Current Research Journal of Biological Sciences 3(3): 240-245, 2011
ISSN: 2041-0778

Received: February 28, 2011 Accepted: March 26, 2011 Published: May 05, 2011

Study of Native Algal Species for Growth Potential and Lipid Yield

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Abstract: The study was aimed to evaluate the potential of some native algal species taking into account their growth rate and the lipid yield as measure of productivity. Following the sampling from some native habitats two algal species were chosen based on the presence of oil and identified as: *Pithophora* sp. (Chlorophyte) and *Botrydiopsis arhiza* Borzi (Xanthophyte). Subsequently maximum specific growth rate was estimated after optimization of some growth factors which came out to be 0.49±0.022 doubling/day of *Pithophora* sp. at light intensity; 25 μmole/m^2/s, temperature; 25±1ºC, pH; 8 while 1.97±0.036 doubling/day of *Botrydiopsis arhiza* at light intensity; 200 μmole/m^2/s, temperature; 30±1ºC, pH; 6. The total lipid content derived using Folch's method was found to be 27.99% ±1.09 in *Pithophora* sp. while 47.99 % ±0.722 in *Botrydiopsis arhiza* on the basis of dry mass.

Key words: *Botrydiopsis arhiza*, growth rate, light intensity, lipid content, *Pithophora* sp., pH, temperature

INTRODUCTION

Worldwide biofuel yield leapfrogged by 11.2 billion gallons from 4.8 billion gallons through 2000 to 2007. Yet this increase has been considered to be less than 3% of the world fuel which is used for transportation (Coyle, 2007). Moreover, there are several obstacles in the production of biofuels including competition with food and fiber production for use of arable land, cost, regional market structure, biomass transport, lack of well managed agricultural practices in emerging economies, water and fertilizer use, conservation of bio-diversity, logistics and distribution networks (IEA, 2007). To resolve the problems, it is crucial that we should find new and innocuous sources of biomass for alternative energy.

Although we may not realize, algae play an important role in our lives by carrying out the process of photosynthesis. They are accountable for the net primary production of ~52,000,000,000 tons of organic carbon per year, which is ~50% of the total organic carbon, produced on earth each year Field *et al*. (1998). These organisms use energy from the sun to combine water with carbon dioxide (CO_2) to create biomass. They are referred as the most promising non-food source of biofuels, containing 10-100 times more oil than other oil producing plants: lipid-rich composition (40-80% in dry weight). Algal oils posses characteristics similar to those of fish and vegetable oils, and can thus be considered as potential substitutes for the products of fossil oil (FAO, 1997). They can provide feedstock for several different types of renewable fuels such as biodiesel, methane, hydrogen, ethanol, among others.

According to the recommendations of Aquatic Species Program the best species as a potential candidate for biofuel production would be the native algal species with rapid growth potential which can harbor the native environmental conditions efficiently thus, minimizing the chances of contamination Sheehan *et al*. (1998). Hence, the aims of present study were (a) screening, selection, identification and culturing of a suitable oil-containing algal species from the native environment (b) Optimization of some important growth parameters of selected algal species.

MATERIALS AND METHODS

The study was conducted at Forman Christian College (A Chartered University), Lahore, Pakistan, during the year 2009-2010.

Sampling of some native algal species: Sampling was conducted at different locations of Lahore and Azad Kashmir during the month of August 2009, Samples were collected using polythene zipper bags, were later shifted to the transparent plastic bottles till further experimentation.

Screening of the oil containing algal species: A simple method was used for the lipid detection based on the use of Sudan IV reagent and found successful for the

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screening of oil containing algal species. Sudan IV ($C_{24}H_{20}N_4O$, MW: 380.49; unichem, Cat. No. 26105) weighing 5 g was dissolved in a total of 100 mL 70% ethanol Creedy (1977). Algal cells were immersed in Sudan IV reagent for about 1 min. and were then observed under the microscope. Red colored droplets were clearly visible in the cells containing prominent amount of lipids.

