CHEMICAL ENGINEERING TRANSACTIONS
VOL. 47, 2016
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.aidic.it/cet
Guest Editors: Angelo Chianese, Luca Di Palma, Elisabetta Petrucci, Marco Stoller

ISBN 978-88-95608-38-9; **ISSN** 2283-9216
DOI: 10.3303/CET1647078

Passive Microalgae Immobilization for Wastewater Treatment: Study of Residual Glycerol Consumption

Vanessa E. Blanco-Suarez[a], Susan S. Villamizar-Sanchez[a], Yeimy Y. Peralta-Ruiz[b], Angel D. González-Delgado[c], Andrés F. Barajas-Solano[d], Ana M. Ardila-Alvarez*[a]

[a] Research Center for Sustainable development in industry and energy, Universidad Industrial de Santander, Colombia
[b] School of Basic Sciences, Technology and Engineering, Universidad Nacional Abierta y a Distancia, Tv 45A, Cartagena de Indias, Colombia.
[c] Progam of Chemical Engineering, Universidad de Cartagena, Av del Consulado # 48-152, Cartagena de Indias, Colombia
[d] Universidad de Santander UDES Progam of Environmental Engineering, Applied Environmental Research Group - GAIA, Bucaramanga, Colombia
anamariaalvarez@gmail.com

The aim of the present study is to evaluate the viability of glycerol as carbon source on immobilized algae cultures coupled with a modification on nitrogen (NO_3-) and phosphate (PO_4) concentration for the production of high valuable compounds (carbohydrates, proteins and lipids).
it was determined that the conditions who favour the deposition of carbohydrates are 3-5% (v/v) of glycerol and 0.37 (g/L) of $NaNO_3$. On The other hand, for obtain higher amounts of lipids, a 3% (v/v) of glycerol coupled with a limitation in the nitrogen source (0.04 g/L) are needed. Finally, in order to improve deposition of total proteins, larger concentrations of glycerol (5% v/v), $NaNO_3$ (>0.37 g/L) and phosphate are needed.

1. Introduction

In recent years, biofuels have gained ground worldwide, within biofuels biodiesel stands out mainly by its chemical similarity to petrochemical diesel which can be distributed using existing infrastructure (Peralta-Yahya, & Keasling, 2010). In the Colombian scenario biodiesel has proven to be a cost effective solution to avoid dependence on fossil fuels. According to the National Biofuels Federation of Colombia "Fedebiocombustibles" in 2014 the country produced over 518,452 Tons of biodiesel, however its production has a major drawback: glycerol which it is a byproduct of the transesterification (with a production ratio 1:10), therefore for the Colombian case it had produced about 51,845 Tons of crude glycerol during 2014. However, the biggest problem is the worldwide fall in the price of glycerol, reducing demand and increasing the volumes to be stored and/or disposed safely, the latter increases dramatically the costs to producers.
Due to this scenario, different studies have been conducted focusing on the responsible use for this by-product (Pyle, 2008), among this alternatives can be highlighted processes such as combustion (Johnson & Taconi, 2007), composting, feed (Cerrate et al., 2006), thermochemical conversion (Alhanash et al., 2008), and finally biological conversion for the production of high valuable compounds such as fatty acids (PUFAs), including docosahexaenoic acid (DHA), Docosapentaenoic acid (DPA) and Eicosapentaenoic acid (EPA) (Speers et al. 2014). Within biological conversion has appeared as a sustainable platform for the consumption of glycerol as sole carbon source (Pyle, Garcia & Wen, 2008; Liang et al., 2009; Heredia-Arroyo et al., 2010), however Estevez *et al* (2013) has proved that *C. vulgaris* can grow on media enriched with up to 10% (v/v) of glycerol.
One of the major drawbacks on the implementation of large-scale microalgae systems is the extensive area to use and the large amounts of water required, one possible solution is the implementation of immobilized cultures, this technique has several advantages over the classic or “suspended” cultures such as: reduction on the amount of water, mechanical and chemical resistance (Silveira et al., 2013) and elimination of post-culture processes like flocculation or centrifugation (Moreno-Garrido, 2007).

