Development of a microalgal automated cultivation system on *Tetradesmus obliquus*

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***Abstract*—An automation system for various applications has been proven to be effective in attaining productivity and efficiency. Moreover, it can be used in monitoring biological culture growth parameters. Microalgae have been a potential source of food, cosmetics, pharmaceutical, and fuel. However, monitoring the growth parameters of microalgae such as the pH level, salinity, dissolved oxygen, and its color density over time has not yet been achieved in previous studies. This paper presents an automated monitoring system for a closed microalgae photobioreactor. Contemporary continuous monitoring of bioprocesses can be challenging, considering parameters crucial to the growth of microalgae, such as pH and dissolved oxygen. Dissolved oxygen, pH, and salinity sensors are installed on the system and are programmed using LabVIEW to take measurements at regular intervals. The set-up includes a vision system to monitor the changes in the color of the solution, corresponding to the population growth of the microalgae cells. Optical density readings are also done to characterize the growth of the microalgae organisms to serve as a benchmark for the experiment results. The system is employed and tested on *Tetradesmus obliquus* (Turpin) M.J.Wynne species, also known as [syn. *Scenedesmus obliquus* (Turpin) Kützing]. Results show that the optical density readings increased at a value of 0.025 in day 1 to the value of 0.27 in day 27 and ended at 0.24 in day 29. This correlates with the increasing values of the RGB values. Hence, the development of a vision system can be used in monitoring algal growth with respect to its change of color.**

***Keywords*: Photobioreactor, Tetradesmus obliquus, microalgae, LabVIEW**

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I. Introduction

World population is tremendously increasing with the current estimated value of seven billion [1]. This event increased the demand for commodities such as food, water, and energy. However, these commodities do not meet the current population demand. Achieving sustainability of these commodities are the focus of stakeholders. One of the popular solutions proposed is to utilize microorganisms that produce high valued products, and one of these microorganisms is microalgae.

The use of microalgae for food, wastewater treatment, and energy has become a widespread research worldwide. Microalgae are a vast group of autotrophic, heterotrophic or mixotrophic organisms which have an extraordinary potential for cultivation as energy crops. They can be cultivated under difficult agri-climatic conditions and can produce a wide range of commercially interesting byproducts such as fat, oil, sugar and functional bioactive compounds [2]. As a group, they are of interest in the development of food sustainability and future renewable energy scenarios. In terms of land area required for cultivation, microalgae are also estimated to have much higher biomass productivity than other energy crops [3]. Aside from biodiesel, microalgae are potentially attractive

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to a wide range of applications such as nutrition and waste water treatment.

Though many microalgae species are easy to reproduce and are relatively resilient to environmental factors, parameters such as pH and dissolved oxygen plays an important role in the growth of the organisms [4]. Extreme levels of pH and dissolved oxygen may result in a declined growth of most microalgae strains. To minimize this effect, commercial cultivation of microalgae on closed environments are mostly done on photobioreactors (PBRs). However, continuous monitoring of the culture conditions is still of utmost importance. The cultivation monitoring of microalgae can be tedious, time-consuming, and is prone to human errors. There are studies that used automation system to improve cultivation performance. Nucleic biosensors were already being used in monitoring phytoplankton in the ocean. [5] Lens-free shadow imaging systems (LSIS) have been used to monitor the floc size analysis using an image sensor. [6] In this study, a web camera will be used to capture images of the photobioreactor set-up. Studies have shown that the use of web camera is viable in finding parameters such as the height of the water level as well as in relating the growth of the organisms with image features such as RGB and grayscale values is feasible. [7] Quantification of microalgae biomass using digital image processing also yielded positive results [8] Automation system was used in the preparation of the algal culture medium, illumination, and control of carbon dioxide (CO_2) condition in optimizing microalgae production conditions [9].

