24thABCM International Congress of Mechanical Engineering
December 3-8, 2017, Curitiba, PR, Brazil

COBEM-2017-0634
PHOTOBIOREACTOR INOCULATION WITH SWINE SEWAGE FOR MICROALGAE CULTIVATION

Gustavo Strauch Wilin Finger
Johana Guadalupe Blanco Martinez
Graduate Program in Materials Science and Engineering, Federal University of Parana (UFPR),CP 19011, 81531-980, PR, Brazil
Postgraduate Program in Mechanical Engineering. Federal University of Parana, CP 19011, 81531-980, PR, Brazil
finger.gustavo@gmail.com, johana.blanco@ufpr.br

YagoKovara
Department of Environmental Engineering. Federal University of Parana(UFPR), CP 19011, 81531-980, PR, Brazil
yagosko@gmail.com

Adalberto Gonçalves
Department of Chemical Engineering. Federal University of Parana(UFPR), CP 19011, 81531-980, PR, Brazil
adalbertodeq@yahoo.com.br

Iago Gomes Costa
Graduate Program in Mechanical Engineering. Federal University of Parana(UFPR), CP 19011, 81531-980, PR, Brazil
iago_gomes_costa@hotmail.com

Dhyogo Miléo Taher
Graduate Program in Materials Science and Engineering, Federal University of Parana(UFPR), CP 19011, 81531-980, PR, Brazil
dhyogomt@gmail.com

José Viriato Coelho Vargas
Graduate Program in Materials Science and Engineering, Federal University of Parana(UFPR), CP 19011, 81531-980, PR, Brazil
vargasjvcv@gmail.com

André Bellin Mariano
Graduate Program in Materials Science and Engineering, Federal University of Parana(UFPR), CP 19011, 81531-980, PR, Brazil
andrebmariano@gmail.com

Abstract. *Microalgae culture has been used to obtain biomass to produced biofuels and other bio-products, such as antioxidants and food. One of the ways of growing microalgae is through compact photobioreactors (PBR), where environmental conditions can be controlled and the culture can be prevented from undesired pathogenic contamination and water evaporation, which could reduce the quantity of biomass obtained. To become cost effectively, the cultivation of microalgae in photobioreactors must be performed in large volumes to obtain enough biomass and, consequently, large amounts of biofuels can be produced. Besides the quantity of biomass produced, researchers are aware of the biomass lipid content, which as high the content the better is to produce biodiesel. This work brings an inoculation of a 10 m^3 PBR to obtain biomass from the microalgae specie Acutodesmus obliquus. The inoculation was performed with 500 L of swine sewage, which contain nitrogen and phosphorus that are important elements for microalgae metabolism and, at the same time, cannot be displaced in the environment without a previous treatment. The PBR was analysed during 19 days in terms of numbers of microalgae cells (number of cells.L^{-1}), biomass concentration (g.L^{-1}) and absorbance of 540 nm light. After the cultivation period, biomass lipid content was analysed and the result found was 16.07 ± 0.43 %.The final biomass concentration in this 10 m^3 PBR was 0.270 ± 0.009 g.L^{-1}.*

Keywords:*Acutodesmus obliquus, Biomass, Microalgae, Photobioreactor, Swine Sewage, lipid content.*

1. INTRODUCTION

The continuous increase in the world population over the last decades has been lead to an increase demand for energy as well. Nowadays, the most common ways of energy sources been used are those derived from fossil fuels, which are not renewable and are consumed at 10^5 higher rate than the nature can replace (Netravali and Chabba, 2003). The concern about the reduction of availability of such fuels in a near future is pushing scientists and researchers to develop alternative energy sources able to meet global demand; furthermore, these new sources must be renewable and cleaner than those being used today.

An alternative source of energy with high potential to replace or complement those derived from fossil fuels is the energy obtained from biomass, especially vegetal and microalgae's. The biomass energy has an estimated potential for the year of 2050 as 150-450 EJ, which can meet Brazilian demand for energy, estimated to grow 23-30 % until that year (Satyanarayana, Mariano and Vargas, 2011; Huber, Iborra and Corma, 2006). Difficulties for obtaining renewable fuel (e.g., biohydrogen, ethanol, biodiesel), in comparison to fossil fuel obtained directly by mineral extraction, are that renewable fuel requires energetically efficient systems to comply with the demand, so that the energy balance is compensatory. Cogeneration, tri-generation or multi-generation systems are possible alternatives to pursue, in order to reach that objective (Vargas *et al*, 2014).

