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To cite this version:

N'goran Urbain Florent Niangoran, David Buso, Laurent Canale, Cisse Theodore Haba, Feng Tian, et al.. Spirulina platensis biomass estimation using reflectance signal and infragram technology. Journal of Algal Biomass Utilization, 2017, 8 (4), pp.69-76. hal-02998753

HAL Id: hal-02998753

https://hal.science/hal-02998753v1

Submitted on 10 Nov 2020

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Spirulina platensis biomass estimation using reflectance signal and infragram technology

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Abstract

A photosynthetic plant reflects a large amount of infrared light and absorbs a lot of red light during the process of photosynthesis. This plant response to the light is due to chlorophyll. Thus, it is possible to use reflectance spectra or visible and infrared images to study the photosynthetic activity of plants. In this paper, dry biomass estimation of *spirulina platensis* using two different approaches are presented. The first one use a contrast function between visible and near infrared reflectance image of vegetation obtained with infragram camera to estimate the spectral indices like the Normalized Difference Vegetation Index (NDVI). The second technique is based on the reflectance spectra to estimate the dry biomass of *spirulina platensis*. The results obtained show that dry biomass from 0.1 to 0.8 g/L are linearly correlated with NDVI values and reflectance signals respectively with R^2 = 0.9881 and R^2 = 0.9934 while the usual spectrophotometry method gives R^2 = 0.9968.

Keywords: Batch culture, Infragram, biomass estimation, NDVI, Reflectance spectra

Introduction

Microalgae are an important source of biomass and biochemical recoverable components. This is the case of *spirulina platensis, a* blue-green microalgae rich in protein. It also contains very interesting special pigments called phycocyanin which may be used as a dye (Sguera, 2008). This microalgue can be cultivated in various form, as outdoor (open tanks) or indoor (photobioreactors). In the two cases, it is important to control the culture to optimize the culture condition (media composition, lighting, pH, temperature, etc.). Nowadays, traditional methods exist for measurements of biological parameters such as biomass, cell size and morphology, pigments and lipid content (Lee et al., 1998, Silveira, 2007). Cells count and spectrophotometry have been widely used to estimate theses parameters as well as turbidity and fluorescence of the culture suspension (Meireles et al., 2002, Chen and Vaidyanathan, 2012). This step in necessary to optimize production process including harvesting and reduces costs. However, these measuring instruments are relatively expensive. More works were performed to monitor and control microalgues. Jia et al designed optical density sensor using multi-wavelength to monitor microalgae growth in real time (Jia et al., 2015). It is also the case of Sandnes et al who used near infrared optical density sensors to monitor algae culture (Sandnes et al., 2006). The reflectance spectrum of the *spirulina* is dominated by chlorophyll. In the near infrared, it presents peaks at 830 nm and 720 nm. The intensity of these peaks varies depending on the biomass (Gitelson et al., 1995, Gitelson et al., 2000).
There are several spectral indices to characterize microalgae (Blondeau Patissier et al., 2014). These indices are used and developed in remote sensing. They integrate several spectral bands, which are generally narrow, in order to differentiate the microalgae studied from other microorganisms present in the medium. Among these indices are the CMI (Cyanobacteria and Macrophyte Index), the FAI (Floating Algae Index), etc. (Hu 2009a, Liang et al., 2017). These indices are calculated from images obtained from multispectral or hyperspectral cameras which are generally expensive. Near infrared photography is one of the latest techniques used in large farms to study plant photosynthesis activity using expensive sensors mounted on satellites and airplanes (Public Lab, 2017b). In small scale farming, the same technology is used but with inexpensive cameras such as Infragram.

In this work, we calculated indices from the obtained infragram images and correlate them to the spirulina biomass concentration. The choice of these indexes is constrained by the infragram camera which provides only two spectral bands (Public lab, 2016a). There are simple indices, based on arithmetic operations between the red bands and near infrared spectra, in particular the differential index of vegetation (DVI), the vegetation index per quotient (RVI) and the NDVI. The disadvantage of the first two indices is that they are very sensitive to atmospheric variations. For the NDVI, the normalization by the sum of the two bands makes it possible to reduce the atmospheric parasitic effects.