However, there is still research gap in the continuous monitoring of the growth of the algal species in terms of algae density with respect to time and its growth parameters such as the pH level, salinity, and dissolved oxygen. Hence, this paper discusses the implementation of a vision system in which the color of the microalgae is monitored with respect to its growth. The objective of this paper is to create a system that monitors the pH, dissolved oxygen, and the color of the algae instantaneously online while observing the algal growth via optical density analysis. The temperature was monitored manually using a thermometer that was dipped inside the reactor. The data gathered in this study can be used to predict the growth of *Tetradesmus obliquus* in a real time setting. Moreover, we can compare this to a study where we inject CO_2 to the algal species while monitoring it real time.

II. AUTOMATED SYSTEM

The methodology of the automation system is of the following: 1) Microalgae Culture, this discusses the nature of the microalgal species used in this study and where it was gathered. Moreover, in this subsection, the overall conditions of the reactor are also discussed., 2) Data monitoring system, this subsection discusses the schematic procedure of the automated system which is used to gather data in real time situation. Table 1 shows the materials and equipment that was used in the system.

TABLE I
Equipment and Specifications Used in the Study

Equipment	**Specification / Model**
Reactor	Measurement (0.61 m long x 0.30 m width x 0.40 m depth)
pH Sensor	Vernier PH-BTA pH Sensor
Dissolved Oxygen Sensor	Vernier DO-BTA dissolved oxygen probe
Salinity sensor	Vernier SAL-BTA salinity probe
Data Acquisition System	LabQuest Data Aquisition
Data Acquisition Software	LabView National Instruments
Light Meter	EXTECH Instruments Easyview ™ 30 Light meter
Optical Density	GENESYS 10 UV spectrophotometer
Culture Media	TMRL

A. Microalgae Culture

The experiment made use of *Tetradesmus obliquus*, a planktonic green alga isolated from Carmona River, Binan, Laguna within the vicinity of the De La Salle University-Science and Technology Complex. The mother cultures were grown for a month in TMRL medium at ambient temperature of 22°C and light intensity of 40µmol $m^{-2}s^{-1}$ continuously provided by cool-white LED fluorescent tubes. *T. obliquus* cultivation was carried out in a photobioreactor (PBR) containing 16L of TMRL culture medium and 10% inoculum. The PBR (0.61 m long x 0.30 m width x 0.40 m depth) was constructed of fiber-glass material fitted with sensors. Mixing of the microalgae and culture medium within the PBR was accomplished using an air compressor that introduced air through spargers. The set-up was observed for 29 days.

B. Data Monitoring Set-up

The automation system consisted of the following: 1) The Tank for microalgae culture, 2) Data Acquisition, 3) Computer LabView Software, 4) sensors (pH, Dissolved Oxygen,Salinity), and 5) image and video collection for image processing. Fig. 1 shows the schematic diagram of the overall experimental setup and Fig. 2 shows the actual experimental set up of the data monitoring system of the algal cultivation system.

Introducing artificial light source to the microalgal species for photosynthesis, a cool-white 16 W fluorescent lamp was used that provides light at an intensity of 2500 lux that was measured using EXTECH Instruments Easyview ™ 30 Light meter and had cycles 15 h light / 9 h dark. The fluorescent lamp was positioned at the front side view of the reactor which has a surface area of 0.244 m^2. The location of the reactor was covered to keep the effect of other light sources to a minimum.

Measuring the value of the dissolved oxygen, salinity, and pH value of the culture system over time sensors were used and are integrated in the data acquisition system. This ensures that the growth environment of the culture is stable with respect to the culture's growth conditions. The tip of the dissolve oxygen, salinity and pH sensors were dipped 10 mm from the surface of the water. Calibration of the sensors was first conducted before their actual use to ensure the accuracy of the data acquired from the developed automated system. Labquest Mini served as the interface to gather data from the sensor, and transfer them to the computer. The sensors measured the three parameters in real-time and were programmed to get measurements at one-hour interval. The measurements were then saved as database in excel format. The dissolved oxygen sensor used can measure dissolved oxygen level from 0 to 15 mg/L and has a tolerance of 02 mg/L. The pH sensor can measure pH values from 0 pH to 14 pH units, which is the maximum range of values for pH. The salinity sensor can measure up to 50 ppt (50,000 ppm) and has an accuracy of