Even though the energy obtained from vegetal biomass are considered renewable, the use of such biomass to produce alternative fuels (e.g., ethanol, biodiesel) has been constantly criticized by part of scientific community because the soil should be used to grow aliments and not fuels. An alternative to contour this problem is to obtain biomass from specific microalgae species that have high lipid content and can be cultivated in photobioreactors (PBR). Besides that, the amount of biodiesel produced from microalgae is higher than those produced from other superior vegetal, such soy, palm and canola, for example; however, the cultivation of microalgae faces challenges to become competitive to fossil diesel (Chisti, 2007).

Nowadays, microalgae biomass can be used to produce biofuels, such as biodiesel by the transesterification process of lipids (Abomohra *et al.*, 2016), syngas by gasification of biomass (Hu *et al.*, 2014), ethanol by biomass fermentation (Silva and Bertucco, 2016) and gasoline by the cracking of hydrocarbons and isoprenoids (Rohmer, 1999). In addition to the production of biofuels, microalgae can also perform other functions, such as the use of residual biomass after the extraction of the oil to produce biogas, biofertilizers or food supplements (Abomohra *et al.*, 2014; Kim *et al.*, 2015). Depending on the microalgae species, other compounds of aggregate values can also be obtained, such as polyunsaturated fatty acids, natural dyes, pigments and antioxidants (Dutta and Pal, 2014; Wang *et al.*, 2015).

The advantages of cultivating microalgae in photobioreactors over open race ponds include protection against undesired contamination and water evaporation that could reduce the quantity of microalgae cells in the PBR (Judd *et al.*, 2015) and, consequently, the biomass obtained from it. The use of PBRs to produced microalgae biomass includes several types of designs in order to maximize the CO_2 gas (naturally presented in the atmospheric air) to the culture medium (Judd *et al.*, 2015).

The microalgae are photosynthetic unicellular organisms that are used from a range of objectives including not only the production of biofuels but also the bioremediation of sewage (Lourenço, 2006). Since the microalgae needs several chemical compounds, especially nitrogenous, phosphorus and carbon, sewage from pig wastes can be used for growing microalgae in PBR at specific concentration/dilution (Pereira *et al*., 2016) and for minimizing the cost with culture medium.

The Self-Sustainable Energy Research and Development Center (NPDEAS) at Federal University of Paraná maintain photobioreactors (PBR) from 500 mL until 10.000 L of culture volume to obtain microalgae biomass and, consequently, obtain bioproducts from it. The schematic diagram of biomass and bioenergy production presented in NPDEAS is shown in Figure 1.

This works aims to inoculate a 10 m^3 photobioreactor with 500 L of sewage from pig waste. The carbon source was supplied from the carbon dioxide presented in the atmospheric air. The culture medium was analysed during 19days through the growth of cells and biomass content per liter. After the experiment, biomass was obtained and analysed in terms of its lipid content.

2. MATERIALS AND METHODS

2.1 Photobiorreactor

The photobioreactor (PBR) designed for this experiment has 742 translucid tubes made of Polyvinyl chloride (PVC). The transparent tubes allow the microalgae perform photosynthesis due to natural exposition of sun light during the daytime and the injection of atmospheric air. The total volume of this photobioreactor is 10 m^3 (Vargas, 2013) and their culture medium stands for approximately 20 days before enough biomass can be obtained from it. Figure2 brings photography of the photobioreactor used in this experiment.