Culturing of different samples on growth medium: Initial culturing of algae from different samples was done in Petri Plates on solidified Bold Basal medium (Bischoff and Bold, 1963). Minimal agar 1.5% was added in the medium before autoclaving for preparation of solid medium. Erlenmeyer flasks 250 mL and empty transparent mineral water bottles (plastic made) of 500 mL to 5 L capacities were used as culture vessels. The cultures of selected algal species, found rich in oil were maintained by regular sub culturing at 14 days interval at 25±1ºC temperature, 25 μmole/m^2/s light intensity, 7.5 pH, (16h: 8h) light: dark cycle. Time to time monitoring of the cultures was done by observing samples under the microscope and photographed.

Identification of the algal species: For the identification of the selected species a list of the morphological features was posted on the following Algae L-list Archive along with the photographs (https://listserv.heanet.ie/cgibin/wa?A0=ALGAE-L).

Growth estimation: The growth rate was estimated on the basis of the rate of increase in fresh weight of biomass. The cell mass was separated via filtration using Whatman filter paper # 1 and then weighed after blotting excess water.

Specific growth rate was calculated using the equation:

$$\mu = 1/X * dX/dt$$

If "t" is expressed in days, then the growth rate μ can be converted to doublings per day (k), according to the equation:

$$k = \mu / 0.6931$$

Doubling time (td) can be calculated using the equation:

$$td = 0.6931/\mu$$

Optimization of different growth parameters: The effect of different factors on the growth rate of two algal species was assessed following completely randomized experimental design in which one factor is variable while all other conditions are kept constant. The effect of three major growth factors was evaluated i.e. light intensity (25, 50, 100 and 200 μmole/m^2/s), temperature (20, 25, 30 and 35ºC) and pH (6, 7, 8 and 9). It is significant to note that the isolated algal species were grown under phototrophic conditions throughout the experiments and no organic carbon source was supplemented.

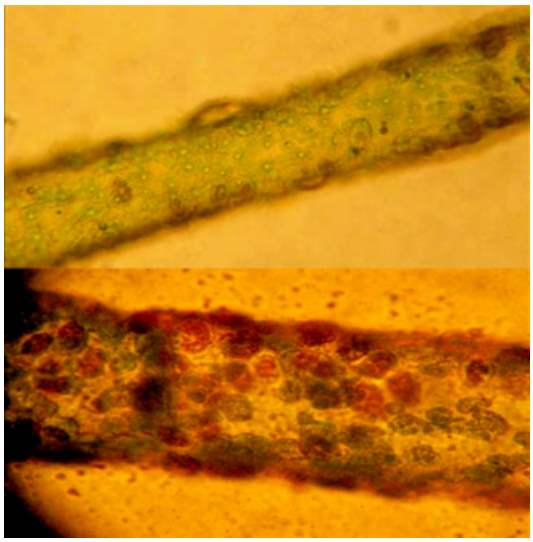

Plate 1: *Pithophora cf. oedogonium,* the part of the filament before and after staining with Sudan IV reagent (X400)

Estimation of lipid content: Estimation of the total lipid content was made as fraction of total dry cell biomass. The extraction of total lipids was performed using Folch's method (Folch *et al*., 1957). The amount of oil was calculated as % of the total cell mass.

Statistical analysis: Experimental design was completely randomized. One way ANOVA (5% confidence limit) and individual confidence intervals test were applied using Minitab version 15.1.

RESULTS AND DISCUSSION

Screening and identification of oil containing algal species: Detectable amount of lipids in the cells was seen as red color oil droplets when Sudan IV reagent was used for screening (Plate 1 and 2). Some of the expert phycologists confirmed the identity of the species in question. The details are given as under:

***Pithophora cf. oedogonia*:** was identified by Christian Boedeker[1] on the basis of the different morphological features i.e., filamentous algae have very few alternate or opposite branches; cask-like akinetes which alternate with vegetative cells or may occur in series; multinucleate cells with reticulate chloroplast containing many pyrenoids (Plate 3).