Please cite this article as: Blanco Suarez V.E., Villamizar Sanchez S.S., Peralta-Ruiz Y.Y., Gonzalez-Delgado Á.D., Barajas-Solano A.F., Ardila A.M., 2016, Passive microalgae immobilization for wastewater treatment: study of residual glycerol consumption, Chemical Engineering Transactions, 47, 463-468 DOI: 10.3303/CET1647078

In the present study we examined on lab scale the viability of glycerol usage as carbon source using immobilized algae coupled with a modification on nitrogen (NO_3-) and phosphate (PO_4) concentration, for the production of high valuable compounds (carbohydrates, proteins and lipids) and glycerol consumption.

2. Methodology

2.1 Microalgae culturing and immobilization

Chlorella vulgaris UTEX 1803 was purchased form the Culture Collection of Algae at University of Texas UTEX, the algae was maintained on 500 mL Bold Basal Culture Media (BBM) (Bischoff and Bold 1963) on 1000 mL flask and mixed using filtered air (0.2 *μ*m membrane filter) with 1% (v/v) of CO_2. After 15 days of culture 100 mL of algae were mixed with 100 mL of BBM on 500 mL flasks, on each flask a polyurethane foam film (5 cm long, 5 cm width, 1 cm thick). The mixture (algae + polyurethane) was mixed using filtered air (0.2 *μ*m membrane filter) with 1% (v/v) of CO_2 until the algae attaches to the material.

2.2 Glycerol/Nitrogen/Phosphorous ratio

In order to prove the effect of Nitrogen and Phosphate on the uptake of glicerol a 3^3 Central composite Design was applied using STATISTICA® 7.0 (Table 1).

Table 1: Variables obtained for the Design of Experiments 3^3

	1	2	3	4	5	6	7	8
Glycerol (% v/v)	1	1	5	5	3	1	1	5
KH_2PO_4 + K_2HPO_4 (g/L)	0,13	0,38	0,13	0,38	0,25	0,13	0,38	0,13
$NaNO_3$ (g/L)	0,12	0,37	0,37	0,12	0,25	0,37	0,12	0,12

	9	10	11	12	13	14	15	16	17
Glycerol (% v/v)	5	3	0	6	3	3	3	3	3
KH_2PO_4 + K_2HPO_4 (g/L)	0,38	0,25	0,25	0,25	0,04	0,46	0,25	0,25	0,25
$NaNO_3$ (g/L)	0,37	0,25	0,25	0,25	0,25	0,25	0,04	0,46	0,25

2.3 Biomass, lipids, carbohydrates and proteins quantification

Every 4 days each of the polyurethane matrix was aseptically removed from flask, the biomass was retrieved by pressing the material into a petri dish, the concentrated algae was filtered using a GF/C 47mm membrane (Whatman) and dry at 60°C overnight and then place in vacuum desiccator until constant weight.
After dry weight the filtered biomass was prepared for lipids, carbohydrates and proteins according to Borowitzka & Moheimani (2013).

2.4 Waste glycerol production and quantification

Glycerol was obtained according to Plata, Kafarov and Moreno (2010). For the characterization of residual glycerol a high efficiency liquid chromatograph with a SUPELCOGELTM C-610 H column coupled to a refractive index detector Agilent Technologies 1200 was used. 10 mL of sample was vacuum filtered using qualitative filter paper, diluted and homogenized for 10 min in an ultrasonic bath. The sample was re-filtered using syringe filters to Olim Peak of Polyvinylidene fluoride (PVDF) 0.45 μm.

2.5 Nitrate and Phosphate quantification

Nitrogen and Phosphate was measured according the methods described by Clesceri et al (1999). For nitrate quantification 50mL of cell-free culture media was mixed with 1mL 1N HCl. The sample was measured at 220 y 275 nm using a spectrophotometer. Eq(1) was used to correct the error due to the presence of organic matter. For phosphate quantification, 3mL of cell-free media was mixed with 1 mL of deonized water and 1mL of Vanadate-molybdate reagent. The solution was mixed and stand for 10 minutes, the absorbance was quantified using a wavelength of 750 nm.

