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RESEARCH ARTICLE

Evaluation of the Suitable Environmental Conditions for Selected Freshwater Microalgae Species with the Potential for the Production of Biodiesel

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Abstract: The importance of using renewable green energies is being debated globally since harmful effects due to continuous combustion of fossil fuels have been realized. Biodiesel is one of the substitutions for fossil fuels. Production of biodiesel from microalgae is categorized under third generation biofuels which are considered to be viable alternative energy resources. This study was focused on investigating growth kinetics of freshwater microalgae, cultivated in synthetic wastewater under laboratory conditions in order to identify the species with the highest potential for the production of biodiesel. Three freshwater microalgal species were isolated from water samples collected from two reservoirs, Victoria reservoir in Central Province (7˚ 14′ N, 80˚ 47′ E) and Ulhitiya reservoir in Uva Province (7˚ 27′ N, 81˚ 3′ E) of Sri Lanka. Light intensity, aeration and temperature were varied during the cultivation of algal species in a synthetic wastewater medium. According to the results, *Chlorella* sp. showed the highest growth rate compared to *Monoraphidium* sp. and *Scenedesmus* sp. under all three environmental conditions provided.

Keywords: Biodiesel, Microalgae, Growth kinetics, Wastewater

INTRODUCTION

The need to reduce the dependence on petroleum based fuels is becoming important because of the global warming and the energy crisis. These apprehensions have increased the interest in developing first and second generation biofuels based upon resources such as vegetable oils and lignocelluloses, respectively (Mata *et al*., 2010). Due to various problems encountered with previous generations of biodiesel, scientists are now aiming for third generation biodiesels where microalgae and sea weeds are widely used as energy sources. The idea of producing methane gas from algae was not recent but goes back to early 1950s. Moreover, the oil crisis reported especially in 1970s resulted in the acceleration of algal research for oil production (Campbell *et al*., 2011). However, due to the high availability of fossil fuel at that time, these concepts may have not realized. After understanding the limitation of fossil fuel resources, idea of searching a sustainable solution for the current energy crisis renewed (Naik *et al*., 2010).

When compared to the other energy sources, freshwater microalgae have a high potential as biodiesel precursors because many of them contain hydrocarbons over 60% of the dry weight. Recent studies have shown that the average lipid content of microalgae varies from 1% -70% on dry weight basis (Fernandes *et al*., 2010). Triacylglycerides (TAGs) generally serve as energy storage compounds in microalgae and once extracted can be easily converted into biodiesel through transesterification. Lipid production and extraction from algae depend on the algal species and also with the extraction solvent system.

One of the important characteristic feature of microalgae is their ability to grow effectively in wastewater as they utilize abundant organic carbon and inorganic nutrients (N and P) present in wastewater (Chen *et al*., 2011), which ultimately provide an added benefit for the production of biofuel. Hence, combining the goals of sustainable fuel production and wastewater treatment through the cultivation of microalgae can be considered as a two-way effort of green energy. The cultivation of microalgae in

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wastewater offers a far more attractive proposition from an environmental point of view, specifically for industrialized and developing countries.

Therefore, our study aimed to evaluate the most suitable environmental conditions for the cultivation of three selected microalgal species isolated from Sri Lankan reservoirs. The selected algal species were cultivated under various conditions of temperature, light intensity and aeration. The results obtained were matched with the Michaelis-Menten kinetic model.

MATERIALS AND METHODS

Sample collection

Algal samples were collected from two selected reservoirs in Sri Lanka, Victoria reservoir in the Central Province (7˚ 14′ N, 80˚ 47′ E) and Ulhitiya reservoir in the Uva Province (7˚ 27′ N, 81˚ 3′ E) during the months of July and August, 2014. A plankton net with the mesh size of 34µm was used for the sampling.

On site measurements were also taken for temperature and conductivity during the time of sample collection. The collected water samples containing algae were preserved in a cool pack (~4ºC) and taken to the Environmental Research laboratory at the Department of Zoology, University of Peradeniya with maximum delay of 24 hrs.

Cultivation of algae

For the cultivation of microalgae, a synthetic wastewater medium was prepared using the method outlined by Zhu *et al*. (2013) (Table 1).

