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Direct lipid and carotenoid extraction from *Rhodosporidium toruloides* broth culture after high pressure homogenization cell disruption: Strategies, methodologies, and yields

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ABSTRACT

Biodiesel produced from oleaginous microorganisms is considered an appealing option since oleaginous yeasts and microalgae can accumulate between 20 % and 80 % lipids of their dry cell weight. However, it is still not economically sustainable since production costs are still high. This work developed a new mild protocol using hexane or sunflower oil as extraction solvents to simultaneously extract lipids and carotenoids from *Rhodosporidium toruloides* biomass directly from the broth culture without using any harvesting technique after performing high pressure homogenization to disrupt the yeast cells. The highest results were obtained for the hexane extractions attaining 55.9 % of total lipids (w/w) and 111.5 μg g^{-1} (eq. torulene) of carotenoids, which are higher values than the ones obtained using lipid extraction traditional methods. The process here presented is an easy, simple, inexpensive and environmentally friendly methodology which involves the simultaneous extraction of carotenoids and lipids directly from *R. toruloides* broth culture which has never been reported before and can greatly improve the economics of the biodiesel production process.

1. Introduction

Oleaginous microorganisms such as yeasts and microalgae can accumulate between 20 % and 80 % lipids of their dry cell weight [1,2] which a significant part can, through a transesterification reaction, be converted into biodiesel [3,4]. However, the cost of the biodiesel produced from oleaginous microorganisms is still not economically sustainable since production costs are still high [5,6].

For biodiesel production from oleaginous microorganisms, several steps have to be performed: biomass production, cell disruption and lipid extraction, followed by a transesterification reaction [7]. From all these processes, the lipid extraction step is reported to be the most expensive [7]. To reduce the overall cost of the process, it is essential to develop more efficient lipid extraction methods to produce low-cost biodiesel from oleaginous microorganisms.

Rhodosporidium toruloides has been widely reported as an oleaginous yeast [5,8,9]. Moreover, *R. toruloides* is able to produce carotenoids with commercial interest: β-carotene, torulene and torularhodin that have very interesting for the food industry [9,10]. If, at the end of the bioprocess, it is possible to extract from the yeast biomass, not only lipids for biodiesel purpose, but also other high-value lipidic biocompounds, such as carotenoids, the overall process cost could be reduced since the value derived from the yeast oil will increase. Such approach is similar to a biorefinery.

One of the biggest challenges to be overcome when developing microorganisms in liquid culture media, is the high liquid quantity present in the broth [11], since most of the lipid extraction protocols use dry biomass. Indeed, the water presence hinders the efficient organic solvent penetration in the cell membranes. However, the microbial biomass drying step (trough freeze-drying or oven) is highly energy demanding and involves high costs. Indeed, harvesting and dewatering techniques such as centrifugation not only represent high energy costs, as well as only can remove efficiently the moisture to a level of 90 % (w/w) [11], being necessary to use drying techniques such as freeze-drying among

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