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## Opponent's review of Doctoral Thesis

Title: "Controlled production of lipids and lipidic substances by selected yeasts and microalgae"

Author: Ing. Martin Szotkowski.

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- Topic of this thesis is related to Biotechnology of Microorganisms, developing new strategies to use waste substrates, mainly from agriculture and food industry, in order to obtain bioactive compounds. Submitted doctoral thesis of Ing. Martin Szotkowski, deals with the study of production properties of carotenogenic yeasts, microalgae and cyanobacteria with a focus on the production of lipids, lipidic metabolites and other metabolites in connection with the utilization of waste substrates originated from agriculture and food industry.
- The objectives and goals set by Ing. Martin Szotkowski at the beginning of the thesis have been achieved with the results, and subsequent discussion, obtained at the experimental part of the dissertation. Results show that it is possible to cultivate carotenogenic yeasts, microalgae and cyanobacteria and also with a co-cultivation of yeasts and microalgae is possible to obtain biomass enriched with carotenoids, lipids and other valuable substances, confirming the huge potential of their biotechnological use.
- This PhD thesis is very well structured with the classic sections of a thesis and with clear set of objectives to elaborate. There is a theoretical part where studied metabolites are described, followed by microorganisms tested, co-cultivation techniques and methods for microorganism cells and biomass analysis. There is also a Materials and Methods section followed by Results and Discussion chapter that is divided into several units according to the tested types of microorganisms.  
In *first unit of results* related to **cultivation of carotenogenic yeasts**, experimental data show that it is possible to optimize cultivation conditions of yeast using treated waste substrates (as animal fat, dairy whey, glycerol and spent coffee grounds), to obtain biomass enriched with carotenoids, lipids and other valuable substances. Best combination obtained, was media with coffee hydrolysate and coffee/frying oil. This first section shows that yeast grown on waste has a huge potential for the processing of waste from the food and agricultural industries and to produce biomass enriched with molecules of interest.



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The *second unit of results*, related to **cultivation of microalgae and cyanobacteria**, indicate the great potential of these strains in a number of experiments, this microorganisms achieved a high production of valuable substances as carotenoids, lipids and sterols. Form all the microalgae tested, *Scenedesmus* and *Desmodesmus* achieved the best results even if and appropriate combination and timing of stress factors should be used to overall behaviour of microalgal cells.

In *third unit of results*, related to **co-cultivation of yeasts and microalgae**, a dealt with pilot tests of symbiotic co-cultivation is showed. The performed tests confirmed that selected microalgae, cyanobacteria and yeast can grow in a symbiotic growth and coexistence.

The thesis concludes with a dissertation conclusions, references section and bibliography of published papers of Ing. Martin Szotkowski.

- The whole work of this thesis is based on number of experiments all carried out by Ing. Martin Szotkowski. All experiments have been performed at the Institute of Food Science and Biotechnology (FCH BUT) and at the laboratory of Biotechnology of Algae at the University of Huelva (Spain) where the author was as an Erasmus predoctoral researcher. Results and discussion shows the ability of the author to conduct independent analysis and individual statements based on solid data. Ing. Martin Szotkowski work demonstrates all the attributes of serious research
- This thesis brings a new insight into Biotechnology of Microorganisms, developing new strategies to use waste substrates, mainly from agriculture and food industry, in order to obtain bioactive compounds. At the same time, it is an interesting contribution to the debate about the problematic of circular economy and re use of by-products as all the elements should be part of an ongoing cycle of being used, transformed and reused and making them more like natural life cycles.  
In addition the results of the experiments with carotenogenic yeasts and microalgae confirmed the huge potential of their biotechnological use. It was confirmed that by suitable optimization of cultivation conditions, it is possible to modulate metabolites' production and increase the profits of the whole process. Co-cultivation is a potentially very effective method of cultivating yeasts and micro-algae, enabling a reduction in the cost of aeration of the medium providing combined biomass enriched with a number of valuable substances. The conclusions offered are well supported and credible.
- All the thesis is written in English, using a formal adjustment free of grammatical errors and adequate for a PhD manuscript.
- This PhD thesis contains original results and some of them have been already published JCR indexed journals as it can be seen in in Bibliography of published papers section. There are 2 articles published in 2019 and 2020 in *Microorganisms Journal*, 1 in *Food and bioproducts processing* in 2020, 1 in the *EuroBiotech Journal* in 2018 and 1 in *Journal of Biotechnolog* in 2017. Ing. Martin Szotkowski has also 1 manuscript, 4 Conference papers with full text and 15 Abstracts of Communication to Congresses.



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- As I have already mentioned, after reading this thesis manuscript, I can affirm that Ing. Martin Szotkowski shows the ability to conduct independent analysis and individual statements are based on solid data. Bibliographic sources are relevant to the chosen topic. From the opponent's point of view, this thesis by its methodological approach and achieved results, fulfils the demands on dissertations of a doctoral study programme. It answers research questions and verify hypothesis and by its contents brings new knowledge related to Biotechnology of Microorganisms. The candidate proves a remarkable knowledge and methodological background, and ability to apply it in practical application. I recommend this thesis to be defended. My grading is excellent (A).