Plate culturing

From the prepared culture medium (synthetic waste water), 200 ml was taken and 2.00 g of agar was added. Then the medium and other glassware were autoclaved under the pressure of 1.5 atm, and temperature (121^{o}C), for about 15-20 minutes. The autoclaved medium was transferred to culture plates under a laminar flow. A dilution series was prepared (10^{-1} to 10^{-3}) by using algae containing water samples obtained from the two reservoirs. An aliquot of 100 µL from each dilution factor was transferred on to the solidified culture medium. The water sample was spread with the help of a sterilized glass spreader. The culture plates were sealed with parafilm and allowed to incubate under ambient temperature and light intensities for 4-10 days.

Cultivation of Microalgae

For the cultivation of microalgae, each algal colony established (after ~10 days) on culture plates was transferred into 100 ml of growth medium (synthetic waste water) using a sterilized loop. All the algal inoculants were kept for 10-14 days under natural conditions.

Table 1: Chemical composition of synthetic wastewater (Source: Zhu *et al*., 2013).

Chemicals	Concentration (mgL^{-1})
Glucose	120
Peptone	90
KNO_3	96
KH_2PO_4	17
$MgSO_4.7H_2O$	24
$MnSO_4.5H_2O$	2.16
$FeCl_3.6H_2O$	0.12
$CaCl_2.2H_2O$	2.4
$NaHCO_3$	300
Yeast extract	12

After the incubation period, three axenic cultures were obtained: *Chlorella* sp. and *Monoraphidiam* sp. from Victoria reservoir and *Scenedesmus* sp. from Ulhitiya reservoir. The three algal species were cultivated in containers (10L in capacity) using the prepared synthetic wastewater as the medium. Three weeks prior to the kinetic study, 10 ml from each algal inoculum was transferred into 250 ml conical flasks containing synthetic wastewater culture medium in order to obtain well grown and healthy algal cells.

Kinetic study

Inoculation

Cultivation of microalgal species for the kinetic study was carried out in 750 ml conical flasks. At the beginning, 5 ml of algal inoculum was added to each container. The remaining was filled with 295 ml of liquid culture medium to make the total volume of 300 ml. Different light intensities, aeration levels and temperatures were used (Table 2).

Growth variables

Fluorescent bulbs (Orange Electronic, Day light, Sri Lanka) with different intensities were used to provide necessary light intensities. Ambient/aquarium air pumps (EJET aquarium pump, 168) were used to provide aeration in different rates and the temperatures were varied with the use of thermostatic water baths (YCW – 01, Gemmy Industrial Corp, Taiwan). The cultivation setup, allowed the major growth variables to be controlled and maintained at constant levels. Control samples were also used.

Monitoring of algal culture

Sample collection was done once in 5 days after introducing the algae into the medium, for a period of 20 days. Daily monitoring of temperature (T), light intensity (L) with the use of lux meter (EXTECH, wide range light meter) and aeration (AE) was done to ensure the maintenance of growth conditions throughout the experimental period

Counting of algal cells

A research microscope (Carl Zeiss, GmbH, Konigsallee, 9-21, Germany) was used for the observation and counting of algal cells. A sedgewick rafter cell (PYSER – SGI Limited, United Kingdom) was used to count the number of cells.

Kinetic model

A kinetic model was developed using the cell counts and optical density of algae. The maximum growth rate was calculated by using regression of cell counts *versus* time. The Equation (1) shows the relationship between cell count (x), growth rate (μ), and time (t). The Equation (2), which is known as the Michaelis-Menten Equation/Monod Model, shows the relationship between specific growth rate (μ), maximum growth rate (μ_{max}), value of non-limiting physical parameter (S), and half saturation constant (K_m).

$$\mu = dx/dt \quad (1)$$

$$\mu = \mu_{max} (S/K_m + S) \quad (2)$$

RESULTS AND DISCUSSION

The overall results of the experiment revealed different growth rates by algal species under different environmental conditions.

Table 2: Growth variables provided for algal cultures.