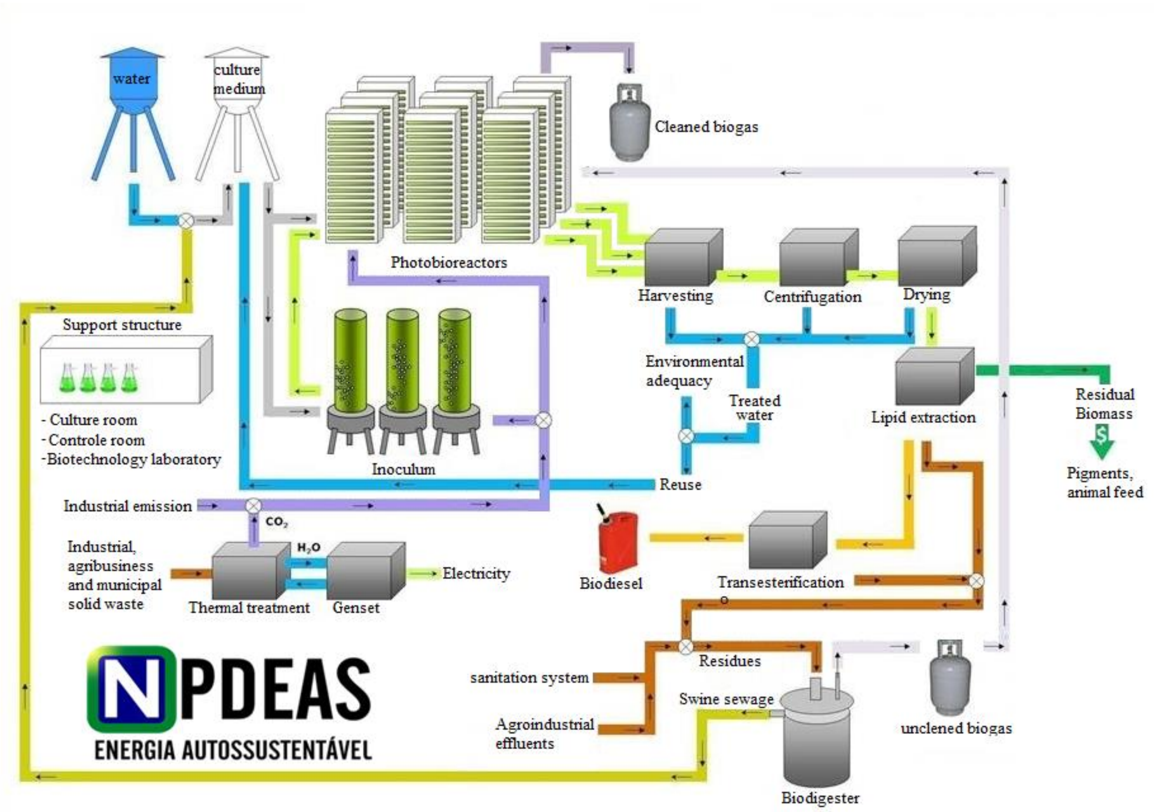

Figure 1. Microalgae Biomass and Energy Production System Conducted at NPDEAS.

Figure 2. Compact Photobioreactor of 10 m^3

2.2 Microalgae Specie, Inoculation and Culture Medium

The selection of microalgae species for cultivation depends on several factors, including lipid content, biomass growth rate, adaptability to environmental changes and future application for biomass acquired (Chisti, 2007). The microalgae chosen for the inoculation is commonly founded at the municipal water supply in Curitiba- PR. Figure 3 shows the specie *Acutodesmus obliquus* (under microscope observation) used in this experiment.

Figure 3. Microalgae *Acutodesmus obliquus* under Optical Microscope (400x times magnification)

The inoculation of the photobioreactor was performed with 500 liters of swine sewage, which is a rich source of nitrogenous and phosphorus. The culture was analysed during 19 days in terms of cell growth (number of cells.L^{-1}), pH and biomass concentration (g.L^{-1}) and photo-spectrometry.

2.3 Culture Growth: Cell Concentration Analyse

For the number of cells, the experimental procedure used is the Neubauer chamber technique. It consists in a rectangular thick sheet of glass for microscopy use, which contains a depression in the center. This depression has two chambers that can be mirrored or not, and have perpendicular lines with quadrants that are used to count cells and determinate the cellular concentration in given volume of a fluid. The cultivation samples were submitted diary to a triplicate on the Neubauer chamber according to the methodology described by Lourenço (2006).

2.4 pH Samples

A pH mPA210 measurer from MS Tecnopon® was used to measure the pH from the samples and a Shimadzu ultra violet spectrophotometer on 540 nm to do the spectrophotometry triplicate both diary.

2.5 Dry Biomass Concentration

The biomass concentration was obtained by filtering a 10 mL of sample in a glass fiber microfilter (47 mm diameter) that was previously taken out of moisture in the stove at 60º C for twenty-four hours and weighted. The samples were put back at the stove at the same parameters. The biomass concentration is then difference between the initial and the final weights.

