Batch cultures of *Y. lipolytica* CBS 2075 on hydrocarbons medium under different conditions of oxygenation

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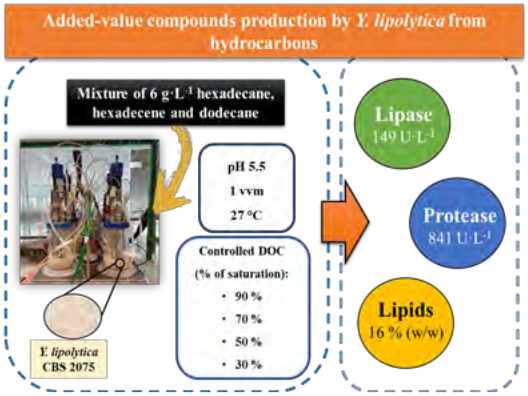

Hydrocarbons are hydrophobic pollutants that can be used as a carbon source in microbial-based processes. *Yarrowia lipolytica* is a nonconventional yeast known for its ability to assimilate hydrophobic substrates to growth and simultaneously produce added-value compounds. The effect of dissolved oxygen concentration on the assimilation of a hydrocarbons mixture, biomass and metabolites production by *Y. lipolytica* CBS 2075 was studied for the first time. The yeast grew in a mixture of hexadecane, hexadecene, and dodecane, producing enzymes (lipase and protease) and intracellular lipids. Though the amount of dissolved oxygen concentration did not affect *Y. lipolytica* CBS 2075 cellular growth, the hydrocarbons consumption rate was slightly dependent on the dissolved oxygen concentration. Low amounts of dissolved oxygen concentration had a clear negative effect on lipase production but no differences were found among all dissolved oxygen conditions for lipids and protease production.

Introduction

Environmental pollution with petroleum hydrocarbons has emerged as a serious ecological and human health concern due to the improper disposal of petroleum-derived effluents. Different techniques have been applied to treat oily wastewaters, but, physicochemical remediation methods are costly and generate other pollutant compounds [1,2]. Therefore, microbial-based processes are environment-friendly technologies and feasible alternatives for the sustainable management of hydrocarbon-polluted effluents [3,4]. Hydrocarbons are hydrophobic pollutants compounds that can be used as a carbon source for microbial growth, but most of the studies are focused on the biodegradation of aliphatic and aromatic hydrocarbons by bacteria species [5]. *Yarrowia lipolytica*, often isolated from oil-contaminated environments, has the ability to assimilate hydrophobic substrates with the simultaneous production of added-value compounds. Although the potential of *Y. lipolytica* to degrade aliphatic hydrocarbons has already been explored, the biotransformation of these compounds into valuable metabolites is much less researched [6]. Since *Y. lipolytica* is a strictly aerobic yeast, the amount of dissolved oxygen in the culture medium is a crucial parameter for bioprocess and may affect intracellular lipids accumulation and the production of enzymes [7]. This work aimed to study the effect of dissolved oxygen concentration (DOC) on the assimilation of a hydrocarbons mixture of hexadecane, hexadecene, and dodecane by *Y. lipolytica* CBS 2075 in bioreactor batch cultures. Furthermore, its impact on yeast growth and the production of added-value compounds (lipids and enzymes) was also evaluated.

Methods

Yeast strain

Yarrowia lipolytica CBS 2075 pre-grow overnight in a YPD medium to prepare cryo-stocks in sterile microtubes (800 µL of yeast culture and 200 µL of glycerol 99.5 %), which were preserved at - 80 °C. Each pre-inoculum was inoculated with one microtube and incubated in an orbital incubator at 27 °C and 200 rpm.