Variables	Light intensity Klux	Aeration (cm^3/min)	Temperature ^oC
	0.71	2	30
	1.77	4	35
	3.92	8	40
Control	0.35	1	27

Growth kinetics of algae with light intensity

All three algal species used for the experiment showed their maximum growth rates at the light intensity of 3.92 Klux. However, the increase of light intensity decreased the growth rates of *Monoraphidium* sp. and *Scenedesmus* sp. with time. Conversely, at the intensities of 1.77 and 3.92 Klux, *Chlorella* sp. showed the highest growth, where *Monoraphidium* sp. had the lowest growth (Figure 1a). Interestingly, *Chlorella* sp. showed the highest growth rates at all three light intensities. Even though initially all three algal species showed their maximum growth rates at the light intensity of 3.92 Klux, a rapid decline of growth rates were observed after five days of incubation This observation can be related to photo-oxidation process. The photo-oxidation takes place when cells are exposed to high light intensity in the presence of high oxygen concentration. As the cultivation process has taken place in a closed system, during the photosynthetic process the containers may get saturated with oxygen. As a result the photooxidative effect could promote the photooxidative death of microalgae.

Growth kinetics of algae with aeration

Chlorella sp. showed the highest growth rate at the aeration rate of 4 cm^3 min^{-1} and the lowest at 8 cm^3 min^{-1}. *Monoraphidium* sp. showed the highest growth rate at 8 cm^3 min^{-1}. Further, the growth rate of *Monoraphidium* sp. was also increased with the increase of aeration rate. However, *Scenedesmus* sp. showed the highest growth rate at 2 cm^3 min^{-1} and the growth rate was slightly decreased when the aeration rate was increased (Figure 1b).

The main purpose of aeration is to supply the CO_2 and to maintain a homogenized culture medium. When CO_2 amount increases, pH can be reduced in the presence of water, due to the formation of carbonic acid. Therefore, the reduction of growth rate of *Scenedesmus* sp. could be related to the presence of carbonic acid. The situation may be related to the high sensitivity of *Scenedesmus* sp. to pH than the other two species. However, as the interpretation of results are based on the microcosm experiments, the macrocosm results could be different as the media is exposed to the natural environment and therefore other environmental factors could have more effect on the growth of the algae.

Growth kinetics of algae with temperature

At 30 °C, the highest growth rate is shown by *Scenedesmus* sp., where *Monoraphidium* sp. showed the lowest. *Chlorella* sp. showed the highest growth rate at 35 °C. All three algal species demonstrated an overall negative growth at 40 °C. Nevertheless, a rapid growth rate was observed within the first five days and with time it decreased and this observation was common to all three species (Figure 1c).

Temperature is one of the important environmental variables that directly affect the algal growth. Temperature influences the physiological processes of organisms, including influences in cellular chemical composition, the uptake of nutrients, CO_2 fixation in photosynthetic organisms, and the growth rates. Generally optimal growth temperatures for most of the algae are in the range of 5 °C – 40 °C. Conversely, at temperatures over 38 °C, the growth inhibits the production of substances such as oleic acid (mono unsaturated omega – 9 fatty acid) (Herman, 1991). In addition, under heat stress condition or with heat shock, the algal protein contents will be decreased and produce abscisic acid (ABA), which is a stress hormone. Production of stress hormones in algae is a key factor in controlling own-stream responses such as growth and gene expressions (Cassidy, 2011). Therefore, it is obvious that the negative growth rates shown by algal species may be due to the stress hormone produced at 40 °C. According to Powell (2008), at 36 °C, there is a 30% increase of chemical composition in algae that helps to improve the growth of the species. Interestingly, when conferring the results of this study, two algal species (*Chlorella* sp. and *Monoraphidium* sp.) have demonstrated their highest growth rates at 35 °C.