Relating algal growth (change of color) to its value when obtaining its optical density, a camera was used as pictures was taken this was processed using LabView software integrating it to the data acquisition system. Recording the color change of the culture strain over time, a camera was used to get the color value of the image which is RGB. The Camera was located five inches away from the reactor, facing the right-side view of the reactor which has a surface area of 0.1239 m^2. The camera was programmed to capture images and videos every hour for the duration of the experiment. The videos were recorded for five seconds at every interval of the video acquisition process. The images and videos were then stored on in specific folders in the computer. The images were saved in Joint Photographic Experts group (JPEG) format at a resolution of 800 pixels by 448 pixels, while the videos were saved in AVI (Audio Video Interleaved) format.

Monitoring the growth of the microalgal species, daily optical density readings at 750 nm wavelength were obtained using GENESYS 10 UV spectrophotometer. A daily optical density was taken for 29 days. There were three points in the reactor where the strain is collected. The optical density value of the three points where then averaged and graphed using Microsoft excel software. Lastly, temperature readings are then recorded manually using a thermometer that is dipped into the reactor.

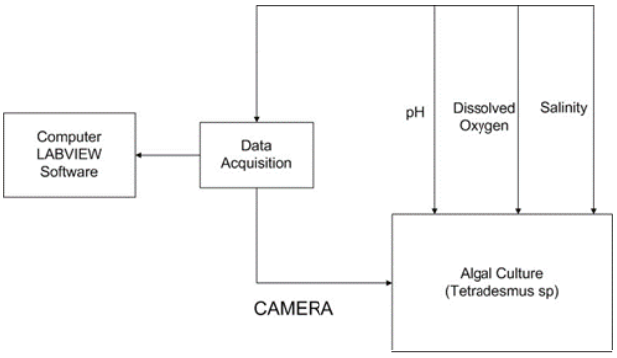

Fig. 1. Schematic diagram of the experimental set-up.

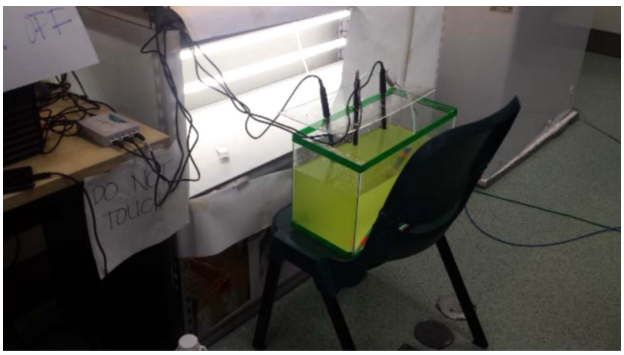

Fig. 2. The actual set-up of the experiment.

III. RESULTS AND DISCUSSIONS

The implementation of an automation system in monitoring a microalgal photobioreactor system was conducted. Dissolved oxygen, pH, and the factors affect the algal growth. Moreover, video and images was captured for images processing using LabView.

The growth of the microalgal species was monitored for 29 days via optical density. Moreover, the algal samples that were taken in the photobioreactor had three replicates; Fig.3 shows the growth curve of the microalgal species. The curve indicates that there is a gradual growth of the species. We can see that from the value of 0.025 in day 1 it increased to the value of 0.27 in day 27 and ended at 0.24 in day 29.

Based on the data acquired, it is observable that the values on day 0 for pH of the culture is higher than the latter days. However, from day 1 onwards, the values of pH normalize. For the rest of the duration of the experiment, the value of pH fluctuates to a maximum of 9.5% from the median which is 8.0135.