In this study, the ability to estimate the biomass of *spirulina platensis* with two inexpensive tools based on the optical properties of the *spirulina* is shown. The first tool is based on infragram imaging and the second one

on reflectance measurement. These two techniques enable measurements without taking samples in the culture. Contrary to the usual method, spectrophotometry, where the medium must be diluted, non-turbid and non-diffuse, in the case of infragram imaging and reflectance measurement, the medium can be diffuse, concentrated and agitated.

Materials and methods

Culture Conditions

The microorganism used was *spirulina platensis* UTEX LB 2340, which was grown in Zarrouk medium (Zarrouk, 1966). The strain culture was grown in a 80 liters glass container with continuous illumination provided by white tubular fluorescent lamps at 32.5±0.5℃, and agitated by a circulating pump. For inoculation, a certain amount of the strain culture was taken, filtered by a 30µm strainer, and then diluted with Zarrouk.

The batch cultures were performed in chamber at 28°C. An aquarium made with transparent glass was selected as the incubator. 5 liters of Zarrouk medium were filled for the cultivation. As shown in figure 1, two LEDs plates were fixed beside the incubator. 8 red LEDs 660 nm (LHCPDP, OSRAM) at 400 $\mu mol \cdot m^{-2} \cdot S^{-1}$ were used to illuminate the culture. The (Photosynthetic Photon Flux Density) PPFD is measured by a spectrophotometer (specbos 1201) in an integrating sphere (diameter: 25cm). Wave maker pumps are used to agitate the culture solution. The flow velocity was 3000 L/h (Sunsun JVO-101A, WilTec Wildanger Technik GmbH, Germany).

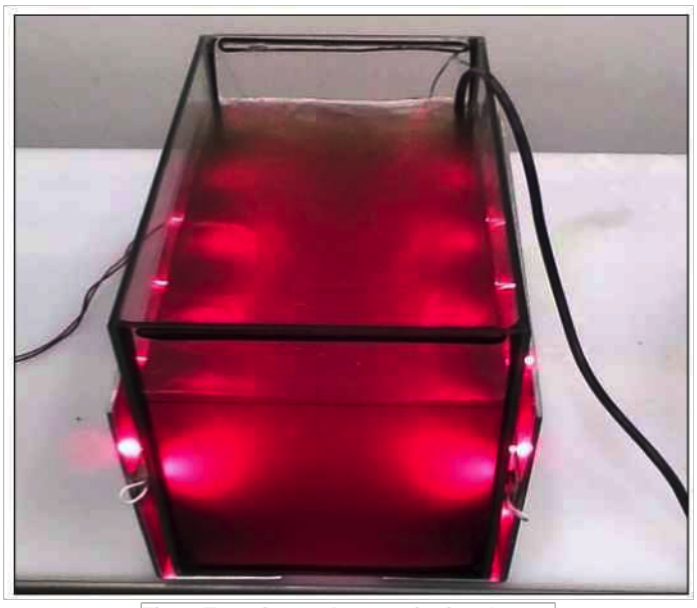

Fig 1. Experimental set up for incubators

Measurement of Biomass Concentration

The biomass of spirulina platensis was determined by measuring the optical density (OD) at 680 nm using UV/Vis/IR spectrophotometer (Spectronic 20 GeneisSYS, Spectronic Instruments, USA). The sample of the culture were filtered through pre-dried, pre-weighed cellulose acetate filter membranes (Sartorius Stedium Biotech, Germany) and washed with distilled water and with 0.9% sodium chloride to remove the non-soluble salts remaining on the filter before drying for 72 h at 35°C. After weighing the dried filter, we obtained the relationship between the biomass dry weight DW (g/L) and optical density OD_{680} with $R^2 = 0.9968$:

$$DW = 0.7247 \cdot OD_{680} + 0.0044 \quad (1)$$

Images acquisition and NDVI calculation

We used an infragram camera Point & Shoot of 5 MPix (2304×1536, Public Lab). It is a modified ordinary webcam (Mobius Action). Indeed the infrared filter was removed, and a red filter called written 25A is added to block the blue and green light (figure 2). This camera captures visible light (red light) on the red channel, and near infrared (NIR) on the blue and green channels.

