Prediction of Microalgae Total Solid Concentration by Using Image Pattern Technique

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Digital image processing have the potential for estimating biomass during cultivation of microalgae in Photobioreactor (PBR). In this research, computer algorithm and non-destructive method were implemented to predict the total concentrated solid of dry microalgae. This research used the native microalgae samples from the experimental facility located at the Minamisoma city of Fukushima Prefecture in Japan. Dry microalgae (DCW) were dissolved in a predetermined concentration range up to 12 g DCW L⁻¹ and proven to be efficiently used up to 3 g DCW L⁻¹. Raw red, green and blue (RGB) values in biomass were extracted and converted into Grayscale (GS) images. Furthermore, GS images were compared with seven conversion methods for determining the most suitable conversion results. The GS methods were investigated: luminance, intensity, monotonic, desaturation, average, minimum decomposition and lightness grayscale methods. The GS method was used to simplify the algorithm, for increasing efficiency in analyzing images. Each GS image of microalgae biomass aimed to derive a special pattern that only each image has in accordance with its concentration. For the result, luminance GS was found the most suitable method for recognizing color pattern identifier to determine total solid concentration using the image taken from the mobile device (R² = 0.9033).

Key Words
Dry microalgae, Image processing, Grayscale methods

1. Introduction

Image processing is a method to perform various operations on an image for extracting useful information. Nowadays, image processing is one of the rapidly growing technologies for non-destructive measurement. It forms core research area within engineering and computer science disciplines. Furthermore, image processing extended to biosystem and bioenergy processing to estimate the amount

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of biomass yield in a growing season.

Microalgae is one of the potential biomass and has been widely used for various human needs, such as pharmaceutical industry and alternative energy source as biodiesel. Technical issues on microalgae cultivation focuses on assessment, quantification encouraging, and many researchers are keen to develop with more precise and non-destructive way of measurement. Technologies and methods for measuring microalgae concentrations in photobioreactor (PBR) have been performed by previous researchers, such as flow cytometry spectroscopy, RGB analysis using CCD camera mode, macroscopic suspension color, and CCD camera with black chamber.

Some of the previous researches were reported the prediction accuracy of biomass concentration using image processing and analysis. Such as, macroscopic image with RGB average and luminescence GS method was used for measuring biomass concentration for 3 types of microalgae: Chlorella, Botryococcus and Ettlia sp ($R^2 = 0.91$). The application was used in Adobe Photoshop. Again, a digital camera and scanner were used for RGB image analysis in another 3 types of microalgae: Trichormus variabilis, $R^2 = 0.88$, Botrydiopsis cf. intercedens, $R^2 = 0.98$, Chlorella vulgaris, $R^2 = 0.95$. By using Rhodococcus, image was taken using COHU camera and fluorescense microscopy. The RGB image analysis method was used ($R^2 = 0.85$). Using Isochrysis galbana (T-ISO) from a CCD camera with RGB average and luminescence method ($R^2=0.99$). However, these microalgae quantification methods take time, higher cost, and inefficient to bring in laboratory for processing. The accurate and appropriate methods are needed for measuring the microalgae concentration within their cultivation systems. Ideally, such methods should be nondestructive, fast, accurate and with good precision. This research carried out a combination of image processing using a mobile camera to calculate the total solid concentration of microalgae content based on correlation between RGB and GS value.

2. Materials and Methods

2.1 Method

Fig. 1 shows the methodology for this empirical study that consists of three phases. In the first phase, an image was captured for microalgae in photobioreactor using the camera. During image acquisition, the images were captured considering the distance of the lens with the objects, defined the Region of Interest (ROI) on the object, temperature and light intensity.

The second phase was to fill 24-bit image or RGB image into computer system, these phase evaluates the color of each image, such as finding values for each RGB channel, finding the RGB minimum and maximum value and normalizing value and laying down the luminance, intensity, monotonic, desaturation, average, minimum decomposition and lightness grayscale methods.

The main reason of grayscale representations are often used for extracting descriptors instead of operating on color images directly is that grayscale simplifies the algorithm and reduces computational requirements.

The third phase was to adjust the image data with...
the values that have been obtained to get a special pattern on each image. Where with this pattern the concentration of each cell can be known accurately. The application of the GS algorithm were performed using MATLAB R2017b® (Math works, USA).