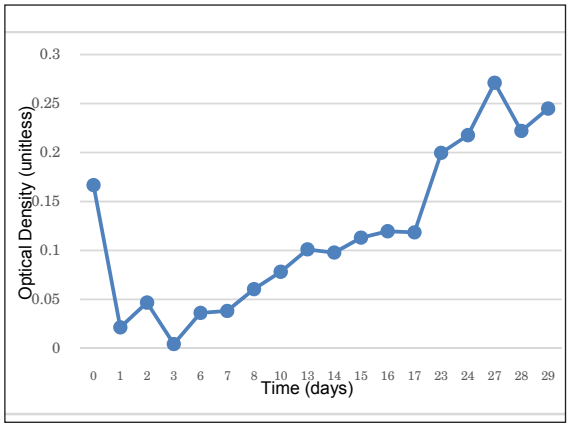

Fig. 3. Optical density growth curve of *Tetradesmus obliquus*.

The highest pH level was acquired on day 23 at a value of 8.7785, while the lowest level of pH was acquired on day 1, which is 7.5175. The highest temperature recorded is 25.4 degree Celsius, taken at day 10, whereas the lowest temperature is recorded on day 29, at a level of 22.5 degree Celsius. The median temperature for the duration of the experiment is 24.5 degree Celsius. The median of the dissolved oxygen values, on the other hand, is 0.6785 mg/L, whereas 0.7190 mg/L and 0.6380 mg/L are the highest and lowest recorded values respectively, taken at day 29 and day 7.

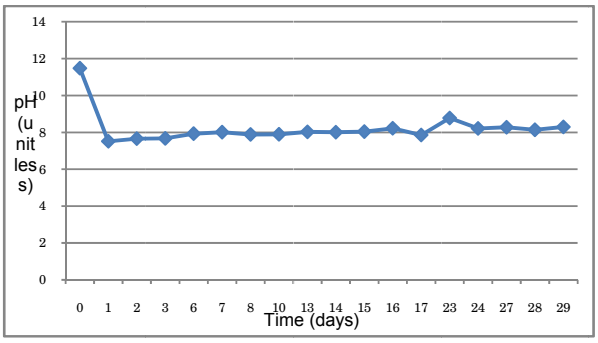

Fig. 4. The resulting pH level versus time.

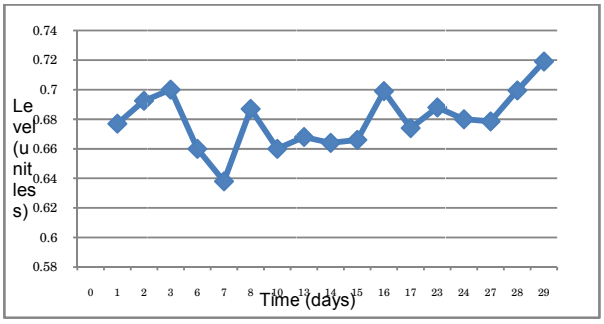

Fig. 5. The resulting dissolved oxygen versus time.

The images captured were processed through an algorithm executed using LabView. The algorithm allows the researchers to select a region of interest on the series of pictures taken by the web camera, extracting the RGB features of the image. The images captured in the daylight cycle; *i.e.,* when the light source is on, are used as the samples for the program. Images in which the culture is not well-mixed is also removed to prevent erroneous results.

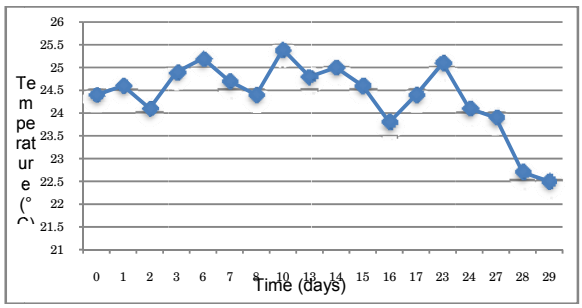

Fig. 6. The temperature versus time (in days).

