PRODUCTION OF DOCOSAHEXAENOIC ACID (DHA) FROM PRETREATED AGRICULTURAL BIOMASS RESIDUES BY THE HETEROTROPHIC MICROALGAE *CRYPTHECODINIUM COHNII*

S. Staikos[1], A. Karnaouri[1], S. D. Stefanidis[2], K.G. Kalogiannis[2], A.A. Lappas[2], E. Topakas[1]

[1] Industrial Biotechnology & Biocatalysis Group, Biotechnology Laboratory, School of Chemical Engineering, National Technical University of Athens, 9 Iroon Polytechniou Str., Zografou Campus, 15780 Athens, Greece

[2] Chemical Process and Energy Resources Institute (CPERI), Centre for Research and Technology Hellas, 6th km Harilaou-Thermi Road, Thermi, 57001 Thessaloniki, Greece.

* savstaikos@gmail.com

ABSTRACT

Biomass residues are an abundant waste stream, disregarded mainly from agricultural industries, which upon proper pretreatment can be valorized towards the rather low-cost production of valuable chemicals. In this study, agricultural residues (straw, bran) from Greek wheat fields were pretreated chemically with aqueous solutions of organic solvents (50% v/v) in the presence of oxygen, in a process called OxiOrganosolv, where no additional catalysts were used [1]. The pretreatment resulted in an almost entirely delignified solid pulp rich in cellulose, and a hemicellulose-rich aqueous liquid fraction, which both were able to be treated further enzymatically. Herein, the pulp and the liquid fraction were subjected to enzymatic hydrolysis using cellulases and hemicellulases, respectively, in order to break down the complex sugars of the biomass to monosaccharides which can be further used as a carbon source for microbial cultures. The desired product of the process is docosahexaenoic acid (DHA), a polyunsaturated fatty acid with undoubted health benefits and an ever-increasing global demand. However, due to fish stocks shortage, it is essential to produce this valuable nutraceutical compound using other eco-friendlier alternatives. Thus, in this study, for the production of DHA, we employed the heterotrophic microalgae *Crypthecodinium cohnii* (ATCC® 30772™) which is able to grow in variable carbon sources such as organic acids [2] and sugars derived from lignocellulosic biomass [3, 4], while leading to great lipid accumulation and DHA yields.

Our results showed that hydrolysates from pretreated agricultural biomass, more specifically wheat straw, is an efficient carbon source for *C. cohnii* batch cultures towards the production of DHA [5]. More samples from various sources were also examined, namely wheat bran and barley straw. Furthermore, fed-batch cultures in bioreactors were established, in order to enhance the production of DHA. The experiments showed that higher lipid accumulation and higher DHA percentagewise resulted in lower cell biomass productivity and vice versa. Hence, different culture conditions are beneficial for cellular growth and for attaining high total lipids and DHA yields.

KEYWORDS: microalgae, docosahexaenoic acid, oxiorganosolv pretreatment, omega-3 fatty acids

REFERENCES

[1] Kalogiannis, K. G., Karnaouri, A., Michailof, C., Tzika, A. M., Asimakopoulou, G., Topakas, E., & Lappas, A. A. (2020). OxiOrganosolv: A novel acid free oxidative organosolv fractionation for lignocellulose fine sugar streams. *Bioresource Technol*. 313.

[2] Chalima, A., Taxeidis, G., & Topakas, E. (2020). *Reney Energ*, 152: 102-109.

[3] Karnaouri, A., Chalima, A., Kalogiannis, K. G., Varamogianni-Mamatsi, D., Lappas, A., & Topakas, E. (2020). Utilization of lignocellulosic biomass towards the production of omega-3 fatty acids by the heterotrophic marine microalga Crypthecodinium cohnii. *Bioresource Technol*. 303 (122899).

[4] Karnaouri, A., Asimakopoulou, G., Kalogiannis, K. G., Lappas, A. A., & Topakas, E. (2021). Efficient production of nutraceuticals and lactic acid from lignocellulosic biomass by combining organosolv fractionation with enzymatic/fermentative routes. *Bioresource Technol*. 341.

[5] Asimakopoulou, G., Karnaouri, A., Staikos, S., Stylianos, Stefanidis, D., Kalogiannis, K. G., Lappas, A. A., & Topakas, E. (2021). *Fermentation*. 7(4): *219*