Optimization of DHA production from the heterotrophic microalga *Crypthecodinium cohnii* utilizing a dark fermentation effluent

Angelina Chalima[1], Anthi Karnaouri[1], Evangelos Topakas[1]

[1]IndBioCat group, Biotechnology Laboratory, School of Chemical Engineering, National Technical University of Athens, 5 Iroon Polytechniou Str., Zografou Campus, Athens 15780, Greece
Keywords: DHA, dark fermentation, optimization, nitrogen, temperature, *Crypthecodinium cohnii*
Presenting author e-mail: achalima@chemeng.ntua.gr

The heterotrophic microalga strain *Crypthecodinium cohnii* is known for its ability to accumulate high amounts of docosahexaenoic acid (DHA; c22:6), which is one of the necessary omega-3 fatty acids. Its industrial cultivation has begun at the early 1990s (Wynn et al. 2010). However, the minimization of the process cost is another goal yet to be achieved.

In order to reduce the cost of the process, a proposed method is the utilization of waste derived substances as carbon sources for the cultivation of heterotrophic microalgae (Turon et al. 2016). It has already been found that *C. cohnii* can grow on acetate (Ratledge et al. 2001). The process of dark fermentation (DF) can be used for the conversion of municipal solid biowaste fractions and sludgy biowaste from other industries, such as food industry or wastewater treatment facilities, into volatile fatty acids (VFA), mainly acetic acid. Based on this, in previous work, we have examined the ability of the strain to grow on other VFA, creating a fermentation protocol for the cultivation of the strain in DF effluents, according to a biorefinery approach, as well as examining the accumulation of DHA by the cells in fed-batch pH –auxostats. The strain *C. cohnii* ATCC 30772 was able to grow on an ultra-filtrated DF effluent and accumulate DHA at a content as high as 29.8 % (w/w) of total fatty acids after 60 h of fed-batch culture.

The purpose of the depicted work is to optimize the DHA production of the cells, by examining how different growth parameters, such as temperature and nitrogen source affect the cells under acetic acid feeding. Batch cultures of *C. cohnii* on acetate as carbon source were performed and the best temperature and nitrogen conditions for DHA accumulation were discovered. The results promoted the use of ammonium as a favored nitrogen source for biomass production, instead of the expensive yeast extract (YE), thus reducing significantly the cost of the process. The final goal is the use of the optimal conditions found in two-stage fed-batch cultures utilizing a DF effluent. The work is introducing a new chapter to the integration of biowaste and microalgae in a biorefinery process.

Results:

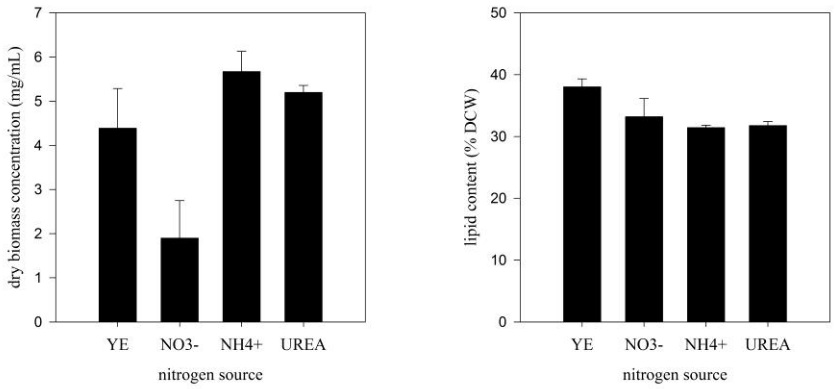

Figure 1. Biomass and lipid production of *C. cohnii* batch cultures with acetate, under different nitrogen source and a ratio C/N= 83 (g/g)

References:

Ratledge, Colin, K Kanagachandran, Alistair J Anderson, David J Grantham, and Janet C Stephenson. 2001. "Production of Docosahexaenoic Acid by Crypthecodinium Cohnii Grown in a PH-Auxostat Culture with Acetic Acid as Principal Carbon Source." *Lipids* 36 (11): 1241–46. doi:10.1007/s11745-001-0838-x.

Turon, V., E. Trably, E. Fouilland, and J. P. Steyer. 2016. "Potentialities of Dark Fermentation Effluents as Substrates for Microalgae Growth: A Review." *Process Biochemistry* 51 (11). Elsevier Ltd: 1843–54. doi:10.1016/j.procbio.2016.03.018.

Wynn, James, Paul Behrens, Anand Sundararajan, Jon Hansen, and Kirk Apt. 2010. "Production of Single Cell Oils by Dinoflagellates." *Single Cell Oils: Microbial and Algal Oils: Second Edition*, 115–29. doi:10.1016/B978-1-893997-73-8.50010-4.