Obtaining microalgae biomass in a small scale reactor

Leonardo García¹, Xavier Álvarez¹, Kenya Bravo¹, Juan Peralta², Alfredo Barriga²

¹Laboratorio de Biotecnología & Genética
Universidad de Guayaquil

Ave. Raúl Gómez Lince s/n y Ave. Juan Tanca Marengo, Guayaquil (Ecuador)

Phone: 04-2253117, e-mail: xalvarezec@gmail.com

²Centro de Desarrollo Tecnológico Sustentable
Escuela Superior Politécnica del Litoral

Campus Prosperina, Km 30.5 vía Perimetral, Guayaquil (Ecuador)

Phone: 04-2269359, e-mail: abarriga@espol.edu.ec

Abstract. In order to industrialize massive production of microalgae with biotechnologically important traits, it is essential to reduce production costs through the design and evaluation of different culture media, which are reflected in the value of the final product. It is important to know and understand the dynamics of growth and behavior of the strain of interest, as well as the response to different environmental factors, in order to obtain an adequate and permanent production of biomass. *Tetraselmis* sp. is an ellipsoidal mobile, compressed alga with four flagella and the acute rear end with the anterior end of four lobes. It is green bright, 7 µm in diameter and 10-16 µm in length. It is a euryhaline species with ability to form spores, whose limits of tolerance to physical variables are very broad. It contains 49.22% protein, 10.60% lipid and 16.38% from carbohydrates. The microalgae *Tetraselmis* sp. was massively cultivated with Guillard media, and their biomass was harvested every 72 h, we obtained an biomass average of 1.35 g/L/d.

Key words: Biomass, microalgae, Guillard media.

1. Introduction

Global energy needs develop exponentially; however, the current reserves of fossil fuels are exhausted rapidly, which has led to explore new alternative and renewable emphasis has been placed on biotechnological development of microalgae for various areas from animal nutrition and cosmetics to the use of biomass for the production of biodiesel. The production of microalgae is complicated and is therefore necessary to use appropriate methodologies and enriched media, to achieve good density cultivation and reduce pollution [1; 2; 3]. Culture at large and medium scale of microalgae and the practical use of its biomass as a source of many products is very import; as an example

of this, the use of the green algae *Chlorella vulgaris*, which can be used as a food supplement in aquaculture, to purify wastewaters in bioremediation [4].

Biodiesel is usually produced from oleaginous crops, such as rapeseed, soybean, sunflower and palm. However, the use of microalgae can be a suitable alternative feedstock for next generation biofuels because certain species contain high amounts of oil, which could be extracted, processed and refined into transportation fuels, using currently available technology; they have fast growth rate, permit the use of non-arable land and non-potable water, use far less water and do not displace food crops cultures; their production is not seasonal and they can be harvested daily [5].

2. Cultivation of *Tetraselmis* sp.

Cultivation took place in sea water (23 ‰) coming from town of Ballenita, filtered and enriched with nutrients from the Guillard f/2 substrate [6], sterilized by autoclaving at 121 °C x 1 atm. for 15 minutes.

Figure 1. Mass culture of *Tetraselmis* sp. in a laboratory setting.

The culture was made in the laboratory of biotechnology and genetics, Faculty of Natural Sciences of the University of Guayaquil, 2° 08' 47.99 "S and 79° 55' 03.69" W, in a location at 3 meters above sea level and with a process temperature of 22°C. The cultivation was continued in flasks of 4000 mL, harvesting 3000 mL every 72 hours. The culture was kept for a period of 15 days, replacing the harvested portion with an equivalent amount of fresh media; maintained with aeration (920 cc/min) and constant lighting of 1000 lux. Initial inoculum from *Tetraselmis* sp. had a concentration of 135 000 cells/mL, every 24 hours cell count was performed in New Bauer chamber which was obtained to growth kinetics.

Table 1. Guillard's f/2 medium composition.

