and filtered water when the emission was set at 368 nm. Therefore, since the fluorescence intensity of the interference from the filtered water (1.59±1.2) and HPLC-grade water containing the MBBM components (1.37±0.8) was almost the same as that from the HPLC-grade water (1.10±0.75) when the emission was set at 368 nm (the difference was less than 5%), the effects of the MBBM components on the fluorescence peak were considered negligible (Fig. 3 and Table 1).

The effects of the MBBM culture medium components on determining the cell mass were also investigated. Thus, the original concentrations of the various MBBM culture medium components were individually added to cell suspensions in HPLC-grade water and the fluorescence intensity measured. The results of the interference study are listed in Table 1, where $FeSO_4 \cdot 7H_2O$, $MgSO_4 \cdot 7H_2O$, H_2SO_4, $ZnSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 5H_2O$, and $Na_2MoO_4 \cdot 2H_2O$ were not found to interfere, as the amplitudes were within ±1% of the fluorescence intensity of the reference. However, $C_{10}H_{16}N_{20}Na$, KOH, H_3BO_3, $MnCl_2 \cdot 4H_2O$, MoO_3, $Co(NO_3)_2 \cdot 6H_2O$, $NaNO_3$, $CaCl_2 \cdot 2H_2O$, K_2HPO_4, KH_2PO_4, and NaCl did interfere with the determination, although less than 3%. Thus, fluorescence spectroscopy was effective in obtaining information on the interactions among the components in the culture medium.

Fluorescence of Cells

Fig. 4 shows the fluorescence spectra for the cells at EX227/EM368 within the range of 320–420 nm in HPLC-grade water, filtered water, and HPLC-grade water containing

Table 1. Effect of various MBBM culture medium components on fluorescence intensity of 1.0 ml of cell suspension.

Compound	Concentration	Corrected fluorescence intensity (mg/l)	Interference	Error (%)
$NaNO_3$	246.5	151.7	1.7	1.1
$CaCl_2 \cdot 2H_2O$	24.99	152.2	2.2	1.5
$MgSO_4 \cdot 7H_2O$	73.95	151.3	1.3	0.9
K_2HPO_4	74.9	152.2	2.2	1.5
KH_2PO_4	175	152	2	1.3
NaCl	25.13	151.8	1.8	1.2
$C_{10}H_{16}N_{20}Na$	49.68	147.6	-2.4	-1.6
KOH	30.86	151.9	1.9	1.3
$FeSO_4 \cdot 7H_2O$	4.98	150.6	0.6	0.4
H_2SO_4	1	148.8	-1.2	-0.8
H_3BO_3	11.13	147.3	-2.7	-1.9
$ZnSO_4 \cdot 7H_2O$	8.83	148.3	-1.7	-1.0
$MnCl_2 \cdot 4H_2O$	1	153.6	3.6	2.4
MoO_3	6.06	147.7	-2.3	-1.5
$CuSO_4 \cdot 5H_2O$	2	150.8	0.8	0.5
$Co(NO_3)_2 \cdot 6H_2O$	0.49	148	-2	-1.3
$Na_2MoO_4 \cdot 2H_2O$	1.19	151.5	1.5	1.0

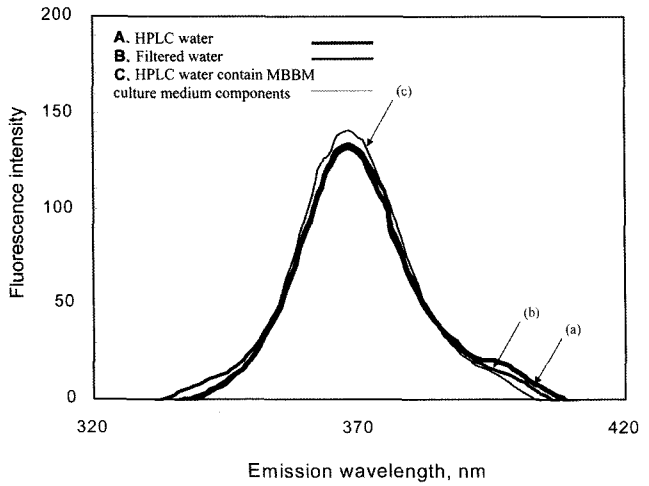

Fig. 4. Fluorescence emission spectra of cell mixture (MX; GN:GB:BR:RD=1:1:1:1) in (**A**) HPLC-grade water (n=10, RSD=1.1%), (**B**) filtered water (n=10, RSD=1.3%), and (**C**) HPLC-grade water plus all the components of MBBM culture medium (n=10, RSD=1.55%).