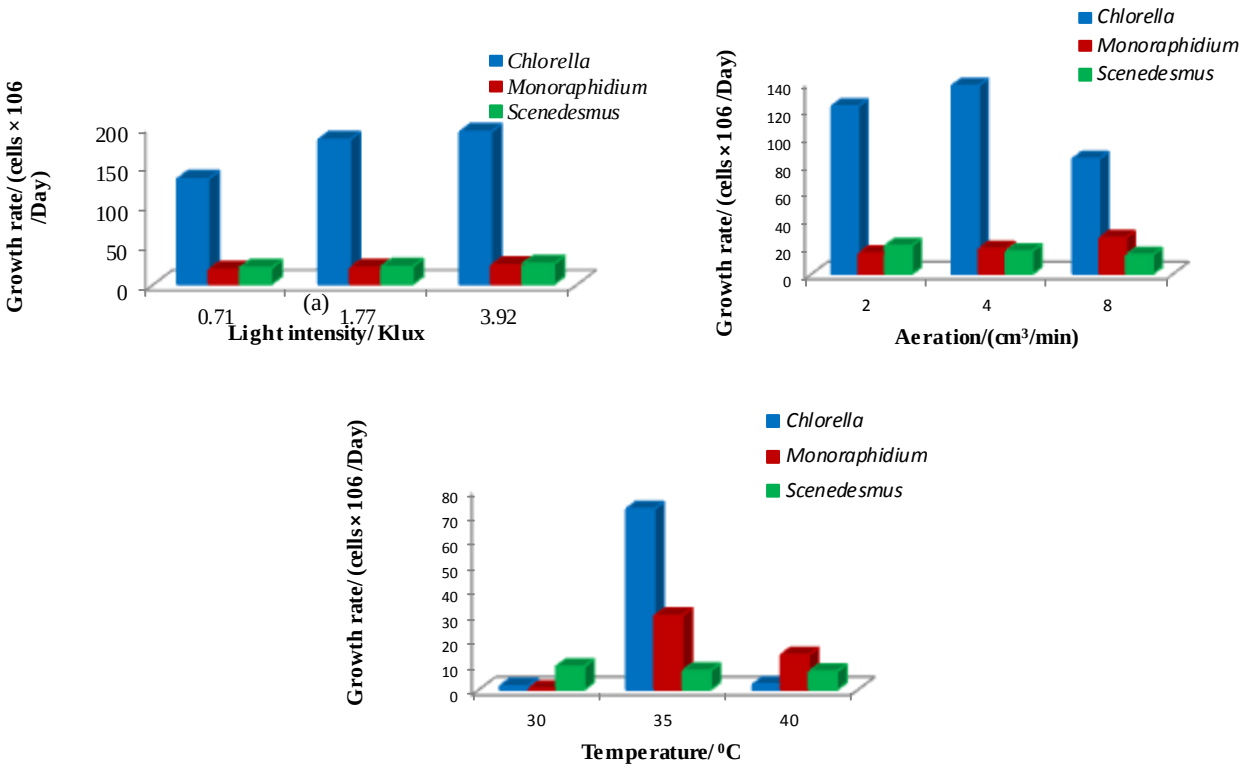

Figure 1: Maximum growth rates of algae species at different a) Light intensities; b) Aeration rates and c) Temperatures.