***Botrydiopsis arhiza* Borzi:** was identified by Allan Pentecost[2] while looking to the features like unicellular

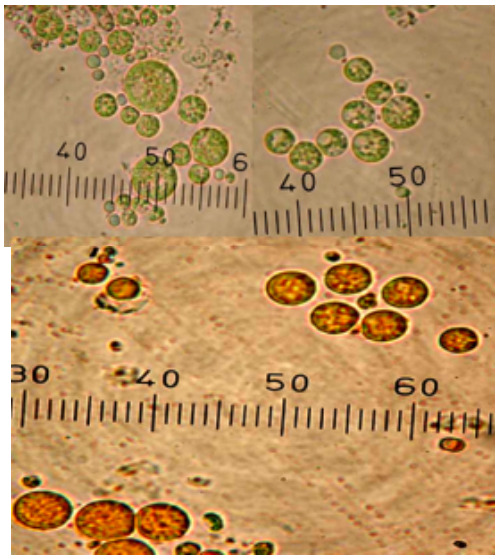

Plate 2: Botrydiopsis arhiza cells before and after staining with Sudan IV reagent (X400)

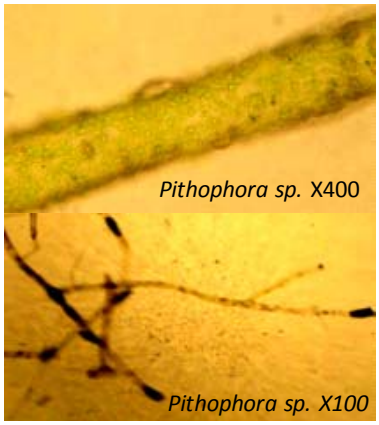

Plate 3: *Pithophora cf. oedogonium*

spherical cells of variable sizes (10-30 microns) young stages contain few chloroplasts than older ones which have numerous peripheral chloroplasts and many nuclei, zoospores and autospores (Plate 2).

Once the selected algal species were identified they were shifted to the suitable culture medium. *Pithophora oedogonia* was shifted to D-11 medium Graham *et al*. (1982) while, *Botrydiopsis arhiza* Borzi was shifted to Bold basal 3N medium Bischoff and Bold (1963) for further culturing and experimentation.

Optimization of different growth parameters:

Light intensity: Light intensity affected the growth rate of both algal species significantly ($p<0.001$). The growth rate of *Botrydiopsis arhiza* was studied under different

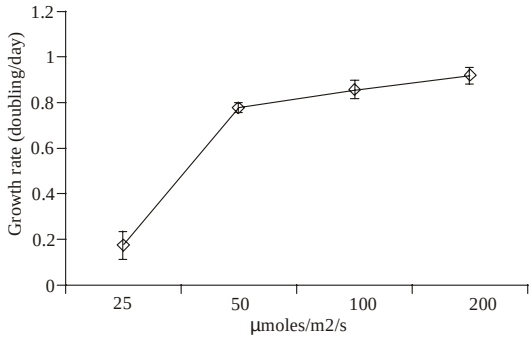

Fig. 1a: Graph showing the effect of light intensity on the growth of *Botrydiopsis arhiza*;

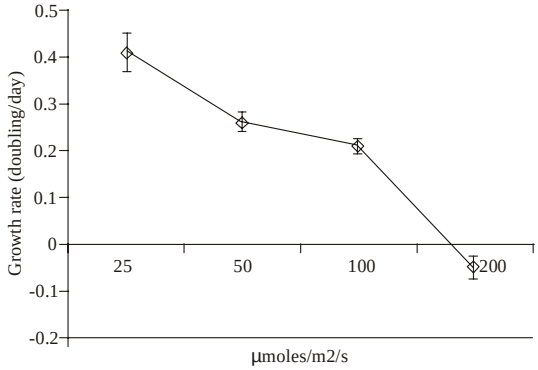

Fig. 1b: Graph showing the effect of light intensity on the growth of *Pithophora* sp.

