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New at-line flow cytometric protocols for determining carotenoid content and cell viability during *Rhodosporidium toruloides* NCYC 921 batch growth

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ABSTRACT

Rhodosporidium toruloides NCYC 921 batch growth was monitored as a means to evaluate the yeast biomass potential as a source for the production of carotenoids and other lipids.

Carotenoid content, cell viability and size were assessed by multiparameter flow cytometry. The saponifiable lipid fraction was assayed by gas–liquid chromatography.

The carotenoid production increased during the stationary phase, reaching 78 μg/g while the total fatty acid content attained 32% (w/w) at the end of the fermentation. The fatty acid profile was suitable for biodiesel purposes.

As the yeast cells entered the stationary phase, the proportion of cells with depolarised mitochondrial membrane and cells with permeabilised cytoplasmic membrane increased, attaining 65% and 14%, respectively. Nevertheless, a high proportion of cells (82%) showed esterase activity.

These results demonstrated that flow cytometry can be a powerful at-line technique to monitor the total carotenoids and cell viability during the yeast growth, being useful for the yeast process optimisation at lab and pilot scales.

1. Introduction

According to recent studies, fossil fuels are not environmentally sustainable as they contribute to the greenhouse effect, their extraction is becoming more expensive, and their sources are depleting, resulting in the increase of their market price. Therefore, alternative renewable energy sources must be found [1,2].

Microbial biofuels (obtained from microbes) are attracting worldwide attention as they do not show the inconveniences of biofuels obtained from food agriculture cultures (first generation biofuels, 1G), such as the competition with food supply, arable land and potable water [1].

Oleaginous microbes such as microalgae and yeasts can be used as biodiesel feedstocks. Indeed, these microorganisms grow faster than crop cultures and their cultivation is non-seasonal. Among oleaginous microorganisms, yeasts have a few advantages over bacteria, moulds and algae, due to their higher growth rate, biomass and lipid productivities [2]. Moreover, biodiesel production from yeasts is of particular interest for countries located at higher latitudes, where the daylight is not as long as in countries closer to the tropics, wherein autotrophic microalgae may be more suitable.

However, at the moment, biodiesel derived from microbes is still economically unsustainable, as its production costs are higher than 1G biodiesel [3,4]. Therefore, new strategies must be attained, in order to reduce the overall costs. If the microbial biomass, beyond its high lipid content, is rich in high value added products such as carotenoids (which have many applications in pharmaceutical, nutraceutical, food and feed industries, with a high market value), their commercialisation may contribute to reduce the overall process cost.

The yeast *Rhodosporidium toruloides* NCYC 921 (which species is an anamorph of *Rhodotorula glutinis* species) has been widely reported as a potential oil producer [2]. In addition, this specie, often called “pink yeast”, has also been reported as a source of carotenoids of high commercial interest which are used as a natural food colourant and feed additive in aquaculture. The major carotenoid pigments produced by *Rhodosporidium* species are β-carotene, torulene (3′-4′-didehydro-β-ψ-carotene), and torularhodin (3′-4′-didehydro-β-ψ-caroten-16′-oic acid). They can be present in several proportions and all of them with commercial interest [5]. Therefore, this yeast may be a source of lipids (for biodiesel production) and carotenoids (*e.g.* for food/feed industries).

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