2.6 Lipid Content

The methodology used in the analysis of total lipid content for the microalgae biomass was described by Folch *et al*., (1957) and adapted by Hosseini *et al*., (2015). In this method, a specific quantity of biomass (P0) was mixed with a solution of chloroform: methanol (2:1 v/v) in a centrifuge tube. The tube was then displaced in an ultrasonic bath during 30 minutes followed by a centrifugation at 4500 rpm during 15 minutes. After centrifugation, the supernatant was removed from the tube and displaced in another tube of known mass (P1). This procedure is repeated three times. The remaining solvent in the tube was evaporated at 60º C. The mass of tube containing the lipid (P2) was then measured. The lipid content could be finally found using Eq. (1):

$$(\%)Lipid = \frac{P2 - P0}{P0} x100 \quad (1)$$

3. RESULTS AND DISCUSSION

3.1 pH

The pH was daily measured and remained between 9-11 as it may be noted in Fig 4. This value is close to the optimal value for microalgae photosynthesis (Kroumov *et al.,* 2016).

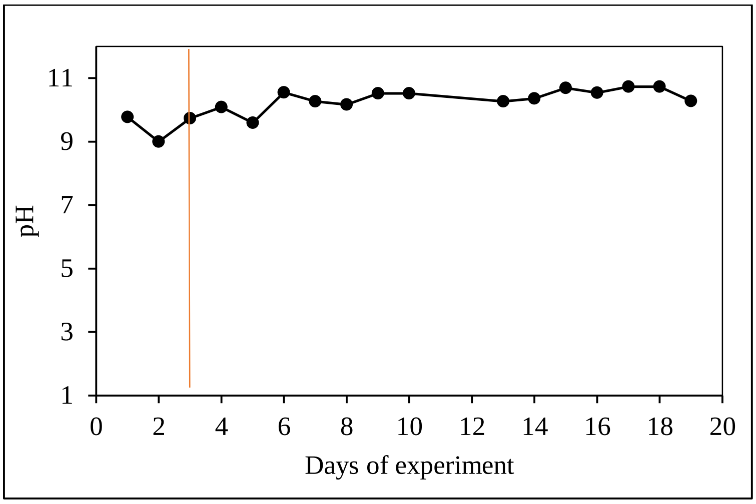

Figure 4. pH

3.2 Cell Concentration

The absorbance (spectrophotometry) and the number of cells are shown in Fig. 5 and Fig. 6, respectively. The reduction in number of cells in the 5th day of experiment is due to the addition of swine swage, when the culture medium became more diluted.

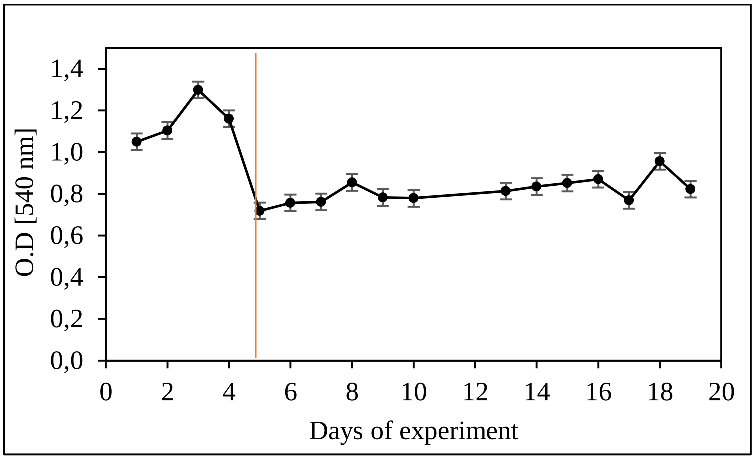

Figure 5. Spectrophotometry (Absorbance)

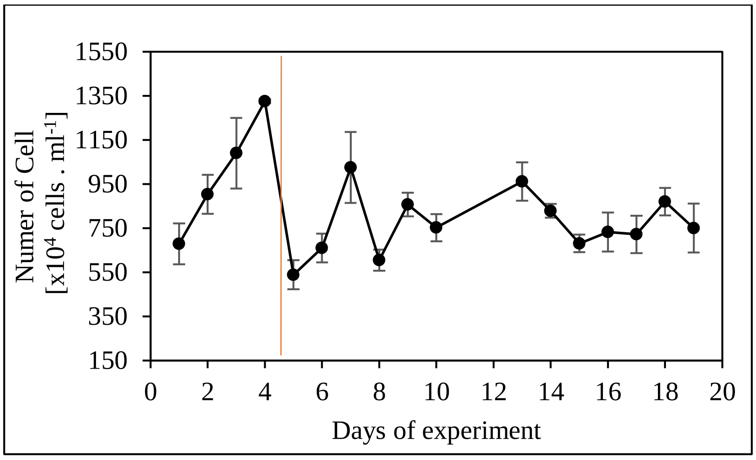

Figure 6. Concentration Cells Throughout the Experiment

The concentration cell on the last day of experiment was 750 ± 110 x10^4cells.mL^{-1},while the first day of experiment the number of cells was 680 ± 95 x10^4 cells.mL^{-1}. The poor growth in the number of cells is possible due to the fact the swine sewage was to dilute or contaminate with high number of pathogenies that compete with microalgae cells.