$$(2 * \lambda 275) - \lambda 220 \quad (1)$$

3. Results and discussion

3.1 Waste glycerol production and quantification

High concentrations of carbohydrates can be obtained with a considerable concentration (1-5% v/v) of glycerol (Figure 1), however the highest amount of carbohydrates (46% of total biomass) was obtained under 5% (v/v) of glycerol and 0.37 g/L of $NaNO_3$; these results agree with those reported by Abreu et al. (2012) and Kong et al. (2013) who report that, under mixotrophic conditions (high amounts of glycerol) *C. vulgaris* increases its accumulation of carbohydrates. On the other hand protein concentration requires larger amounts of glycerol (>3 %v/v) and nitrogen (>0.3 g/L). The above allows verify the direct proportionality between the carbon and nitrogen source. The best protein concentration was achieved in treatment 9 working under a high initial concentration of glycerol (5% v / v) combined with a high initial concentration of nitrogen (0.37 g/L), thus achieving a protein concentration corresponding to 20% of the total biomass.

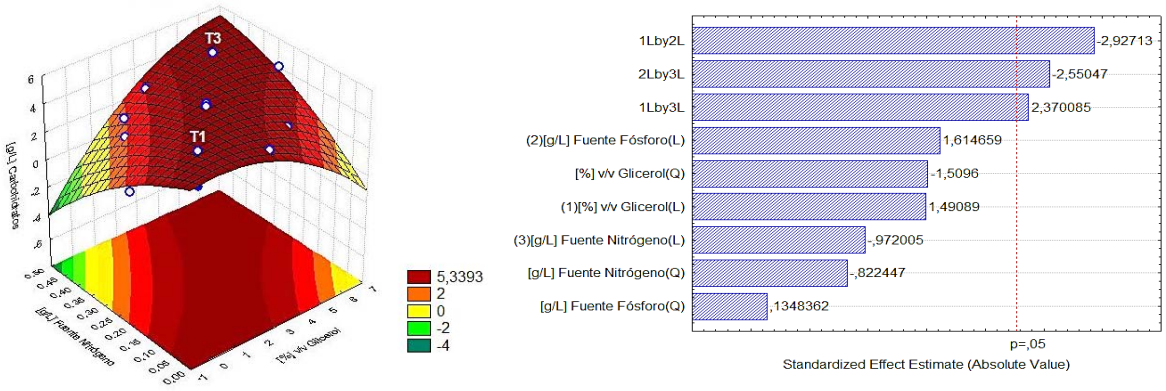

Figure 1: Surface response for the effect of Nitrogen and Glycerol for Carbohydrates accumulation

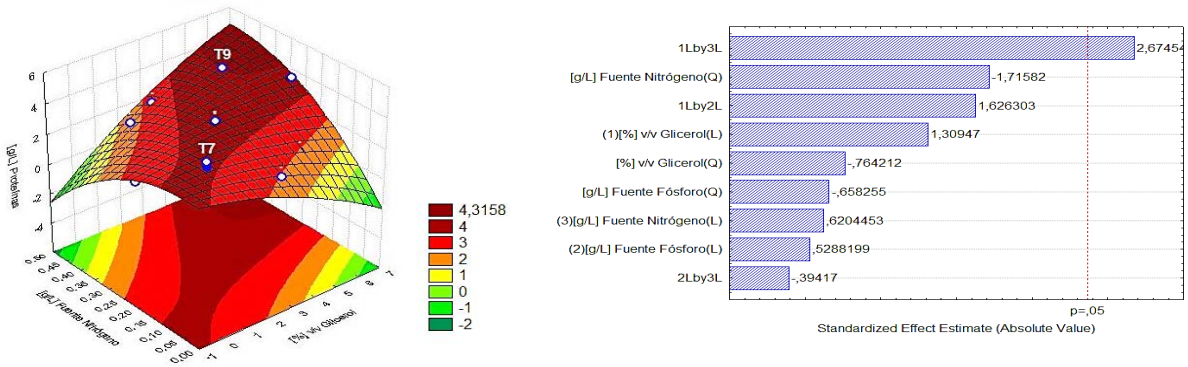

Figure 2: Surface response for the effect of Nitrogen and Glycerol for proteins accumulation