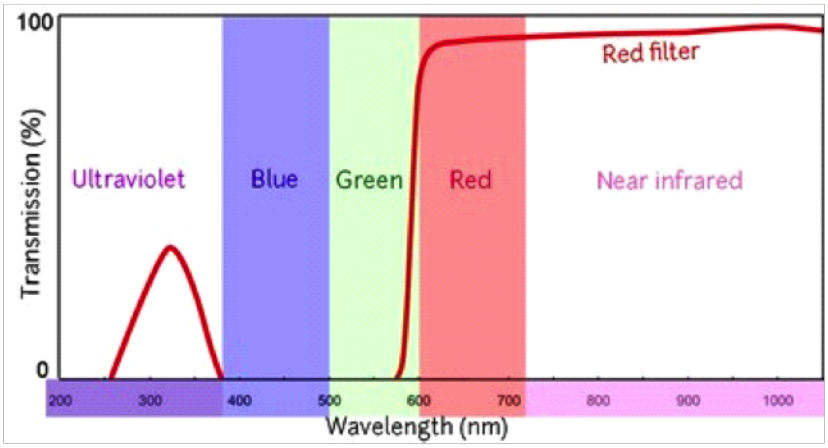

Fig 2. Spectral response of camera redfilter
(Source: https://publiclab.org/notes/cfastie/11-24-2015/dual-band-pass-filters)

The red channel will capture NIR, but also red light and the two will be mixed in an unknown proportion. So although this modified camera can capture a mostly pure NIR image (in the blue channel) it cannot capture a mostly pure visible image. Infragram technology requires reflectance from both visible and infrared region for assessing the amount of photosynthesis in plants. Each day, a sample of collected spirulina culture is exposed to sunlight for image acquisition (figure 3). This sample was homogenized in a small tank to maximize uniformity of the solution.

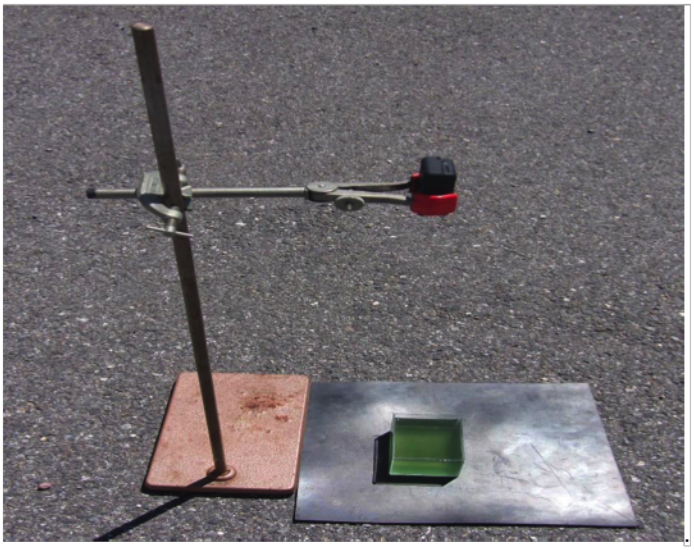

Fig 3. Experiment setup for infragram image acquired.

The NDVI is calculated by following equation:

$$\mathrm{NDVI} = \frac{R_{NIR} - R_{red}}{R_{NIR} + R_{red}} \qquad (2)$$

Where R_{NIR} is reflectance in the range from 700 to 1000 nm and R_{red} is reflectance in the range 600 to 700 nm respectively. An image in false color NDVI is then obtained and the average value of NDVI is calculated.
The calculation principle for this index is as follows:

