Simultaneous degradation of hydrocarbons and production of valuable compounds by *Yarrowia lipolytica*

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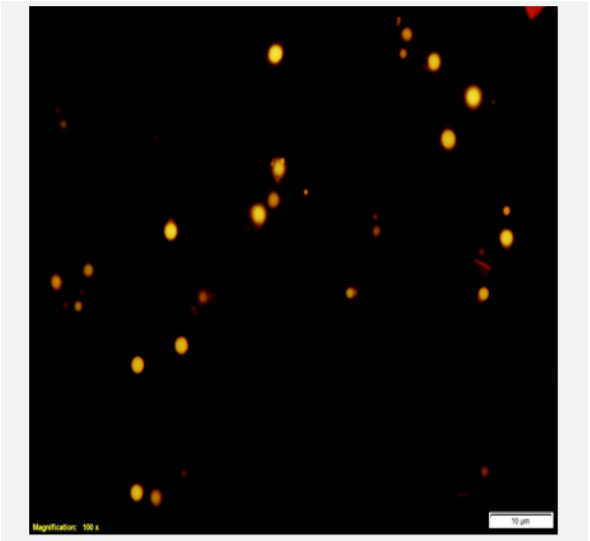

Hydrocarbons are dangerous pollutants and great amounts of these compounds are released to the environment, due to the inadequate handling in the petroleum extraction or in the effluents of industries that use petroleum or its derivatives. The ability of *Yarrowia lipolytica* to efficiently degrade hydrocarbons (herein demonstrated with hexadecane and hexadecene) and use them as carbon source to grow and produce valuable compounds was demonstrated. In hexadecane-based medium, *Y. lipolytica* W29 cells were able to accumulate up to 16 % of their cellular dry weight as intracellular lipids. Due to its composition, similar to that of vegetable oils, these microbial lipids can be used as feedstock for biodiesel production. Moreover, the simultaneous production of lipase (2730 $U \cdot L^{-1}$), which market demand is increasing due to its application in the field of bioenergy, represents an economic advantage. Thus, it is possible to valorize wastes contaminated with hydrocarbons with this bioprocess.

Introduction

Hydrocarbons have become one of the most important types of organic pollutants due to splits during the processes of exploration, extraction, refining and transporting. Moreover, the marketing of petroleum products and the inadequate release of hydrocarbons on effluents of various industries that use petroleum or its derivatives have contributed to the increase of pollution by hydrocarbons. A high negative impact on ecosystems is expected by the release of these compounds into the environment [1,2]. Although several physical and chemical processes have been developed to treat hydrocarbon contaminated environments or industrial effluents, some disadvantages are recognized to these techniques. Biological methods are an attractive alternative because are environmentally-friendly, less expensive and less hazardous and can be applied *in situ* (bioremediation) and *ex situ* (in bioreactors) [3]. Several microorganisms possess the ability to secrete enzymes that degrade hydrocarbons [4]. The non-conventional yeast *Yarrowia lipolytica* is an example of microorganism that is able to degrade hydrocarbons [5]. This yeast strain is known as an industrial workhorse because is capable of producing important industrial metabolites and can grow in agro-industrial by-products or wastes [6,7]. In this work, the ability of *Y. lipolytica* W29 to degrade hydrocarbons (hexadecane and hexadecene) and them as carbon source to produce biomass and added-value products (microbial lipids and lipase) was evaluated in batch microplate and flask experiments.

Methods

Yeast Strain. *Yarrowia lipolytica* W29 (ATCC 20460) was maintained on YPDA medium, composed by ($g \cdot L^{-1}$): yeast extract 10 $g \cdot L^{-1}$, peptone 20 $g \cdot L^{-1}$, glucose 20 $g \cdot L^{-1}$ and agar 20 $g \cdot L^{-1}$, at 4 °C to a maximum of two weeks.

Microplate experiments. The ability of *Y. lipolytica* W29 to grow in hydrocarbons-based medium was assessed in 96-wells microplates during 48 h. Cells were pre-grown in YPD medium for approximately 19 h and 30 µL of pre-inoculum were transferred to each microplate well with 270 µL of sterilized hydrocarbon medium. The hydrocarbons media were composed by hexadecane or hexadecene (1 $g \cdot L^{-1}$ – 10 $g \cdot L^{-1}$) as carbon source, YNB without aminoacids (6.7 $g \cdot L^{-1}$) and Tween 80 1 % (w/v). A control experiment (YNB 6.7 $g \cdot L^{-1}$), was also carried out.

Erlenmeyer flask experiments. Batch experiments with hexadecane (10 $g \cdot L^{-1}$), hexadecene (10 $g \cdot L^{-1}$) and a mixture of both hydrocarbons (5 $g \cdot L^{-1}$ of each hydrocarbon) as carbon source were carried out in 250-mL and 500-mL Erlenmeyer flasks filled with 100 mL of each culture medium (hydrocarbons, YNB without aminoacids 6.7 $g \cdot L^{-1}$ and Tween 80 1 % (w/v)).