Trace elements stock solution	L
Na ₂ EDTA	4.160 g
FeCl ₃ ·6H ₂ O	3.150 g
CuSO ₄ ·5H ₂ O	0.010 g
ZnSO ₄ ·7H ₂ O	0.022 g
CoCl ₂ ·6H ₂ O	0.010 g
MnCl ₂ ·4H ₂ O	0.180 g
Na ₂ MoO ₄ ·2H ₂ O	0.006 g
Vitamin mix stock solution	100 mL
Cyanocobalamin (Vitamin B ₁₂)	0.0005 g
ThiamineHCl (Vitamin B ₁)	0.1000 g
Biotin	0.0005 g
Media composition	
NaNO ₃	0.07500 g
NaH ₂ PO ₄ ·2H ₂ O	0.00565 g
Vitamin mix stock solution	1. 0 mL
Trace elements stock solution	1. 0 mL

Figure 2. Growth kinetics during 144 h of *Tetraselmis* sp.

3. Microalgal biomass harvest

The 3 000 mL harvested were withdrawn from the growth chamber, and added to them 925.13 µmol of KAl(SO₄) to flocculate the biomass. The precipitate retrieved from the flocculation process was subjected to centrifugation for 10 minutes at 3 000 rpm. The pellet obtained in

each harvest was weighed on a Sartorius Analytical scale, model: BL 210 S, with an accuracy of 0.01 mg.

Figure 3. Flocculation of microalgae biomass with KAl(SO₄)

4. Biomass obtained

A total of five harvests was carried out, with a mean value of 12.16 g; this is equivalent to 1.35 g/L/d, generating a total mass of 60.8 g during the massive production period.

Table 2. Biomass obtained in different cultures in continuous cropping of *Tetraselmis* sp.

	C 1	C 2	C 3	C 4	C 5
Weight (g).	11.7	11.4	12.8	12.4	12.5
Media	3.9	3.8	4.26	4.13	4.16

Figure 4. Microalgae biomass obtained.

5. Conclusions and Recommendations

The small-scale reactor of the present study maintained the conditions for continuous culture of *Tetraselmis* sp. and biomass was harvested periodically without affecting the physiological development of microalgae.

The biomass data obtained agree with work done previously [7]. Response remained within the exponential region. More extended work should reach the stabilization and saturation stage.

The flocculation and centrifugation methods combined gave a total utilization of the biomass obtained.

Future research should evaluate the kinetics of growth of *Tetraselmis* sp. with culture media formulated with agricultural fertilizers in order to reduce costs. Care should be taken to balance the N/P ratio

The need to determine culture response to the supply of CO₂ still remains, specifically the effect of concentration and diffusion of the gas in the culture media.

Another important parameter to evaluate is the effect of intensity and characteristics of the applied photosynthetically active radiation

The effect of other culture stressors still remains to be ascertained.

6. Acknowledgment

This research was partially supported by a grant from the Agencia Española para la Cooperación Internacional – AECI (International Cooperation Agency of Spain).

Special thanks to the Faculty of Natural Sciences, University of Guayaquil, the Escuela Superior Politécnica del Litoral and the University of Santiago de Compostela

References

- [1] T. Pillay, “Acuicultura: Principios y Prácticas. Editorial LIMUSA.S.A. 1^{era} Ed. México (1997), pp. 35-55.
- [2] R. Abibidi, “Use of probiotic in larval rearing of new candidate species”. National Bureau of Fish. Genetic Resources (2003), Vol. 8(2), pp. 15-16.

- [3] V. Morales, and A. Develotti, “Phytoplankton” PRADEPESCA. UNION EUROPEA Y OLDEPESCA (1990), Vol. 2, pp. 1-21.

- [4] R.D. Andrade, R. Torres, E.J. Montes and A.C. Fernández “Obtaining of flour to leave of the cultivation of *Chlorella vulgaris* and their protein analysis”. Temas Agrarios (2007), Vol. 12:(1), pp. 50-57.

- [5] L. Gouveia and A.C. Oliveira, “Microalgae as a raw material for biofuels production”. J Ind Microbiol Biotechnol (2009), Vol. 36, pp. 269-274.

- [6] R. Guillard and J. Ryther, “Studies of marine planktonic diatoms. *Cyclotella nana* Hustedt and *Detonula confervaceae* (Cleve)” Gran.Can. J. Microbiol (1962).

- [7] J. Santos, y X. Álvarez, “Evaluación del medio de cultivo Guillard modificado F2 para la producción masiva de *Tetraselmis* sp. como fuente de biocombustible. Laboratorio de Biotecnología y Genética, Facultad de Ciencias Naturales, Universidad de Guayaquil, Guayaquil, Ecuador, (2011).