Figure 3 to 5 shows the behavior in the concentration of carbohydrates, proteins and lipids from the first to the last day corresponding to the biomass obtained from the consumption of the source of glycerol (Figure 3) nitrate (Figure 4) and phosphate (Figure 5) in the best treatments. Carbohydrate concentration increases exponentially, this occurs due to complete consumption of nitrogen and phosphate after 5th day. This exponential accumulation of specific metabolites is consistent with the results reported by Bránykiová et al. (2011) and Belotti et al. (2013), however, the nitrogen and phosphorous limitation is not favorable for protein synthesis, because after 8th day its concentration decreased gradually over time.
Is worth noting that crude glycerol was completely consumed by *C.vulgaris* in all treatments, proving its viability as a carbon source. Likewise, it is proved that the detention is a fundamental part in the adaptation of the microalgae to this substrate, while preventing potential inhibition processes (Silveira et al. 2013).

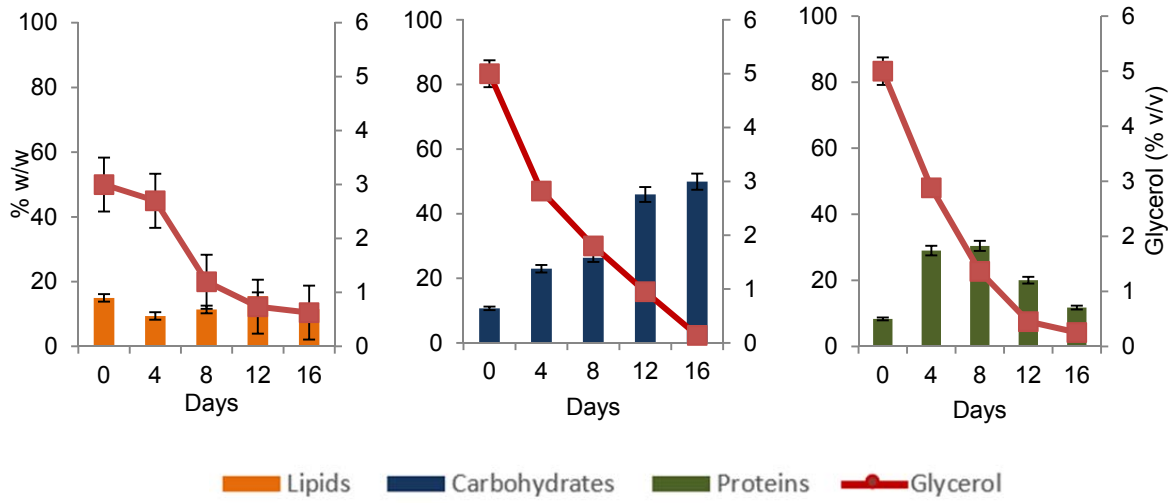

Figure 3: Change on concentration of Carbohydrates, proteins, lipids and glycerol for best experiments through time.