3.3 Culture Dry Biomass Concentration

The concentration of dry biomass analysed throughout the experiment is shown in Fig 7. The final dry biomass concentration found on 19^{th} day of experiment was 0.270 ± 0.009 g.L^{-1}. From the first day until the last day of experiment, no significant variation of microalgae biomass has been observed. This could occur due to the low photosynthetic process or due low nutrient content in the culture medium. For industrial application, the microalgae biomass should be higher.

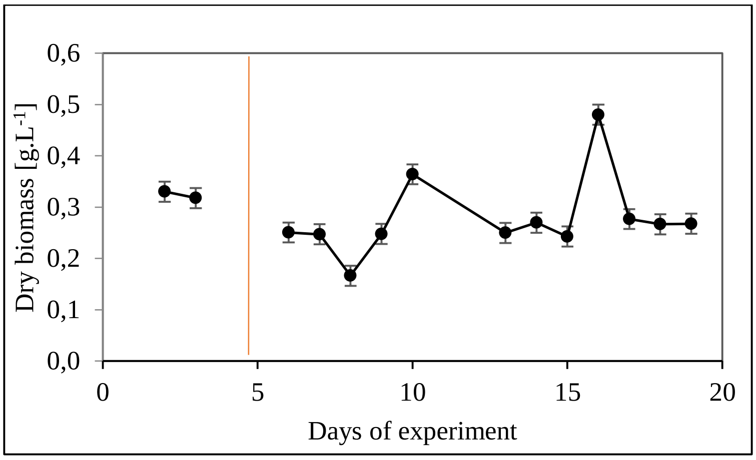

Figure 7. Dry Biomass Concentration

3.4 Total Lipid Conted

After the 19 days of experiment, the biomass was recovered from the photobioreactors and agitated with an organic flocculant (TANFLOC®) at a concentration of 210g.L^{-1}. For the homogenization of culture and flocculant, an agitator attached to an electrical motor was applied. After 24 hours, the biomass decantation occurs and the residual water can be removed. Finally, the humid biomass is put into a drier (Model: Pardal Peg 100) during 36 h at 60 °C. The dry biomass is used to theanalyses of lipid content, which is an important parameter for biodiesel production. The total lipid content found in the biomass was 16.07 ± 0.43 %. This value is similar to the results found by Nascimento *et al*, 2013, when they found a lipid content of 16.73 % on microalgae biomass from the specie *Scenedesmus obliquus,* which its nomenclature has recently changes to *Acutotodesmus obliquus.*

4. CONCLUSION

The use of wastewater from swine sewage for microalgae cultivation has been proposed by several authors over the last decade in order to minimize the cost with culture medium and, at the same time, bioremediate the nutrients presented in the wastewater. This paper work has demonstrated the possibility of growing microalgae in a large scale photobioreactor of $10m^3$volumeusing swine sewage. However, biomass productivity and lipid content found was lower than those encountered by other researches. The final dry biomass concentration obtained was 0.270 ± 0.009 g.L^{-1} with 16.07 ± 0.43 % of lipid content in the microalgae biomass from specie *Acutodesmus obliquus*. The low lipid content could disqualify this microalgae specie as potential source for biodiesel production since there are other species with higher lipid content.

5. ACKNOWLEDGEMENTS

The authors would like to thank CAPES (Coordination for the Improvement of Higher Education Personnel, the Graduate Program in Materials Science and Engineering (PIPE), and the UFPR (Federal University of Paraná) for their financial and physical support to development of this study.

6. REFERENCES

Abomohra, A.E.F., El-Sheekh, M. and Hanelt, D., 2014. “Pilot cultivation of the chlorophyte microalga *Scenedesmus obliquus* as a promising feedstock for biofuel”. *biomass and bioenergy*, 64, pp.237-244.