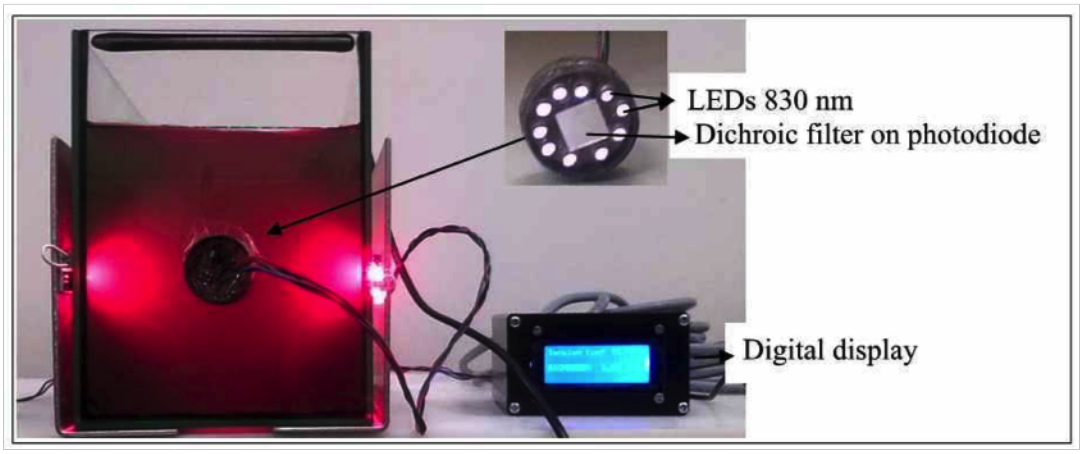

Fig 4. Experimental setup for reflectance signal measurement.

The greyscale image NDVI consists of pixels having the same intensity. This intensity is obtained by averaging the pixels of the calculated gross gray level image with the near infrared and red bands of the infragram image. The value of the NDVI index associated with the biomass of a given sample is obtained by the difference between the NDVI index of the sample and the NDVI of the Zarrouk culture medium. The experiments were carried out for 8 days over the period from 25 July to 01 August 2016 in Auzeville-Tolosane (France) 43.5327°N and longitude 1.4912°E. The global solar radiation allowed us to study the influence of the intensity of the solar illumination on NDVI values. Global radiation data were obtained from *meteo France* for the station located in Auzeville-Tolosane.

This station (latitude 43.529°N and longitude 1.504°E) is located at 1.6 km from the site were the experiment were carried out. Infragram images of two spirulina samples were taken with a biomass concentration equal to 0.2 g/L and 0.5 g/L at different times over two days. These samples were kept away from light to minimize biomass variations. For each measurement, the optical density at 680 nm is measured to ensure that there is no concentration variation.

Biomass measurement by reflectance signal

We designed a sensor to measure the reflectance signal at 830 nm. This sensor includes a photodiode (OPT101 from Texas Instruments) peak sensitivity is around 850 nm surrounded by 10 near infrared LEDs at 830 nm (TSHG5510 from Vishay Semiconductors). A polyethylene tube separates the Photodiode and LEDs. We used infrared filter with a cut-on wavelength at 750 nm to cut the light of 660 nm (Dichroic longpass filter, Edmund optics) (figure 5).

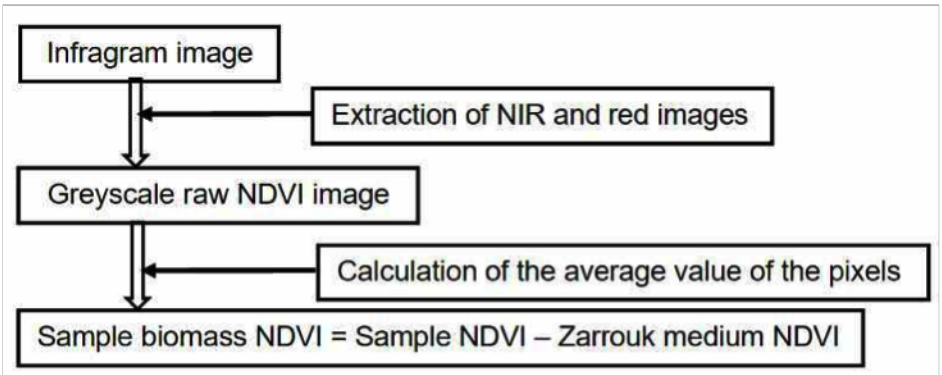

Fig 4. NDVI calculation principle.

The reflectance normalized value of sample is given by equation 3:

$$R\,(\%) = \frac{R_{Sample} - R_{CultureMeduim}}{R_{Maximum} - R_{CultureMedium}} \qquad (3)$$

In which R_{Sample}, $R_{CultureMedium}$ are respectively the voltages corresponding to the reflectance signals of the sample **and the culture medium. $R_{Maximum}$ is the voltage corresponding to the sample which have a maximum reflectance signal (in Volt).**

Results and Discussion

Biomass evolution as a NDVI function

The infragram images of the culture medium and spirulina samples of the 6 first days of spirulina culture are shown in figure 6. We can see differences in color between the images. The infragram images take a light blue color as the biomass increases. The infragram image of a vegetation containing a certain amount of chlorophyll is sky blue in color (Pubic Lab 2016c).