Based on the data gathered, the RGB levels of the culture changes as the microalgae thickens. The green component of the images has a level that is noticeably higher than the red and blue values. As time passes,

the RGB values vary, but in all of the images that are used as samples, the green component is always higher than the red and blue components, which is akin to the green tint of the culture. A more appropriate observation is that as the days pass, the red and blue component of the images taken diminishes at a faster rate than the green component. However, the growth of the microalgae are not well-represented by the changes in the RGB values due to the variation of the image features used as samples. Changes in camera position, as well as with the tank orientation, can result to discrepancies in image features; *i.e.,* different image brightness, different image perspectives. A more appropriate parameter to consider in this matter would be the difference between the red and blue levels with respect to the green levels.

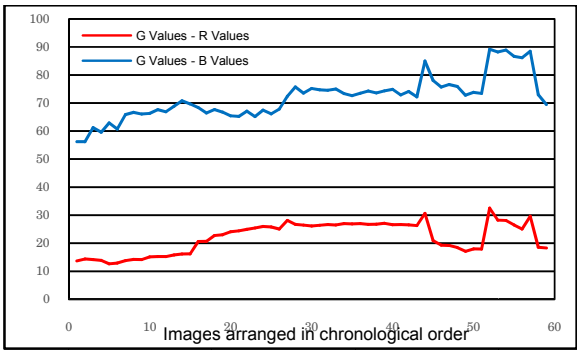

Fig. 7. The RGB values and their differences.

As shown in Fig. 7, it can be observed that the difference between the red and blue level with respect to the green level of the images is increasing as the time passes. It affirms the decreasing values of red and blue components of the culture in comparison with the green color component.

By visual inspection of the videos taken by the system, the bubbles caused by the aeration of the air pump can be observed. It was also observed that as the solution becomes darker, the bubbles become less visible. From day 8 onwards, the bubbles are no longer visible on the web camera when the culture is well-mixed.

IV. CONCLUSION

An automated monitoring system for closed bioreactors has a huge potential when it comes to the commercial production of microalgae. The use of vision system on the set-up can be a potential substitute to optical density readings. The changes in the pigment of the culture constitute to the population of the organisms. However, based on the optical density readings, a very high growth rate of the *Tetradesmus obliquus* is not observed and can be further be explored in future studies.

The videos that are recorded by the system can be used for analysis of the bubbles. Algorithms for tracking such as mean-shift and Kalman filter can be implemented to the system to analyze the behavior of the bubbles as the culture grows. However, the visible observation of the pattern of the bubbles gets harder as the solution darkens. Without supplemental image processing algorithms, analysis of the bubbles may not be viable.

After analysis of the captured images, it can also be concluded that the green pigment of the culture is caused not by the increase of the green component of the algae, but rather by the diminishing red and blue levels, resulting to a greener culture pigment. Although the green component of the images also varies, the rate at which it increases or decreases is different with the changes in the red and blue components. As the culture darkens, the RGB level decreases, however, the red and blue levels decrease at a faster rate. The general decrease of the RGB level results to a darker culture, while the faster rate of decrease in the blue and red level with respect to the green level results to the green pigment of the culture.

For future work, several points can be improved. The number of aerators can be increased to ensure that the solution is well agitated and the organisms will not settle at the bottom of the tank. Changing the design of the tank may also be feasible, to allow better circulation of the microalgae in the culture. Further isolation of the system can also be done to eliminate the effect of other light sources completely. Securing the web camera to prevent any changes on its orientation can be done to avoid changes in image perspective. Isolation of the system from light sources and securing the web camera position will preserve the homogeneity of the images captured by the web camera and may account to better parallel analysis of the images. This means that direct analysis of the RGB levels of the captured images alone may become sufficient. Further processing of the images can also be done. Extraction of the brightness levels can supplement the RGB levels for better analysis of the sample images.

ACKNOWLEDGEMENTS

This research was funded by the University Research Coordinating Office (URCO) Interdisciplinary Research Project with the project number 03IR3TAY14-3TAY15 of De La Salle University. Acknowledgement also goes to the USAID STRIDE CARWIN funding with the fund number 0213997-G-2016-012-00 for research engagement with the Philippine algae industry.

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