The RGB color model was implemented in different ways, depending on the capabilities of the system was introduced. Colors in computer processing are usually held as a combination of values of Red, Green and Blue for each pixel. Typically, in an 8-bit data scheme, each of these can range from 0 to 255. For example, Red=0 means ‘no red in color’ and Red=255 means (in 8-bit color) ‘maximum, full-saturated, red’.

For calculations, these values are often normalized to a range between 0 and 1 (calculated as Red/255, Green/255 and Blue/255). It also can be used any number of bits for each RGB color like 12, 14, 16, 20, 24 or 32 bits. To normalize these to 0 to 1, again simply divide by the maximum value they can have. When RGB values are equal, then the resultant color is gray. When they are all zero, the result is black, and when they are all 1 then the result is white. Hence, in an 8-bit color scheme, the values of 0 to 255, giving 256 black/white shades, which are normalized to the range from 0 to 1. In a 16-bit scheme, each RGB color can have 65,536 shades, which can be normalized to the range 0 to 1 (by dividing by 65,535). This gives many more color possibilities than 8-bit color, but results in much larger files and requires much more computer power to process.

Each image captured in 3,024 rows x 4,032 columns pixels. The photographic setting is arranged according to Table 1. After confirmation of few tests, were selected after several tests. The ISO level was set to 100 aims to avoid the effect of noise on the image, where the noise greatly affects the image, the direct effect makes the RGB expression reduced and lower quality.

### 2.2 Methodology

The microalgae models for this research are native microalgae that were collected in experimental facility at Minamisoma city. Cultivation experiments were conducted at the Minamisoma center for microalgae biomass production, Minamisoma city. The dominant microalgae general in the samples were identified to be Desmodesmus spp, Scenedesmus spp, Dictyosphaerium spp and Klebsormidium spp.

The images were captured while ASTM glass cylinders contained with dry microalgae and dissolved through continuous circular movement. The dissolved solution was placed just below the light source. ASTM glass cylinders were varied in size, shape, reflectivity and transparency even though they may be of the same volumetric volume. ASTM E-1272 (ASTM International, USA) glass cylinders were used. The material with a capacity of 500 ml.

Camera lens were placed precisely attached to Region of Interest (ROI) which has been predetermined in glass cylinders, to avoid varying illumination value for all images, the same ROI rectangle was processed (Fig. 2). The camera lens was attached directly to the glass cylinders to avoid the occurrence of light bias, such as the initial bias reflections on the surface of the camera lens and the glass cylinders.

Hard to get an image with fixed intensity value if the image takes directly from the surface, because the presence of air bubbles in the solution and microalgae solution flows continuously. So much light reflection and refraction occurred.

| Table 1 Photographic settings |
|-------------------------------|
| Parameter | Settings |
| Mode | Aperture priority |
| ISO | 100 |
| HV resolution | 72 dpi |
| Exposure time | 1/4s |
| F-stop | F/18 |
| Focal length | 4mm |
| Metering mode | Pattern |

![Fig. 2 The proposed scheme to analyze the microalgae image consisting of two interconnection sections: (i) a light source and (ii) the arrangement of tool components](image-url)
Data were taken using camera device iPhone 8+ with 12MP wide-angle and telephoto cameras and Wide-angle: f/1.8 aperture, Telephoto: f/2.8 aperture (Apple, Inc., USA) using 3-way panhead tripod WT-3110A. The resulting digital image was analyzed with MATLAB R2017b® (Mathworks, Inc., USA).

2.3 Digital image processing

Image is a two-dimensional function f(x,y), where x and y are the spatial (plane) coordinates, and the amplitude of f at any pair of coordinates (x,y) is called the intensity of the image at that level. If x,y and the amplitude values of f are finite and discrete quantities, it calls a digital image. A digital image is composed of a finite number of elements called pixels, each of which has a location and value.

Grayscale is a range of shades of gray without apparent color. The darkest possible shade is black, which is the total absence of transmitted or reflected light. The lightest possible shade is white, the total transmission or reflection of light at all visible wavelength. Intermediate shades of gray are represented by equal brightness levels of the three primary colors (red, green and blue) for transmitted light, or equal amounts of the three primary pigments (cyan, magenta and yellow) for reflected light.