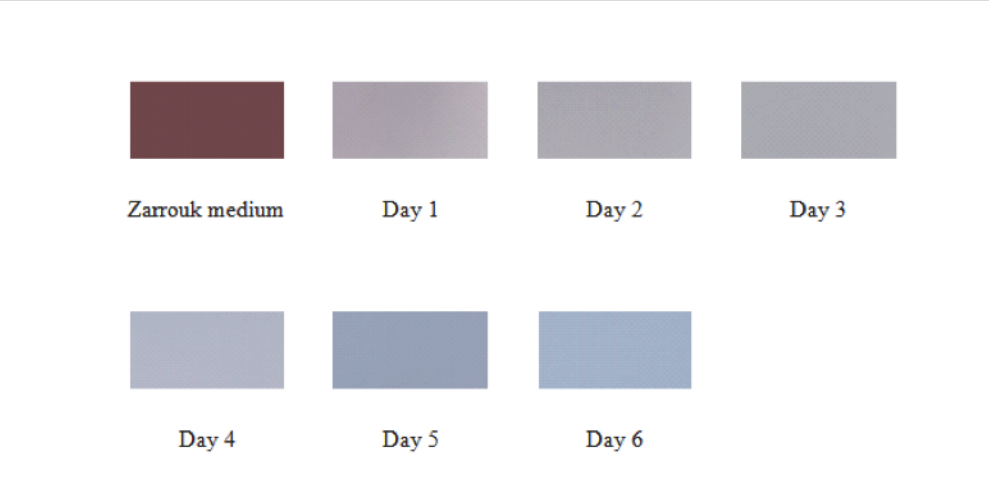

Fig 6. Infragram images of culture medium and spirulina samples.

Figure 7 shows the NDVI gray level images obtained from the of infragram images. Grayscale allows to see contrast differences between infrared and red components of infragram images. For example, in figure 7 the increase in biomass by observing the NDVI images can be appreciated.

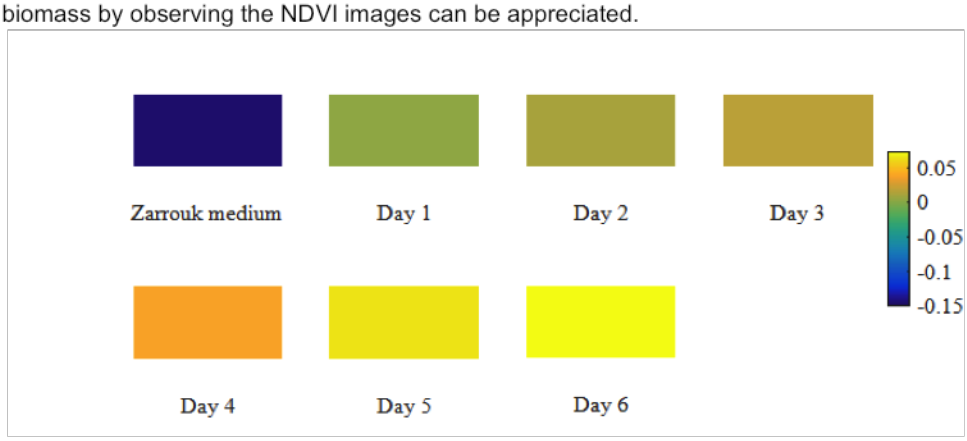

Fig 7. NDVI images of culture medium and spirulina samples.

In table 1, different average NDVI values corresponding to the three cultures are presented. There is an average standard deviation of ± $19.58 \cdot 10^{-5}$ to the pixel values composing the NDVI grayscale image. This standard deviation value indicates a good homogenization of the samples of spirulina. During the first six days of culture, NDVI values are consistent. These values increase with the biomass concentration under relatively high solar illumination (between 217 and 310 $W \cdot m^{-2}$). For the last two days, low NDVI values are found which is in contradiction with the increase in biomass concentration. This could be justified by the low solar illumination (below 160 $W \cdot m^{-2}$). We can therefore assert that the intensity of the solar irradiance has an influence on the NDVI values in our study.