Abomohra, A.E.F., Jin, W., & El-Sheekh, M., 2016. “Enhancement of lipid extraction for improved biodiesel recovery from the biodiesel promising microalga *Scenedesmus obliquus*”. *Energy Conversion and Management*, 108, 23-29.

Chisti, Y. 2007. “Biodiesel from microalgae”. *Biotechnology Advances*Vol. 25(3), p. 294–306.

de Farias Silva, C.E. and Bertucco, A., 2016. “Bioethanol from microalgae and cyanobacteria: a review and technological outlook”. *Process Biochemistry*, Vol. 51(11), pp.1833-1842.

Folch, J., Lees, M. and Sloane Stanley, G.H., 1957. “A simple method for the isolation and purification of total lipids from animal tissues”. *J biolChem*, Vol., p. 497-509.

Hosseini, N.S., Shang, H., Ross, G.M. and Scott, J.A., 2015. “Microalgae cultivation in a novel top-lit gas-lift open bioreactor”. *Bioresource technology*, Vol 192, p.432-440.

Hu, Z., Ma, X., Li, L., & Wu, J., 2014. “The catalytic pyrolysis of microalgae to produce syngas”. *Energy Conversion and Management*, Vol 85, p. 545-550.

Huber, G.W., Iborra, S. and Corma, A., 2006. “Synthesis of transportation fuels from biomass: chemistry, catalysts, and engineering”.*Chemical reviews*, 106(9), pp.4044-4098.

Judd, S., van den Broeke, L.J., Shurair, M., Kuti, Y. and Znad, H., 2015. “Algal remediation of co 2 and nutrient discharges: A review”. *Water research*, 87, pp.356-366.

Kim, S.S., Ly, H.V., Kim, J., Lee, E.Y. and Woo, H.C., 2015. “Pyrolysis of microalgae residual biomass derived from Dunaliella tertiolecta after lipid extraction and carbohydrate saccharification”. *Chemical Engineering Journal*, Vol. 263, p.194-199.

Kroumov, A.D., Módenes, A.N., Trigueros, D.E.G., Espinoza-Quiñones, F.R., Borba, C.E., Scheufele, F.B. and Hinterholz, C.L., 2016. “A systems approach for CO 2 fixation from flue gas by microalgae—Theory review”. *ProcessBiochemistry*, Vol. 51(11), p.1817-1832.

Lourenço, S.O., 2006. *Cultivo de microalgas marinhas: princípios e aplicações*. São Carlos: RiMa.

Nascimento, I. A., Marques, S. S. I., Cabanelas, I. T. D., Pereira, S. A., Druzian, J. I., de Souza, C. O., & Nascimento, M. A., 2013. “Screening microalgae strains for biodiesel production: lipid productivity and estimation of fuel quality based on fatty acids profiles as selective criteria”. *Bioenergy research*, Vol. 6(1), p. 1-13.

Netravali, A.N. and Chabba, S., 2003. “Composites get greener”. *Materials today*, Vol. 6(4), p .22-29.

Pereira, V.D; Da Costa, M.V.P; Almeida, G.H.G; Taher, D.M.; Mariano, A.B; Vargas, J.C.V. “Análise da concentração de lipídios em biomassa de microalgas em diferentes quantidades de substrato”, In *Proceedings of*6° Congresso da Rede Brasileira de Tecnologia de Biodiesel. Natal, 2016.

Rohmer, M. (1999). The discovery of a mevalonate-independent pathway for isoprenoid biosynthesis in bacteria, algae and higher plants. *Natural product reports*, Vol. 16(5), p. 565-574.

Satyanarayana, K.G., Mariano, A.B. and Vargas, J.V.C., 2011. “A review on microalgae, a versatile source for sustainable energy and materials”.*International Journal of energy research*, 35(4), p.291-311.

Vargas, J.V.C, 2013. *Modelagem e Simulação de Processos de Geração de Hidrogênio via Cultivo de Microalgas em Fotobiorreatores Compactos*. Monografia de Conclusão de Curso de Ciência Biológicas. Universidade Federal do Paraná.

Vargas, J.V.C., Mariano, A.B., Correa, D.O. and Ordonez, J.C., 2014. “The microalgae derived hydrogen process in compact photobioreactors.”*International Journal of Hydrogen Energy*, 39(18), p.9588-9598.

7. RESPONSIBILITY NOTICE

The authors are the only responsible for the printed material included in this paper.