The flowchart of the software is shown in Fig. 3. The delay step explaining how the program continuously get the RGB value and convert into gray color using GS algorithm. In this research, grayscale images were utilized rather than RGB color images. The connection was established between the gray value and light intensity. The advantage for this method allowed us to avoid problems relating to various colors in light sources. Image capture for each sample repeated ten times 3. Camera lens attached directly to the cylindrical glass wall (Fig. 4).

The developed algorithm was proposed to recognize the concentration color of Native algae communities in Minamisoma, Japan.

The images were captured from 0.1% to 2.4% concentration and shows the color concentration that causes the difference of RGB value. The varying color concentration can be recognized using camera device.

The color differences were occurred due to the different concentrations affecting the light velocity. At concentrations of 0.1% to 2.4%, the density of the microalgae composition with the solution was very high and caused interruptions of light reflectance.

2.4 RGB channel values extraction

To extract the RGB values for every image, the image tool feature of MATLAB2017b® was used to display the pixel info (x,y) [R,G,B] and pixel region. The result (Fig. 5) shows the value of each RGB channel in 4032 x 3024 pixels, referred the total number, 1907638472 pixels overall for each channel. To extract the value of each channel used command,

The extracted channel value was used to obtain the normalization values (r,g,b), this normalization value was required for the grayscale algorithm and to minimize the effect of excessive light on the image and reduced the shadow effect to facilitate the process of image analysis.
2.5 Grayscale algorithm conversion

From the entire set of images captured, in a controlled environmental conditions (light source, light intensity, temperature). Light intensity was ranged from 79-90 μmol s⁻¹ m⁻² and chosen to reduce the possible influence of the external illumination on the light distribution profile. Once, the GS algorithm is applied, an image value was obtained, and the last step was displayed in the converted image. A pre-developed image analysis algorithm was then utilized to convert the raw image from RGB space into luminance, intensity, monotonic, desaturation, average, minimum decomposition and lightness GS.

The luminance GS based on the equation (1) and recommended by ITU (International Telecommunications Union), technically luminance of light is a measure of the luminous intensity per unit area of light travelling in a given direction. Luminance is designed to match human brightness perception by using a weighted combination of the RGB channels.

This research takes the same amount of light energy and concentrated at a point or spread over a wider area. The point seems brighter than the area because the intensity per unit area was higher, even though the total energy was same. In other words, luminance was a measure of light not over time but over an area, luminance GS (LGS):

\[
LGS = 0.222 \times R + 0.707 \times G + 0.071 \times B
\]  

\[
GRAY_{new}^{XY} = \frac{1}{25} \sum_{x=-2}^{x=2} \sum_{y=-2}^{y=2} GRAY_{old}^{XY}
\]  

Where LGS stands for luminance using GS algorithm for RGB values and GRAY is stands calculation of luminance stands using normalized RGB values. X and Y are the axial positions of the pixel in the image. The gray scale images were filtered by a 5 x 5 neighborhood averaging filter (equation (2)) to remove noises.

GS based (equation (3)), intensity method is the simplest one. The equation takes the average of three colors. Since it's an RGB image, it means have to average the RGB in the GS image and can be expressed as:

\[
AGS = \frac{(R + G + B)}{3}
\]

Intensity and average algorithm is the smallest discernible change in intensity level. Stated with 8 bits, 12 bits, and 16 bits. The equation that is often used for average can be expressed as:

\[
AGS = 0.333 \times R + 0.333 \times G + 0.333 \times B
\]

The monotonic grayscale was based on the equation (5). Monotonic Grayscale (MLi) can be expressed as:

\[
MLi = (0.299 \times Ri) + (0.587 \times Gi) + (0.114 \times Bi)
\]

Where i is a color index and Ri, Gi, Bi represent values of the red, green and blue channels, respectively. This expression is likely that most software use to convert a color image into grayscale. Considering 24-bit color space (8 bits per channel), the maximum value of the channel is 255.