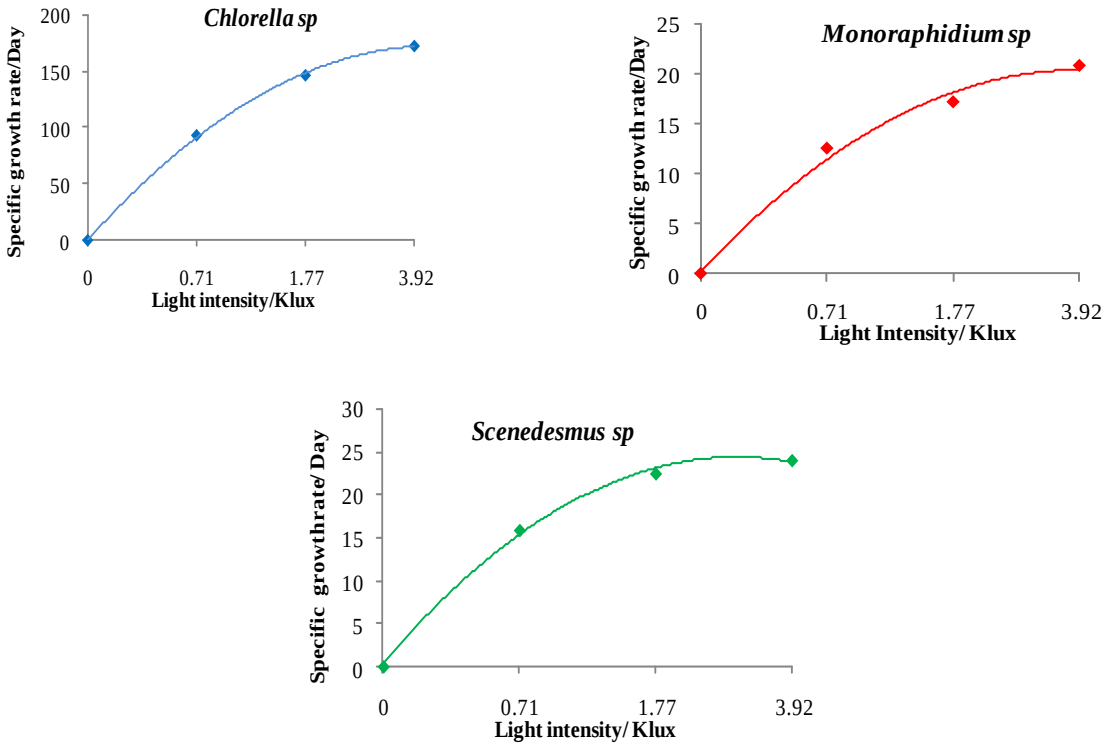

Figure 2: Michaelis-Menten plots for three algal species at different light intensities.

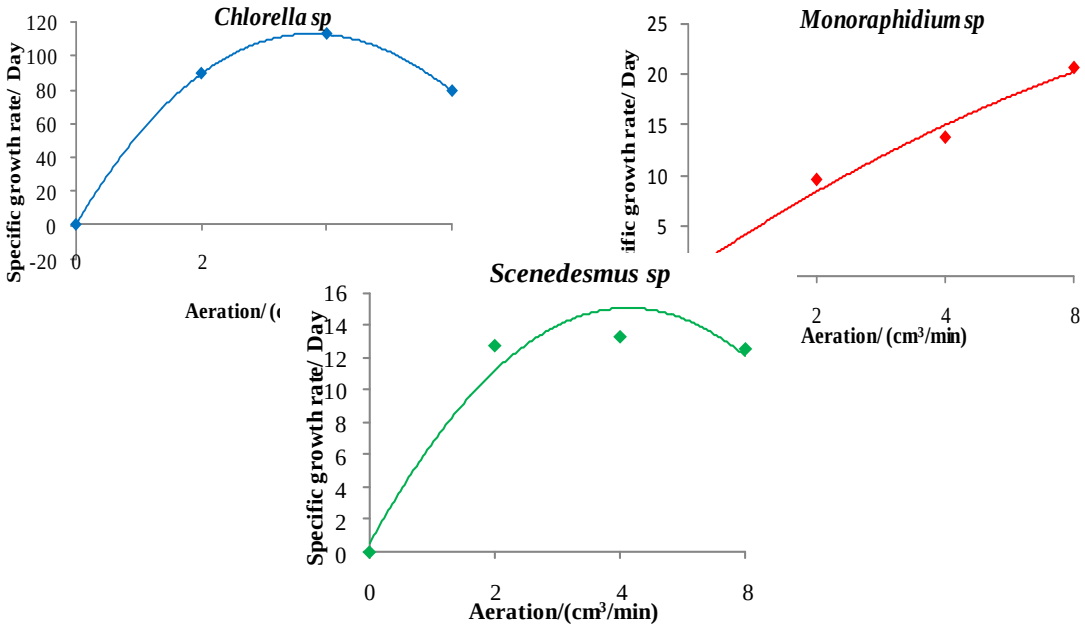

Figure 3: Michaelis-Menten plots for three algal species at different aeration rates.