the MBBM culture medium components at 80 μg/ml. The difference in the fluorescence emission of the algal cells in the HPLC-grade, filtered water, and HPLC-grade water containing the MBBM components was less than 5% after subtracting the background peak intensities. Thus, based on these results, a fluorescence emission wavelength of 368 nm was selected for the cell-mass sensing. The wavelength accuracy of the spectral data (using the wavelength intensity) was determined by repeated scanning (×10) and found to be ±0.5 nm. Therefore, the excitation wavelength was fixed at 227 nm and the emission spectra of the cell suspensions recorded within a range of 320–420 nm.

Fig. 5 shows the fluorescence spectra for the green, green-brown, brown-red, and red cell suspensions. The fluorescence emission spectra at 368 nm with the excitation set at 227 nm showed similar intensities for the green, green-brown, brown-red, and red cells at a concentration of 100 μg/ml, where the reproducibility was determined using 10 separate individual measurements, giving a coefficient of variation (CV) of 1.21%. As such, since the fluorescence intensity of the various morphological forms of *H. pluvialis* did not change with the same concentration, this demonstrates that the fluorescence at the 368 nm wavelength was independent of the chlorophyll and astaxanthin pigments, and also NADPH, which has a maximum peak at 425 nm, thereby facilitating the convenient sensing of various *H. pluvialis* cells. Although the identity of the peak at 368 nm is not known, when comparing with references, the most possible candidate is the amino acid tryptophan, since this fluorophore exhibits similar fluorescence properties [20].

Calibration Graph and Statistical Analysis of Proposed Method

Fig. 6 reveals the obvious effect of the algal concentration on the fluorescence emission intensity. In the calibration experiments, the high background interference peak was subtracted from the cell peak to overcome the water interference peak. The intensity of the normalized fluorescence values for the standards was then drawn versus the cell mass, and a linear calibration curve obtained based on the 0–140 μg/ml concentration range of the tested samples.

The highest fluorescence intensity of 231 was observed with the 140 μg/ml cell suspensions (Fig. 6). The calibration curve for the cell mass using fluorescence spectroscopy had the following equation between a 0 and 140 μg/ml cell mass:

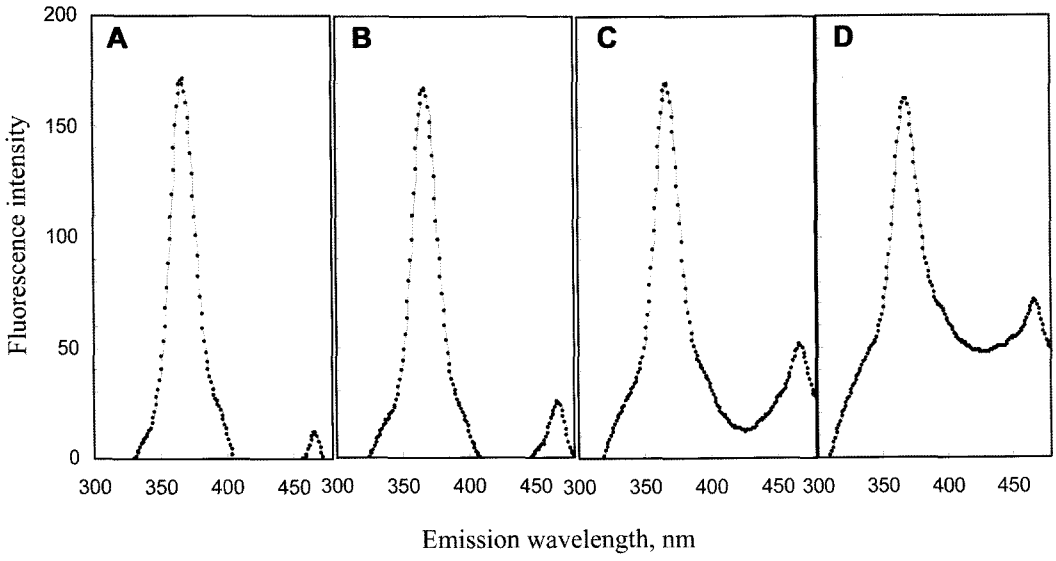

Fig. 5. Fluorescence emission spectra of (**A**) green, (**B**) green-brown, (**C**) brown-red, and (**D**) red cells in HPLC-grade water (cell mass=100 μg/ml).