For the next grayscale equation, Desaturation grayscale was considered. Desaturating image works by converting an RGB triplet to an HSL triplet, then forcing the saturation to zero. Basically, this method takes a color and converts the image to its least saturated variant. The mathematics of this conversion are more complex. A pixel can be desaturated by finding the midpoint between the maximum of (R, G, B) and the minimum of (R, G, B). In the HSI color model, S is the saturation component of the color. Saturation describes the intensity (purity) of that hue. When
color is fully saturated, the color is considered in purest (truest) version. Primary colors: red, blue or yellow are considered truest version color as they are fully saturated.

Desaturating an image means setting the S component to 0. This can be done by averaging the minimum and the maximum values or R, G, and B. Desaturation Grayscale (DGS) can be expressed as:

\[
DGS = \frac{(\text{Max}(RGB) + \text{Min}(RGB))}{2}
\]  

Lightness is a perceptually uniform grayscale representation used in the CIELAB and CIELUV color spaces. This means an increment in lightness should more closely correspond to human perception, which can be achieved using a nonlinear transformation of the RGB color space and can be expressed as:

\[
LGS = (\text{max}(R,G,B) + (\text{min}(R,G,B)) \times 0.5
\]  

For the image processing, the variables of class uint8 (data type) value is 0-255, when 0 is the minimum for any RGB color and 255 is the maximum for any RGB color. To define any color, the Red (R), Green (G), and Blue (B) values must be specified. Grayscale images only need one value (0 = black, 255 = white).

The luminance is a good parameter to describe the brightness as it reflects the response of the human eye to different colors, but it is not perfect. It is possible to design a color image whose luminance is quickly increasing but the image appears to have darker parts, which leads to unnatural look in the presented data. Thus, another parameter, which must be considered, is a normalized luminance which can be defined as:

\[
L' = \frac{L}{255}
\]

Intensity and average GS is the most common grayscale conversion routine, fast and simple. This formula generates a reasonably nice grayscale equivalent and its simplicity makes it easy to implement and optimize. However, this formula is not without shortcomings while fast and simple but low quality for representing shades of gray relative to the humans perceive the brightness.

Monotonic GS has excellent results in representing color brightness and becomes one of the good parameters also. In many different cases, monotonic GS is often combined with luminance techniques to better expose bright colors in the image. For Desaturation GS, in terms of the RGB color space, desaturation forces each pixel to a point along the neutral axis running from (0, 0, 0) to (255, 255, 255). Desaturation results in a flatter, softer grayscale image. For lightness method, the image is much brighter than 6 methods, but this brightness causes the color pattern and color descriptor for each image to be faint and hard to recognize.

Minimum decomposition, this method forces each pixel to the lowest of its red, green, and blue values. Decomposition only focus for which color value is lowest not which channel it comes from, so the result is very darker.

For MATLAB software, the seven algorithm formulas are applied to the command form. The initial logic command to call the image in RGB form then processed using grayscale algorithm. After this step, the RGB image will be converted into grayscale form. The pseudocode using MATLAB can be traced in Fig. 6. The command to display the converted image results are also combined into this grayscale algorithm.

3. Normalizing

To get rid of the distortions caused by lights and shadows in an image. Normalizing the RGB values of an image is an effective way. Each raw RGB value is normalized (r, g, b) with the pixel has the intensities R, G, and B in the respective channels, its normalized values will be R/S, G/S and B/S (where, S=R+G+B) or the common equation is:

\[
f = \frac{R}{R + G + B}
\]

\[
g = \frac{G}{R + G + B}
\]

\[
b = \frac{B}{R + G + B}
\]

For normalized RGB, some key changes in the image like the shadows with different color have vanished.
4. Results and Discussion

From the datasets of images, the comparison of samples results using seven methods of grayscale algorithm, luminance GS required an extra computation time. However, results were found in a more dynamic grayscale image. The results listed in Table 2.

Red color values at concentrations of 0.1% to 0.6% move normally in accordance with the cell concentration on the dissolved microalgae samples, this is due to the greater concentration then the density was increased.

Furthermore, the incoming light was reflected and absorbed without forwarding (opaque), but for blue colors indicate fluctuations in values. In Fig. 7, the range of linearity was obtained up to concentration of 12 g L\(^{-1}\) with \(R^2=0.9103\).