Table 1. NDVI values by biomass

Days	DW (g/L)	NDVI	SD	SI (W/m^2)
0	0.098	0.1515	$28,93 \cdot 10^{-5}$	217
1	0.199	0.1604	$22.88 \cdot 10^{-5}$	221
2	0.323	0.1674	$10.49 \cdot 10^{-5}$	248
3	0.504	0.1961	$43.15 \cdot 10^{-5}$	315
4	0.636	0.2139	$13.00 \cdot 10^{-5}$	325
5	0.728	0.2204	$10.62 \cdot 10^{-5}$	310
6	0.790	0.2005	$15.35 \cdot 10^{-5}$	122
7	0.855	0.2097	$12.20 \cdot 10^{-5}$	160

SD: Standard deviation. SI: Solar irradiation

The NDVI values of days 6 and 7 were not correlated with their concentrations
in biomass, the first 6 values of the biomass concentration as a function of the NDVI for the 3 crops averaged (figure 8) are represented. It can already been noticed an almost identical NDVI values for the 3 cultures, which shows a good repeatability (standard deviation average is equal to ± 0.38%) of our measurement system (camera infragram). The adjustment of the mean (blue point) shows that the biomass concentration changes linearly as a function of the NDVI biomass according to the following equation with $R^2 = 0.9881$:

$$\mathrm{DW(g/L)} = 8.5534 \cdot \mathrm{NDVI} - 1.1697 \qquad (4)$$

The intensities of the reflectance in the red and the near infrared depend on the physiological state of the spirulina individuals but more particularly to their number. Thus, when the spirulina biomass concentration is high (spirulina population), *spirulina* reflects a large amount of infrared light and a very small amount of red light, which makes it possible to have a high NDVI. Sajith et al., studied aquaponics plant health using infragram technology. They showed that NDVI is high when photosynthetic activity increase. (Sajith et al., 2016).

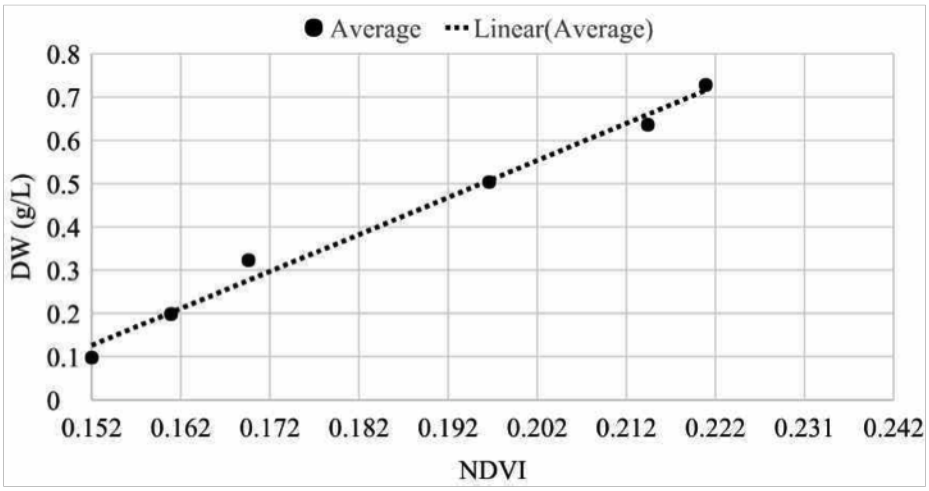

Fig 8. Evolution of dry biomass according to NDVI.

Figure 9 shows the influence of the intensity of the solar irradiance on the NDVI for the two samples with biomasses equal to 0.2 g/L and 0.5 g/L.

The NDVI values have been measured over two days in order to obtain different illumination values (time labeled in red corresponds to measurements made on July 28, 2016 and those labeled in green on July 27, 2016). The Biomass concentration values of 0.2 g/L and 0.5 g/L have mean values of NDVI respectively equal to 0.1755 ± 0.0112 and 0.2083 ± 0.0133. For some Solar irradiance values below 160 $W \cdot m^{-2}$, NDVI values remain relatively low for the 2 samples. Beyond this illumination value, high NDVI values are found increasing with illumination. However, on July 27th, at 10 am, the NDVI values are lower than those of 9 am contrary to the intensities of solar illumination (shadow effect). These observations may be justified either by the solar angle or by the physiological state of the microalgae (Hu 2009). For the measurements carried out, the maximum NDVI values were obtained at 12 pm. In general, at this time the sun is at the zenith, which therefore avoids the effect of the solar angle on infragram images.