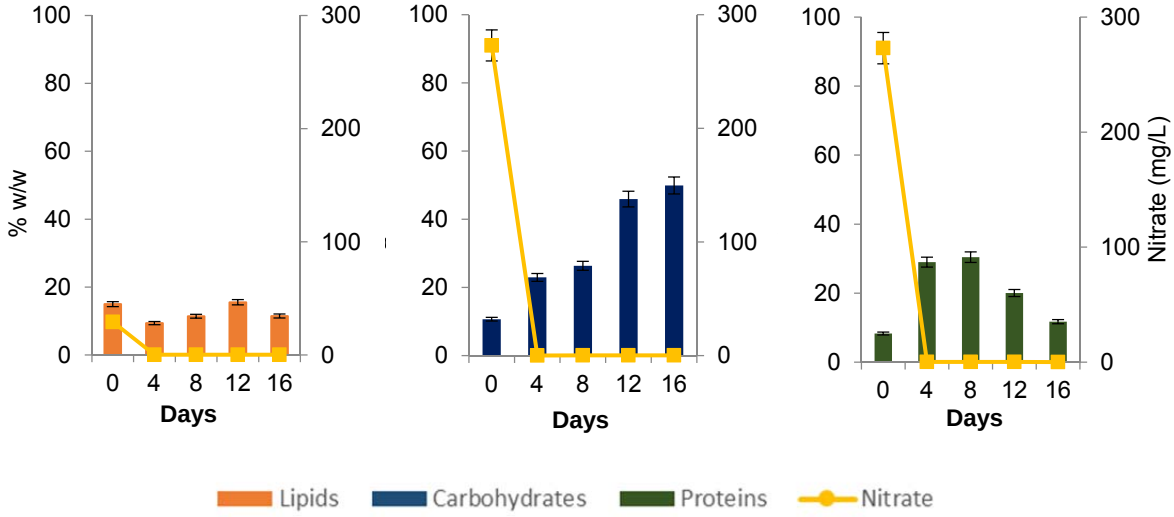

Figure 4: Change on concentration of Carbohydrates, proteins, lipids and nitrate for best experiments through time.

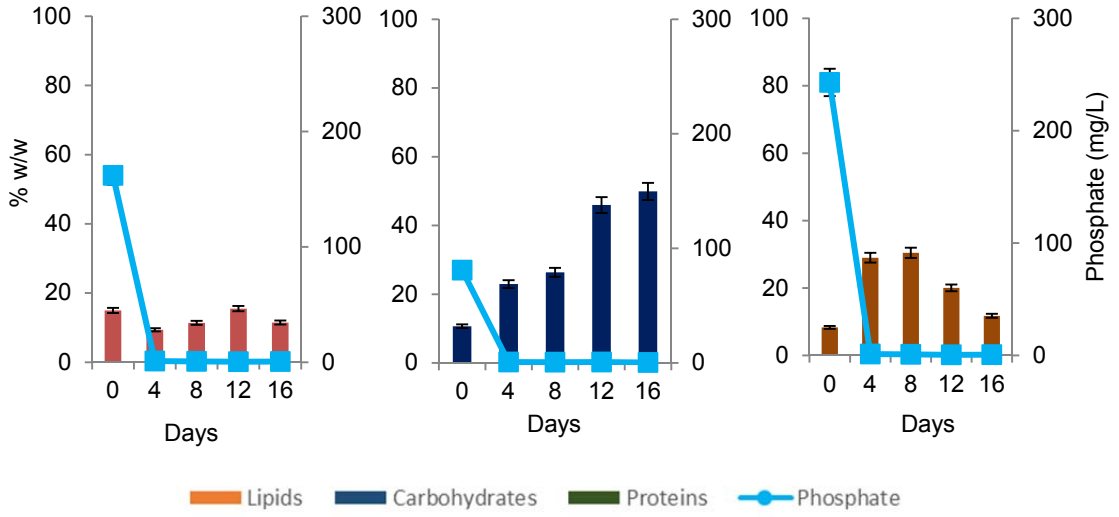

Figure 5: Change on concentration of Carbohydrates, proteins, lipids and phosphate for best experiments through time

4. Conclusions

The results of this study showed that the use of raw glycerol as a carbon source increases the production of metabolites when it consumes larger amounts of the same (between 3 and 5% v/v), compared to treatments at low concentrations of glycerol and absence, it was also found to stimulate the accumulation of lipids and carbohydrates, there should be a limitation on the nitrogen and phosphorus sources. However, to encourage the accumulation of proteins must fully apply the opposite, on the other hand you can highlight that passive restraint favored adaptability from the micro to the substrate and significantly increased the concentration of biomass, managing to surpass the amount of it in Compared with previous studies.

All this results allows to establish an interesting way to reduce the cost in the production of biofuels from microalgae; this use of a low cost substrate such as glycerol and cost reduction in the use of nutrients in the culture medium.

Acknowledgments

The Authors thank to Universidad Industrial de Santander UIS, Universidad de Santander UDES, University of Cartagena and University UNAD for providing materials and equipment for successfully conclude this research.

References

Abreu A.P., Fernandes B., Vicente A.A., Teixeira J., Dragone G. 2012. Mixotrophic cultivation of *Chlorella vulgaris* using industrial dairy waste as organic carbon source. Bioresour Technol. 118, 61–66.