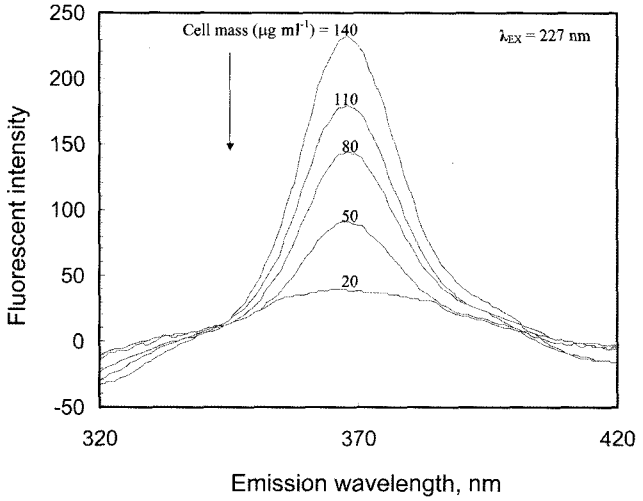

Fig. 6. Fluorescence emission spectra of cell mixture (MX; GN:GB:BR:RD=1:1:1:1) for cell mass of 20, 50, 80, 110, and 140 μg/ml.

$$F=1.6333\ C_{mass}+1.35 \qquad (R^2=0.9938).$$

where the constant 1.35 corresponds to the background interference. The reproducibility was determined using 10 separate individual measurements, giving a CV of 1.02%.

A statistical analysis of the proposed method and the DCW are compared in Table 2. The mean values were compared with the null hypothesis and retained a good agreement. The data from the fluorescence spectra also exhibited a greater variance than the data obtained from the DCW method, thereby demonstrating a higher sensitivity. The interference of the MBBM culture medium components had no significant effect on the fluorescence intensity of the cell measurements, yet increased slightly with the MBBM culture medium components during the cultivation of *H. pluvialis*. The detection limit obtained for the various kinds of cell was 20 μg/ml.

A simple and rapid fluorescence spectroscopic method has been presented for determining the various kinds of *H. pluvialis* cells in water and a real cultivation broth. The proposed whole-cell-based sensing system does not require any pretreatment, and has potential use as a noninvasive method for online cell-mass determination. The detection limit obtained for the various kinds of cell were in the microgram per liter range, which is at least a couple orders of magnitude lower than the cell-mass detection limit with the DCW. The analytical characteristics of the proposed method, including the detection limit and coefficient of variance, were determined, plus an interference analysis of the cell mass was also performed. The proposed method was applied to determine the cell mass in a culture broth of *H. pluvialis*, and achieved a high degree of accuracy and sensitivity. The proposed method is also suitable for routine analyses, simple, rapid, and reliable. A good agreement was achieved between the results obtained with the proposed and a comparative method. It was demonstrated that a variety of cells did not affect the model, making it an effective alternative to the existing methods used for detecting the *H. pluvialis* cell mass. Since the spectrofluorophotometer operation is as simple as a spectrophotometer operation, the proposed method is suitable for routine analyses. The proposed whole-cell-based sensing system also does not require any pretreatment, and has potential use as a noninvasive method for the online cell-mass determination of *H. pluvialis*, as well as phytoplankton.

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Table 2. Precision comparison of DCW and proposed method for measuring cell mass in 1.0 ml of *H. pluvialis* culture broth.

	DCW				Proposed method			
	G	GB	BR	R	G	GB	BR	R
Mean	1.84	1.79	1.801	1.59	1.80	1.81	1.80	1.79
SD	0.267	0.259	0.310	0.303	0.195	0.211	0.182	0.142
Minimum value	2.12	2.08	2.15	1.95	2.05	2.02	1.07	2.01
Maximum value	1.22	1.21	1.25	1.20	1.57	1.43	1.62	1.62
Variance	0.192	0.182	0.245	0.267	0.166	0.16	0.152	0.112
Number of measurements (N)	10	10	10	10	10	10	10	10
t-test (95% confidence level)					0.383	0.297	-0.880	-1.84

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