light intensities. It was found that at light intensity of 25 µmole/m^2/s growth was slow while four folds increase in the growth rate was observed at 50 µmole/m^2/s. Although, the growth rate increased somewhat beyond 50 µmole/m^2/s but this increase was not significant (Fig. 1a).

The growth rate of *Pithophora* sp. was found maximum at the light intensity of 25 µmole/m^2/s and with increase in the light intensity the growth rate decreased significantly rather became negative at 200 µmole/m^2/s as the cell mass started degrading (Fig. 1b).

(Brand and Guillard, 1981; Vonshak *et al*., 1996; Renaud *et al*., 2002; Kitaya *et al*., 2005; Jeon *et al*., 2006; Ota *et al*., 2009; Yeisang and Cheirsilp, 2011) also indicated that light had significant impact on the growth of different microalgae. Sada *et al*. (1989) correlated the overall growth rate of *Porphyridium cruentum* with the mean light intensity available in the culture vessels.

Temperature: The effect of temperature was highly significant on growth of *Botrydiopsis arhiza* and *Pithophora* sp. ($p<0.001$). Maximum growth rate (1.33 doublings/day) of *Botrydiopsis arhiza* was recorded at a temperature of 30ºC. The growth rate exhibited a decline when the temperature was raised to 35ºC (Fig. 2a)

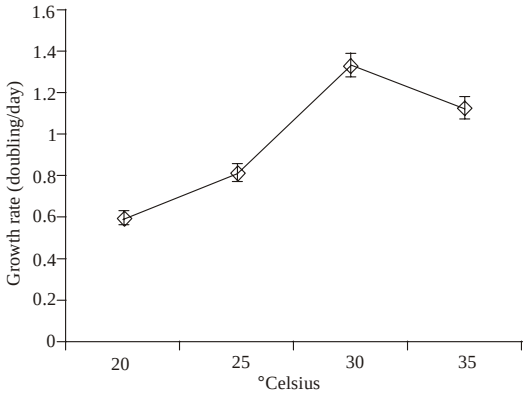

Fig. 2a: Graph showing the effect of temperature on the growth of *Botrydiopsis arhiza*

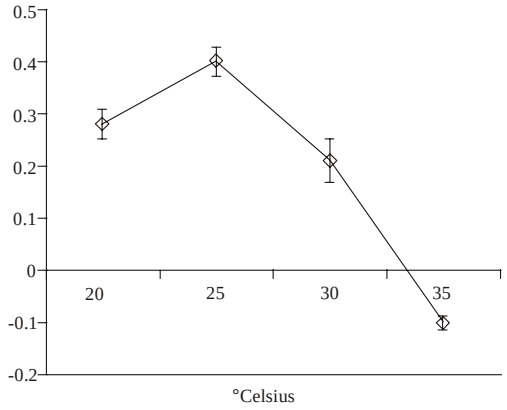

Fig. 2b: Graph showing the effect of temperature on the growth of *Pithophora* sp.

The pattern of growth of *Pithophora* sp. was quiet different with regards to the effect of temperature. From data it appears that *Pithophora* sp. exhibited highest growth rate at 25ºC and increment of 5ºC resulted in substantial decrease in growth and at 35ºC the species tend to show negative growth rate (Fig. 2b).