Alhanash A., Kozhevnikova E.F, Kozhevnikov I.V. 2008. Hydrogenolysis of Glycerol to Propanediol over Ru: Polyoxometalate Bifunctional Catalyst. Cat Letters. 120(3), 307-11.

Belloti G., Bravi M., De-Caprariis B., De-Filippis P., Scarsella M. 2013. Effect of Nitrogen and Phosphorus Starvations on *Chlorella vulgaris* Lipids Productivity and Quality under Different Trophic Regimens for Biodiesel Production. Am J Plant Sci. 4, 44-51.

Bischoff H., Bold H. 1963. Phycological Studies. IV. Some Algae from Enchanted Rock and Related Algae Species. University of Texas Publications. 1-95.

Brányiková I., Maršálková B., Doucha J., Brányik T., Bišová K., Zachleder V., Vítová M. 2011. Microalgae novel highly efficient starch producers. Biotechnol Bioeng. 108(4), 766–76.

Cerrate S., Yan F., Wang Z., Coto C., Sacakli P., Waldroup P.W. 2006. Evaluation of glycerine from biodiesel production as a feed ingredient for broilers. Int. J. Poult. Sci, 5(11), 1001-7.

Estévez-Landazábal L.L., Barajas-Solano A.F., Barajas-Ferreira C., Kafarov V. 2013. Improvementof lipid productivity on *Chlorella vulgaris* using waste glycerol and sodium acetate. CT&F - Ciencia, Tecnología y Futuro. 5(2), 113-126.

Heredia-Arroyo T., Wei W. Hu, B. 2010. Oil accumulation via heterotrophic/mixotrophic *Chlorella protothecoides*. Appl. Biochem. Biotechnol. 162(7), 1978-95.

Johnson D., Taconi K. 2007. Glycerin glut: options for the value-added conversion of crude glicerol resulting from biodiesel production. Environ Prog. 26(4), 338-48.

Kong W., Yang H., Cao Y., Song H., Hua S., Xia C. 2013. Effect of Glycerol and Glucose on the Enhancement of Biomass, Lipid and Soluble Carbohydrate Production by *Chlorella vulgaris* in Mixotrophic Culture. Biotechnol. 51(1), 62–9.

Liang Y., Sarkany N., Cui Y. 2009. Biomass and lipid productivities of *Chlorella vulgaris* under autotrophic, heterotrophic and mixotrophic growth conditions. Biotechnol Lett. 31, 1043-49.

Moheimani N.R., Borowitzka M.A., Isdepsky A., Fon Sing S. 2013 Standard methods for measuring growth of algae and their composition. In: Borowitzka MA, Moheimani NR (eds) Algae for biofuels and energy. Springer, Dordrecht, pp 265–284.

Moreno-Garrido I. 2008. Microalgae immobilization: Current techniques and uses. Bioresour Technol. 99, 3949-64.

Peralta-Yahya P.P., Keasling J.D. 2010. Advanced biofuel production in microbes. *Biotechnol. J.* 5(2), 147−62.

Plata V., Kafarov, V. Moreno N. 2010. Optimization of third generation biofuels production: biodiesel from microalgae oil by homogeneous transesterification, Chem. Eng. Trans. 21, 1201-1206.

Pyle D. (2008). Use of biodiesel-derived crude glycerol for the production of omega-3 polyunsaturated fatty acids by the microalga *Schizochytrium limacinum*. M. Sc. Thesis, Faculty of Biological Systems Engineering, Virginia Polytechnic Institute and State University, Virginia, 72pp.

Pyle D., García R. Wenn, Z. 2008. Producing docosahexaenoic acid (DHA)-rich algae from biodiesel-derived crude glycerol: Effects of impurities on DHA production and algal biomass composition. J. Agricul. Food Chem. 56(11), 3933-9.

Silveira M.S., Martins C., Cidrao L., Santaella S. 2013. Immobilization of microbialcells: A promising tool for treatment of toxic pollutants in industrial wastewater. Afr J Biotechnol. 12(28), 4412-18.

Speers A.M., Young J.M., Reguera G. 2014. Fermentation of Glycerol into Ethanol in a Microbial Electrolysis Cell Driven by a Customized Consortium. Environ. Sci. Technol. 48, 6350−58.