

**Use of flow Cytometry as a quick way to select low cost carbon sources  
for biodiesel and carotenoid production by the red yeast  
*Rhodospiridium toruloides* NCYC 921**

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**1. Introduction** - Some yeasts like *Rhodospiridium toruloides* are oleaginous and can be used for biodiesel production. In addition, some strains are able to produce valuable carotenoid pigments which are of great commercial interest, contributing significantly to reduce the biodiesel production costs. Carotenoid extraction and quantification are time consuming, generate high amounts of waste and require the use of considerable amounts of biomass. Flow Cytometry (FC) can monitor carotenoids accumulation in yeasts, at line, and with a high statistical resolution, during growth. Moreover, during the cultivation it is important to assess cell physiological states, since injured or dead cells decrease the bioprocess yield. If this information is obtained near real time, it is possible to change the process control strategy in order to achieve the maximum lipid and carotenoid productivities.

To obtain a more sustainable biodiesel production, it is also important to use low cost substrates, such as industrial by-products. In the present work, different low cost substrates were used as carbon sources [glycerol as a biodiesel byproduct, sugarcane molasses and carob pulp (syrup) as a cheap by-product of the food industry in the European south countries] to produce lipids and carotenoids by the yeast *R. toruloides* NCYC 921, and a comparison was made. Flow cytometry was used to monitor carotenoid content and cell viability (enzymatic activity, membrane potential and membrane integrity) during all the yeast cultivations.

**2. Methods** – The yeast was grown in shake flasks containing a basal nutrient medium (salts) with different carbon sources. In a first step, glycerol and saccharose (present in molasses) were studied. Fructose and glucose were also studied because they resulted from the saccharose hydrolysis. In a further step, sugarcane molasse and carob pulp (syrup) were used as low cost carbon sources. All the yeast cultivations were monitored by optical density (cell growth), Kjeldahl method (nitrogen detection), HPLC (carbon source consumption), GC (fatty acid analysis) and FC (carotenoid content, membrane potential and integrity and enzymatic activity).

**3. Results and discussion** – In the first stage, data revealed that the carbon source that produced more lipids and carotenoids was saccharose, followed by glucose, fructose and glycerol. These results were confirmed by FC in terms of cell physiological states. Based on these results, sugarcane molasse and carob pulp were studied and results showed that carob pulp syrup, at a dilution of 75, gave the best results in terms of lipids (17% w/w) and carotenoids (21.7 µg/g).

#### **4. Conclusions**

Carob pulp is an abundant and cheap food industry byproduct in Portugal that can be used as carbon source in media formulations for lipids (biodiesel) and carotenoid production by yeasts. This will reduce the overall process costs, making the biodiesel production economically sustainable.

It should be emphasized that this is the first work using flow Cytometry to assess carotenoid and cell viability near real time, when the yeast was grown on different carbon sources. Having this at-line information, it is possible to change process control strategies during the yeast cultivations, in order to achieve the highest carotenoid productivities. This represents a step forward comparing to the conventional techniques currently used for yeast growth monitoring (dry cell weight and optical density that do not give any information on cell physiological states and viability) and carotenoid detection (the results are available after the process is ending, too late for changes to process control).