Kitaya *et al*. (2005) reported the growth of *Euglena gracilis* significantly varied with the change in temperature and the optimal temperature for this unicellular alga was found to be 27 to 31ºC. (Zhu *et al*., 1997; Oliveira *et al*., 1999; Renaud *et al*., 2002; Koru and Cirik, 2003; Araujo and Garcia, 2005) revealed that temperature not only affects the growth of the different algal species but also changes the proportion of different biochemical constituents of the cells.

pH: The growth was significantly affected by pH ($p<0.001$). The growth rate of *Botrydiopsis arhiza* was highest at pH 6 and at pH 7 it was slightly less but at pH 8 a reduction of 48% was observed and at pH 9 it further decline by 35 % (Fig. 3a).

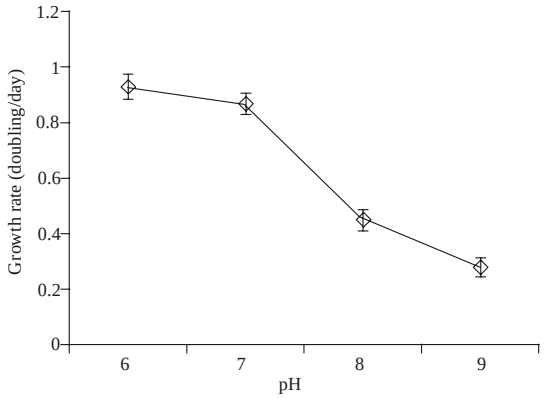

Fig. 3a: Graph showing the effect of pH on the growth of *Botrydiopsis arhiza*

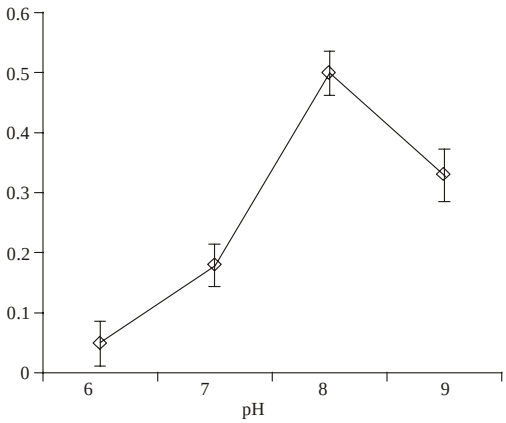

Fig. 3b: Graph showing the effect of pH on the growth of *Pithophora* sp.

pH 8 was found to be best for growth of *Pithophora* sp. and on either side of this pH exhibited decline in growth rate. Minimum growth rate was observed at pH 6 (Fig. 3b).

In the present study it was noticed that change in pH has significant impact on culture maintenance. A slight change in the pH was found to be quiet drastic in terms of invasion by other algal species. Rachlin and Grosso (1991) reported the change in the growth of *Chlorella vulgaris* due to changes in the pH of the medium. Danilov and Ekelund (2001) reported changes in the growth and photosynthetic efficiency of *Euglena gracilis* due to change in the pH. Wang *et al*. (2010) revealed that pH not only affects the growth rate but also the lipid content of the *Chlorella vulgaris.*

Specific growth rate: Specific growth rate of both algal species was investigated after optimizing conditions of light, temperature and pH (Table 1). Maintaining the optimal conditions for growth of *Botrydiopsis arhiza*;

Table1: Specific growth rate of isolated algal species

Species	Division	Specific growth rate μ(mg/mg.day)	Doubling/day
Botrydiopsis arhiza Borzi	Xanthophyta	1.37±0.0249	1.97±0.036
Pithphora sp.	Chlorophyta	0.336±0.015	0.49±0.022

30ºC temperature, 200 μmoles/m^2/s light intensity, 6 pH specific growth rate was found to be 1.37±0.0249 mg/mg.d. The specific growth rate for *Pithophora* sp. at 25ºC temperature, 50 μmoles/m^2/s light intensity, 8 pH was found to be 0.336±0.015 mg/mg.d. Barclay *et al*. (1985) reported that *Boekelovia* (Chrysophyte) showed maximum growth rate of 3.5 doubling/day while *Ankistrodesmus* sp. (Chlorophyte) exhibited 3 doubling/day. Sommerfeld *et al*. (1987) reported maximum growth rates of some chlorophytes i.e., *Nannochloris* sp.; 2.78 doubling/day, *Chlorella* sp. 2.66 doubling/day, *Eremosphaera* sp. 3.47 doubling/day.

