Micro-cultivation of Oleaginous Fungi and High-throughput Estimation of Fatty Acid Profiles by FT-IR spectroscopy

Gergely Kosa[1, 2], Valeria Tafintseva[1], Achim Kohler[1], Volha Shapaval[1, 2]

[1]Dept. Mathematical Sciences and Technology, Norwegian University of Life Sciences
Drøbakveien 31, 1430 Ås Norway
[2]Nofima AS, Osloveien 1, 1430 Ås, Norway
e-mail: gergely.kosa@nmbu.no

Oleaginous microorganisms, which include bacteria, yeasts, molds and microalgae can accumulate lipids which account for more than 20% of their dry weight (Ratledge, 1991). During the past decades, microbial oils or single cell oils (SCO) have been produced as an alternative, sustainable source of biodiesel (plants) and high-value added polyunsaturated fatty acids (fish). Today, arachidonic acid (20:4, ω-6; ARA), eicosapentaenoic acid (20:5, ω-3; EPA) and docosahexaenoic acid (22:6, ω-3; DHA) single cell oils are produced in industrial scale with filamentous fungi and microalgae for nutraceutical applications [1]. Currently the utilization of various low-cost substrates (food rest material and lignocellulosic sugars) is investigated, since it might lead to an economically more viable production of single cell oils.

The aim of the present study was to develop and validate a high-throughput screening system for optimization of SCO-PUFA production. This goal may be achieved by combining micro-cultivation and rapid, high-throughput FT-IR spectroscopy of the microbial biomass and substrate. Several cultivation conditions affect lipid accumulation (pH, temperature, carbon- and nitrogen-source, C/N ratio, morphology, dissolved oxygen concentration). In the present work, the effect of two different temperatures (20°C and 30°C) on lipid accumulation was investigated. Two oleaginous fungi from the phylum Zygomycota (*Mucor circinneloides* and *Mortierella isabellina*) and a reference strain from Ascomycota phylum (*Penicillium glabrum*) were cultivated in 24-square deep well microtiter plates for 12 days using a nitrogen-limited medium. Fermentations were subjected to daily monitoring by FT-IR spectroscopy, gas chromatography (GC-FID) and high-performance anion-exchange chromatography (HPLC). Fatty acid profile and total oil content was determined with GC-FID (reference method) after extraction of the lipids from the mycelium. As a quick and inexpensive alternative measurement, the mycelium was also analyzed directly after sonication by high-throughput Fourier transform infrared spectroscopy. Subsequently the two dataset were calibrated using sparse-PLS regression combined with cross-validation. Sparse PLSR allows better interpretation of the calibration models since it is very selective in choosing relevant parts of FT-IR spectra. Good calibration models were obtained for SAT (saturated fatty acids), MUFA (monounsaturated fatty acids), PUFA (polyunsaturated fatty acids) and for individual fatty acids.

In conclusion, the micro-cultivation system combined with the established FT-IR versus GC-FID calibration model enable rapid screening of low-cost substrates using various microorganisms for the following larger scale optimization of SCO - PUFA production.

References

[1] C. Ratledge, ″Microbial production of polyunsaturated fatty acids as nutraceuticals″, *Microbial Production of Food Ingredients, Enzymes and Nutraceuticals*, p. 531-558, Woodhead Publishing Limited (2013).