The conversion results using seven methods of grayscale is listed in Table 3. The comparison of the seven grayscale methods show the brightness and darkness sides. For luminance and monotonic methods, the grayscale images spreaded uniformly.

| Solid Content of DCW (%) | Size/pixels | Normalize R channel value (r) | Normalize G channel value (g) | Normalize B channel value (b) |
|-------------------------|-------------|------------------------------|------------------------------|------------------------------|
| 0.10                    | 3024 x 4032 | 158.53                       | 160.89                       | 18.11                        |
| 0.11                    | 3024 x 4032 | 156.15                       | 159.70                       | 14.12                        |
| 0.12                    | 3024 x 4032 | 155.59                       | 159.07                       | 14.48                        |
| 0.13                    | 3024 x 4032 | 153.10                       | 158.44                       | 16.19                        |
| 0.14                    | 3024 x 4032 | 149.30                       | 155.21                       | 15.67                        |
| 0.15                    | 3024 x 4032 | 143.61                       | 149.38                       | 15.18                        |
| 0.16                    | 3024 x 4032 | 142.58                       | 152.51                       | 21.45                        |
| 0.17                    | 3024 x 4032 | 129.56                       | 139.82                       | 19.15                        |
| 0.18                    | 3024 x 4032 | 122.18                       | 134.30                       | 13.48                        |
| 0.19                    | 3024 x 4032 | 117.31                       | 128.52                       | 15.97                        |
| 0.20                    | 3024 x 4032 | 112.05                       | 121.62                       | 23.35                        |
| 0.60                    | 3024 x 4032 | 82.28                        | 114.59                       | 13.13                        |
| 1.00                    | 3024 x 4032 | 66.68                        | 67.92                        | 31.26                        |
| 1.40                    | 3024 x 4032 | 49.71                        | 50.33                        | 28.88                        |
| 1.80                    | 3024 x 4032 | 45.21                        | 46.31                        | 24.55                        |
| 2.40                    | 3024 x 4032 | 41.84                        | 42.34                        | 21.37                        |

Fig. 7 RGB image value of DCW
Table 3  RGB image transform into grayscale using seven algorithms

| No. | Microalgae Image | Grayscale Image         | Value          |
|-----|------------------|-------------------------|----------------|
| 1   | ![RGB Image](image1.png) | R: 1.36  
G: 1.48  
B: 284   
r: 112.05  
g: 121.62  
b: 23.35 |               |
| 2   | ![Luminance GS](image2.png) |                  | 112.30         |
| 3   | ![Monotonic GS](image3.png) |                  | 1.04           |
| 4   | ![Intensity GS](image4.png) |                  | 1.31           |
| 5   | ![Desaturation GS](image5.png) |                  | 133.30         |
| 6   | ![Average GS](image6.png) |                  | 1.04           |
| 7   | ![Minimum Decomposition GS](image7.png) |                  | 23.35         |
| 8   | ![Lightness GS](image8.png) |                  | 208            |

For the luminance and monotonic GS, the gray level was evenly distributed and spread very well, the brightness was good and not too dark. In the intensity method, the visible brightness level was excessive, but overall the color pattern could be recognized well when the color pattern recognition was applied for each microalgae concentrated, as well as the average method, the distribution grayscale was quite well in color pattern recognition. For the minimum decomposition, it looked very dark and this method was not suitable for the color pattern recognition. In the lightness
method, a fluctuation was occurred for each concentration and had a bad effect on the microalgae image for color pattern recognition. Image conversion value (Table 4) was implemented with seven GS algorithms. The color pattern recognition is observed using $R^2$ in line chart for each microalgae concentration.

From Fig. 8, it can be concluded that luminance, monotonic, intensity, desaturation and average grayscale methods had differences, but they were not significant. In luminance GS, noise could be avoided on the image and average GS seem darker.

In range between 0.1% - 0.6%, the luminance method proved to be very effective in predicting the value of total solid concentration with a value of $R^2 = 0.9033$ (Fig. 9), more than 0.6%-2.4% was decreased in accuracy due to the thicker and darker solution of microalgae.