Estimation of total lipid content:

The amount of total lipids in the cell biomass of the selected algal species is as follows:

Pithophora sp.; 27.99 %±1.09
Botrydiopsis arhiza; 47.99% ±0.722

Chisti 2007 had reported the oil contents of different microalgae on % dry weight basis, the average of which is equivalent to 33.82 % ±3.76.

CONCLUSION

Specific growth rate of *Pithophora sp.* was found to be 0.49±0.022 doubling/day with lipid content of 27.99%±1.09 while, the growth rate of *Botrydiopsis arhiza* was found to be 1.97±0.036 doubling/day with lipid content of 47.99 % ±0.722. Present efforts were fruitful regarding the isolation of native algal species that could be suitable candidate for the production of algal fuel. But extensive research is needed for the optimization of the concentration of some important mineral nutrients and up scaling of the process.

ACKNOWLEDGMENT

Present research work was partially supported by WWF-Pakistan under the project code 50044001.

REFERENCES

Araujo, S.C. and V.M.T. Garcia, 2005. Growth and biochemical composition of the diatom Chaetoceros cf. wighamii brightwell under different temperature, salinity and carbon dioxide levels. I. Protein, carbohydrates and lipids. Aquaculture, 246(18): 405-412.

Barclay, B., N. Nagle, K. Terry and P. Roessler, 1985. Collecting and screening microalgae from shallow, inland saline habitats. Aquatic Species Program Review: Proceedings of the March 1985 Principal Investigators' Meeting, Solar Energy Research Institute, Golden, Colorado, SERI/CP-231-2700, pp: 52-68.

Bischoff, H.W. and H.C. Bold, 1963. Phycological Studies IV. Some Soil Algae From Enchanted Rock and Related Algal Species. University of Texas, Austin, 6318: 1-95.

Brand, L.E. and R.R.L. Guillard, 1981. The effects of continuous light and light intensity on the reproduction rates of twenty-two species of marine phytoplankton. J. Exp. Mar. Bio. Eco., 50(16): 119-132.

Chisti, Y., 2007. Biodiesel from microalgae. Biotechnol. Adv., 25: 294-306.

Coyle, W.A., 2007. Global Perspective: The Future of Biofuels, Economic Research Service. US Department of Agriculture, Washington, DC, Retrieved from: http://www.ers.usda.gov/Amber Waves/November07/PDF/Biofuels.pdf.

Creedy, J., 1977. A Laboratory manual (Heinemann Educational, 1977). A mine of information on the management of the school laboratory and the preparation of material for practical work.

Danilov, R.A. and N.G. Ekelund, 2001. Effects of pH on the growth rate, motility and photosynthesis in Euglena gracilis. Folia Microbiol (Praha), 46(6): 549-554.

Field, C.B., M.J. Behrenfeld, J.T. Randerson and P. Falkowski, 1998. Primary production of the biosphere: integrating terrestrial and oceanic components. Science, 281: 237-240.

Folch, J., M. Lees and G.H.S. Stanley, 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem., 226: 497.

Graham, J.M., M.T. Auer, R.P. Canale and J.P. Hoffmann, 1982. Ecological studies and mathematical modeling of Cladophora in Lake Huron: 4. Photosynthesis and respiration as functions of light and temperature. J. Great Lakes Res., 8: 100-111.

FAO, 1997. Corporate document repository Renewable biological systems for alternative sustainable energy production. Chap. 6, Oil production. Agricultural Services Bulletin. 128. Retrieved from: http://www.fao.org/docrep/w7241e/w7241e0h.htm.