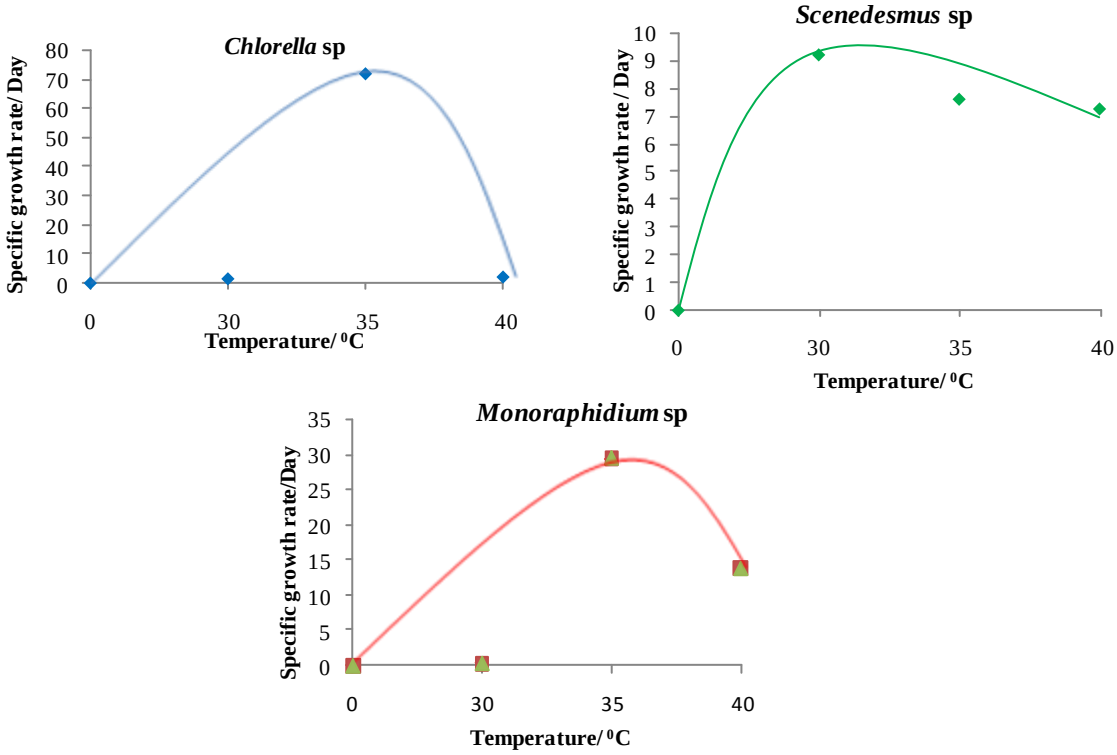

Figure 4: Michaelis-Menten plots for study algal species at different temperatures.

Table 3: Optimum growth conditions for the maximum growth rate of three algal species under laboratory conditions.

Species	Maximum growth rate / (Cells × 10^6/ Day)		Maximum Specific growth rate (μ_{max}) / Day)	Non-limiting physical parameters (S)		
				Light intensity/ Klux	Aeration/ (cm^3/min)	Temperature / °C
Chlorella	Light intensity	192.87	173.40	3.92	-	-
	Aeration	137.03	113.22	-	4	-
	Temperature	71.76	37.37	-	-	35
Monoraphidium	Light intensity	24.28	20.83	3.92	-	-
	Aeration	26.34	20.70	-	8	-
	Temperature	29.46	15.16	-	-	35
Scenedesmus	Light intensity	25.88	24.08	3.92	-	-
	Aeration	20.58	13.36	-	2	-
	Temperature	9.21	4.81	-	-	30

Specific growth rates of algae species

Specific growth rates were estimated using Equation (2). The calculated specific growth rate values were used to construct a kinetic model for each algal species at different growth conditions (Figures 2 to 4). The constructed models were fitted with the Michaelis-Menten plot.

The maximum and specific growth rates of the three algal species and their optimum growth conditions are given in Table 3.

CONCLUSION

This experiment illustrates the ability of microalgae to adapt and survive under different environmental conditions. Among the three algal species used in the experiment, *Chlorella* sp. showed the highest tolerance and growth rate under different environmental conditions created by altering the light intensity, aeration and temperature. As *Chlorella* sp. is known to contain the highest amount of lipid among the three species, the present results further enhances its potential as a renewable and sustainable feedstock for the production of biofuel.

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