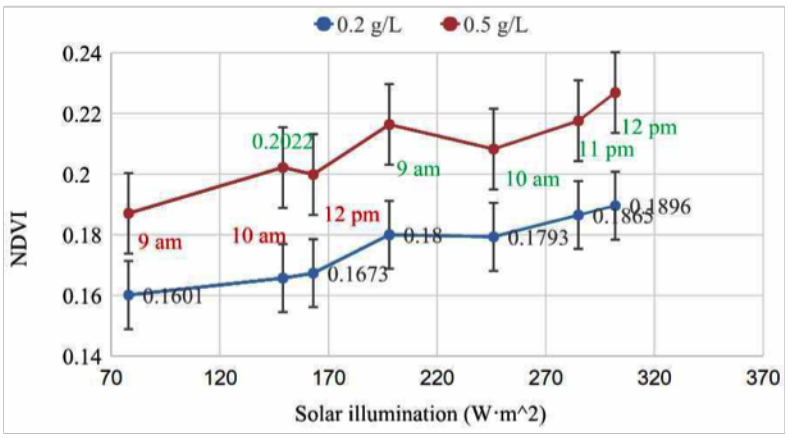

Fig 9. Evolution of the NDVI as a function of the intensity of the solar irradiance.

Evolution of the biomass as a function of the reflectance signal

The concentration of spirulina biomass changes linearly as a function of reflectance signal (figure 10). The voltage supplied by the reflectance signal is linked to the chlorophyll content contained in spirulina. Moreover, this content of chlorophyll depends on the culture conditions and especially on the population of spirulina.

The spirulina biomass concentration is proportional to the population of Spirulina: the more the population increases the more biomass increases. Measured data are almost identical with an error of 0.47%. The relationship between the dry weight of biomass and the reflectance signal is given by the following equation with $R^2 = 0.9934$:

$$\mathrm{DW(g/L)} = 8.231 \cdot \mathrm{R(\%)} + 0.0104 \qquad (5)$$

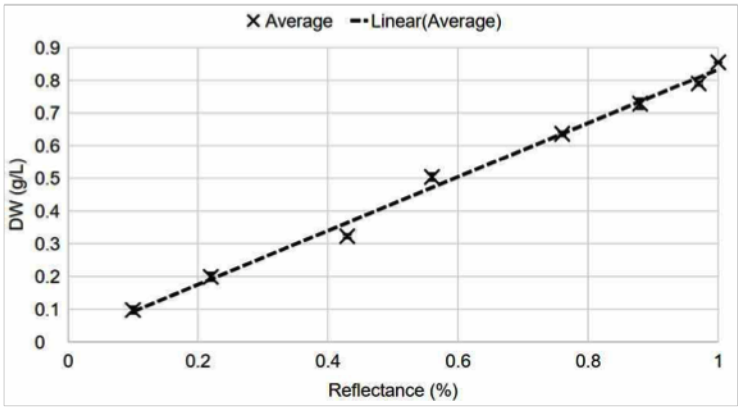

Fig 10. Evolution of biomass according to reflectance.

Conclusion

In this paper, we presented two simple and efficient methods to estimate spirulina platensis biomass. One consists to measure the reflectance signal of spirulina solution at 830 nm and the other is based on the analysis of infragram images contrast. These two methods make it possible to estimate the spirulina biomass with R^2 above 0.9. However, the culture must be well homogenized. Moreover, the infragram images reliability depends on solar illumination. Measurements should be perform when solar irradiation is high (typically above 160 $W \cdot m^{-2}$) and when the sun is at the zenith to avoid shadow effect. The reflectance sensor can be used to monitor spirulina growth in photobioreactors. On the other side infragram imaging can be used in open systems because it requires solar illumination. Compared to time consuming conventional measurement techniques using expensive devices, those two fast and low cost techniques have also the advantage to monitor spirulina growth without taking sample of the culture.

Acknowledgments

The authors would like thank Biosentec company for experiments on *spirulina platensis*, LAPLACE electronic service in particular Nordine Ouahhabi for reflectance sensor display design and Dr Bagui Olivier for image processing explanation.

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