International Energy Agency (IEA), 2007. Key World Energy Statistics 2007. Retrieved from: http://www.iea.org/textbase/nppdf/free/2007/key_stats_2007.pdf.

Jeon, Y.C., C.W. Cho and Y.S. Yun, 2006. Combined effects of light intensity and acetate concentration on the growth of unicellular microalga *Haematococcus pluvialis* Enz. Microbial Tech., 39: 490-495.

Kitaya, Y., H. Azuma and M. Kiyota, 2005, Effects of temperature, CO_2/O_2 concentrations and light intensity on cellular multiplication of microalgae, Euglena gracilis. Adv. Space Res., 35(9): 1584-1588.

Koru, E. and S. Cirik, 2003. Spirulina platensis (Cyanophyceae) mikroalg'inin effect of temperature on growth and some biochemical properties. E.U. J. Fish. Aquatic Sci., 20(3-4): 419-422 (in Turkish).

Oliveira, M.A.C.L. de, M.P.C. Monteiro, P.G. Robbs and S.G.F. Leite, 1999. Growth and chemical composition of spirulina maxima and spirulina platensis biomass at different temperatures. Aquacul. Int., 7(4): 261-275.

Ota, M., Y. Kato, H. Watanabe, M. Watanabe, Y. Sato, R.L. Smith and H. Inomata, 2009, Fatty acid production from a highly CO_2 tolerant alga, *Chlorocuccum littorale*, in the presence of inorganic carbon and nitrate. Bioresour. Technol., 100: 5237-5242.

Rachlin, J.W. and A. Grosso, 1991. The effects of pH on the growth of Chlorella vulgaris and its interactions with cadmium toxicity. Arch. Environ. Contam. Toxicol., 20(4): 505-508.

Renaud, S.M., H.C. Zhou, D.L. Parry, L.V. Thinh and K.C. Woo, 2002. Effect of temperature on the growth, total lipid content and fatty acid composition of recently isolated tropical microalgae *Isochrysis* sp., *Nitzschia closterium*, *Nitzschia paleacea*, and commercial species Isochrysis sp. (clone T.ISO). Aquaculture., 211(1-4): 195-214.

Sada, E., S. Katoh, A. Kheirolomoon and H. Sawada, 1989. Effects of light intensity on the growth rate of the red alga *Porphyridium cruentum*. J. Ferment. Bioeng., 67(2): 135-137.

Sheehan, J., T. Dunahay, J. Beneman, and P. Roessler, 1998. A look back at the U.S. Department of Energy's Aquatic Species Program-biodiesel from algae. U.S. Department of Energy. Office of Fuels Development.

Sommerfeld, M., S. Ellingson and P. Tyler, 1987. Screening microalgae isolated from the southwest for growth potential and lipid yield. FY 1987 Aquatic Species Program Annual Report, Solar Energy Research Institute, Golden, Colorado, SERI/SP-231-3206, pp: 43-57.

Vonshak, A., G. Torzillo, P. Accolla and L. Tomaselli, 1996. Light and oxygen stress in Spirulina platensis (cyanobacteria) grown outdoors in tubular reactors. Physiol. Plant., 97(1): 175-179.

Wang, C., H. Li, Q. Wang and P. Wei, 2010. Effect of pH on growth and lipid content of Chlorella vulgaris cultured in biogas slurry. Sheng Wu Gong Cheng Xue Bao., 26(8): 1074-1079.

Yeisang, C. and B. Cheirsilp, 2011. Effect of nitrogen, salt, and iron content in the growth medium and light intensity on lipid production by microalgae isolated from freshwater sources in Thailand. Bioresour. Technol., 102(3): 3034-3040.

Zhu, C.J., Y.K. Lee and T.M. Chao, 1997. Effects of temperature and growth phase on lipid and biochemical composition of Isochrysis galbana TK1. J. App. Phycol., 9(5): 451-457.

End Notes:

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