### Table 4 Image conversion values that have implemented for seven grayscale algorithms

| Solid content of DCW(%) | Luminance Grayscale (LGS) | Intensity Grayscale (IGS) | Monotonic luminance (increasing= i+1) | Desaturation Grayscale (GS) |
|-------------------------|---------------------------|---------------------------|--------------------------------------|----------------------------|
| 0.10                    | 149.91                    | 1.33                      | 1.70                                 | 169.95                     |
| 0.11                    | 148.27                    | 1.34                      | 1.73                                 | 166.77                     |
| 0.12                    | 147.72                    | 1.33                      | 1.72                                 | 166.31                     |
| 0.13                    | 146.85                    | 1.33                      | 1.71                                 | 166.54                     |
| 0.14                    | 143.70                    | 1.30                      | 1.67                                 | 163.06                     |
| 0.15                    | 138.29                    | 1.25                      | 1.63                                 | 156.97                     |
| 0.16                    | 140.72                    | 1.28                      | 1.64                                 | 163.24                     |
| 0.17                    | 128.72                    | 1.17                      | 1.49                                 | 149.40                     |
| 0.18                    | 122.79                    | 1.09                      | 1.42                                 | 141.05                     |
| 0.19                    | 117.81                    | 1.06                      | 1.36                                 | 136.51                     |
| 0.20                    | 112.30                    | 1.04                      | 1.31                                 | 133.30                     |
| 0.60                    | 72.09                     | 75.3                      | 86.1                                 | 93.85                      |
| 1.00                    | 65.35                     | 68.2                      | 78.0                                 | 84.32                      |
| 1.40                    | 48.57                     | 50.2                      | 57.8                                 | 64.77                      |
| 1.80                    | 44.43                     | 38.3                      | 43.8                                 | 58.59                      |
| 2.40                    | 40.66                     | 39.5                      | 42.6                                 | 53.03                      |

| Solid content of DCW(%) | Lightness Grayscale (LGS) | Average Grayscale (AGS) | Minimum decomposition |
|-------------------------|---------------------------|-------------------------|-----------------------|
| 0.10                    | 224                      | 1.33                    | 18.11                 |
| 0.11                    | 215                      | 1.33                    | 14.13                 |
| 0.12                    | 205                      | 1.33                    | 14.48                 |
| 0.13                    | 215                      | 1.33                    | 16.20                 |
| 0.14                    | 224                      | 1.30                    | 15.68                 |
| 0.15                    | 218                      | 1.25                    | 15.18                 |
| 0.16                    | 216                      | 1.28                    | 21.45                 |
| 0.17                    | 215                      | 1.17                    | 19.74                 |
| 0.18                    | 193                      | 1.09                    | 13.49                 |
| 0.19                    | 190                      | 1.06                    | 15.98                 |
| 0.20                    | 208                      | 1.04                    | 23.35                 |
| 0.60                    | 165                      | 75.1                    | 33.13                 |
| 1.00                    | 153                      | 68.1                    | 31.26                 |
| 1.40                    | 84                       | 50.1                    | 28.89                 |
| 1.80                    | 82                       | 38.2                    | 24.55                 |
| 2.40                    | 91                       | 39.5                    | 21.37                 |

### 5. Conclusions

The research was conducted for biomass microalgae analysis using digital image processing for quantification, prediction of total solid concentration of microalgae. The method does not require a complex procedure for sample preparation since it is empirical and directly on physical-based measurement.

The application of image pattern technique with the grayscale method was implemented on the microalgae solution with total solid concentration 12 g DCW L⁻¹ and total solid prediction was effective up to 3 g DCW L⁻¹, with Luminance GS as the most suitable method compared to the six grayscale methods that have been investigated.

It can be concluded that the luminance GS method was suitable to be applied for total solid of microalgae measurement in photobioreactor by using camera device in the controlled environmental conditions specified in this
Fig. 8  Microalgae concentration using seven grayscale algorithms methods (a) luminance, (b) intensity, (c) monotonic, (d) desaturation, (e) average, (f) minimum decomposition and (g) lightness GS.
Fig. 9 Microalgae concentration with seven grayscale algorithms methods with total solid concentration up to 3 g DCW L^{-1} (a) luminance, (b) intensity, (c) monotonic, (d) desaturation, (e) average, (f) minimum decomposition and (g) lightness GS.
research. The method was also noninvasive, nondestructive, and could measure microalgae biomass range up to 3 g DCW L⁻¹.

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