Analytical methods. Biomass concentration was quantified by cell counting in the microscope and converted to cell dry weight by a calibration curve. Lipase activity was measured in the cultures supernatant by enzymatic reaction (absorbance measurement at 410 nm during 10 min at 37 ºC), using 1 mM p-nitrophenyl butyrate dissolved in 4 % (v/v) acetone and phosphate buffer 50 mM (pH 7.3) as substrate. One unit of activity was expressed as the quantity of enzyme that produces 1 µmol of *p*-nitrophenol per minute in the assay conditions. Microbial lipids were quantified by the phospho-vanillin colorimetric method, after extraction with methanol and chloroform (1:1, v/v) from lyophilized cells as described by Lopes et al. [8]. The visualization of microbial lipids by fluorescence microscopy was performed after staining with Nile red (0.1 mg/mL in acetone).

Results

Microplate experiments. The ability of *Y. lipolytica* W29 to grow on hydrocarbons-based media was evaluated in microplate batch cultures. A considerable yeast growth was obtained in media with hydrocarbons comparatively to the control (without carbon source) (Figure 1). Independently of hydrocarbon used as carbon source, *Y. lipolytica* was able to grow even in media with 10 $g \cdot L^{-1}$ of hexadecane or hexadecene. In fact, as the hydrocarbons concentration

increased, highest values of absorbance were attained. These results demonstrated the ability of *Y. lipolytica* W29 to degrade hexadecane and hexadecene and use them as carbon source to growth.

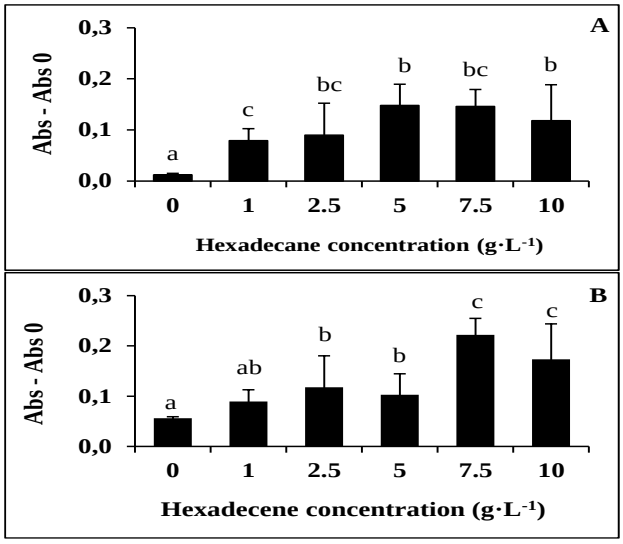

Figure 1. Growth of *Y. lipolytica* W29 on several concentrations of hexadecane (A) and hexadecene (B) obtained in microplate experiments. Values are the average of three independent replicates ± standard deviation.

Erlenmeyer flask experiments. As no growth inhibition was observed with 10 g·L^{-1} of each hydrocarbon, yeast growth and metabolites production in 250-mL Erlenmeyer flask experiments were carried out with this concentration. Additionally, an experiment with a mixture of both hydrocarbons was performed. No differences on biomass concentration were observed for hexadecane and hexadecene-based media, but a higher cellular density was attained with the mixture of both hydrocarbons. The highest lipid content and lipase activity were obtained with hexadecene and hexadecane as carbon source, respectively. The effect of oxygen mass transfer was studied increasing the volume of flask and consequently the headspace. This result in an improvement of biomass concentration in both experiments (hexadecane alone or mixture of both hydrocarbons). Also microbial lipids content and lipase activity was considerably augmented with the increase of oxygen transfer, particularly with in experiments with hexadecane as sole carbon source. Microbial lipids were perfectly observed by fluorescence microscopy after staining with Nile red and lipids accumulated in lipid bodies has an intense yellow color (graphical abstract).

Conclusions

This work demonstrates the ability of *Y. lipolytica* W29 to efficiently use hydrocarbons (hexadecane and hexadecene) as sole carbon source to grow and to produce added-value compounds, such as lipase and microbial lipids. Both metabolites have industrial interest: (a) lipase is exploited for several applications, such as additives in food industry, in detergent industry and in biodiesel production; (b) microbial lipids, due to its composition similar to that of common vegetable oils can be used to obtain biodiesel, a renewable fuel. The bioprocess developed herein has an important impact both from an economic and environmental point of view, since simultaneously occurs the degradation of highly pollutant compounds and the production of added-value metabolites.

Table 1. Values of maximum biomass, microbial lipids content, microbial lipids concentration and maximum lipase activity of *Y. lipolytica* W29 batch cultures obtained in flask experiments.

Flask	Hydrocarbon	Xmax (g·L^{-1})	Microbial lipids (%, w/w)	Lipids concentration (g·L^{-1})	Lipase (U·L^{-1})
250 mL	Hexadecane	3.5 ± 0.02	8.7 ± 0.7	0.3 ± 0.03	1260 ± 125
	Hexadecene	3.0 ± 0.01	9.9 ± 1.4	0.3 ± 0.04	610 ± 112
	Mixture	5.1 ± 0.04	8.5 ± 0.8	0.4 ± 0.003	567 ± 134
500 mL	Hexadecane	4.7 ± 0.03	15.6 ± 1.1	0.7 ± 0.02	2730 ± 304
	Mixture	7.3 ± 0.05	6.6 ± 0.9	0.5 ± 0.01	868 ± 270

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