Bioreactor batch cultures

Several experiments were carried out in a 2-L DASGIP Parallel Bioreactor System at 27 ºC and pH 5.5 with 1200 mL of culture medium composed of 6 $g \cdot L^{-1}$ hydrocarbons mixture (2 $g \cdot L^{-1}$ dodecane, 2 $g \cdot L^{-1}$ hexadecane and 2 $g \cdot L^{-1}$ hexadecene), 0.5 $g \cdot L^{-1}$ ammonium sulfate and 3.4 $g \cdot L^{-1}$ corn steep liquor. To evaluate the effect of DOC, four conditions of controlled DOC, from 30 % to 90 % (±10 %) were studied by automatic variation of stirring rates between 200 rpm and 700 rpm. All experiments were conducted at a specific airflow rate of 1 vvm.

Analytical methods

Biomass concentration was quantified by optical density and converted to cell dry weight by a calibration curve. Lipase and protease activities were quantified in accordance with the methods described by Lopes et al. [8]. 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 Pereira et al. [9]. Hydrocarbons were extracted from the culture medium samples by liquid-liquid extraction, using hexane as solvent (1:6, v/v) and undecane as the internal standard.

Results

Y. lipolytica CBS 2075 grew in a 6 $g \cdot L^{-1}$ hydrocarbons mixture without inhibition, and, regardless of DOC in the culture medium, no differences were found in the yeast growth profile, final biomass concentration (Figure 1) and specific growth rates (values ranged between 0.068 h^{-1} to 0.072 h^{-1}). By contrast, the assimilation of hydrocarbons by *Y. lipolytica* CBS 2075 was dependent on the concentration of dissolved oxygen in the culture medium. All hydrocarbons were completely assimilated after 24 h of cultivation, but in the lowest DOC value, total assimilation of hydrocarbons was reached only at 48 h (Figure 1). Although hydrocarbons were assimilated faster in the cultures with 90 %, 70 % and 50 % of DOC when compared to experiments conducted at 30 %, no improvement was attained in biomass yield ($Y_{x/s}$) (values ranged from 1.0 $g \cdot g^{-1}$ and 1.3 $g \cdot g^{-1}$) since hydrocarbons were totally consumed.

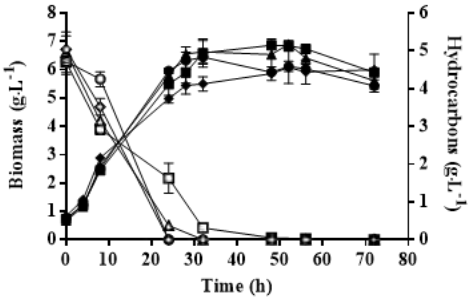

Figure 1. Time course of biomass (closed symbols) and total hydrocarbons (open symbols) concentration obtained in batch cultures of *Y. lipolytica* CBS 2075 carried out in bioreactor at different DOC: 90 % (♦, ◊), 70 % (●, ○), 50 % (▲, Δ) and 30 % (■, □). The error bars represent the standard deviation of two independent replicates.

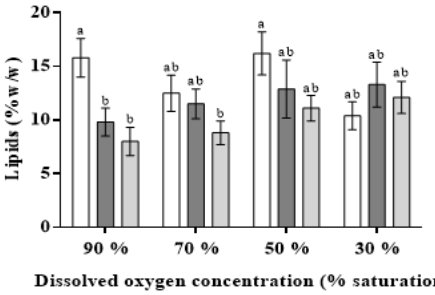

Figure 2. Lipids content of *Y. lipolytica* CBS 2075 at 24 h (white bars), 48 h (dark grey bars) and 72 h (light grey bars) in different DOC (% saturation): 90 %, 70 %, 50 % and 30 %. The error bars represent the standard deviation of two independent replicates.

The effect of DOC on added-value compounds production by *Y. lipolytica* CBS 2075 was also studied. As observed for biomass concentration, lipids content (%, w/w) and lipids concentration (values ranged between 0.8 $g \cdot L^{-1}$ and 0.9 $g \cdot L^{-1}$) were not affected by DOC conditions, and a similar pattern for lipids accumulation was observed in all DOC conditions (Figure 2). At DOC conditions of 90 %, 70 % and 50 %, a higher amount of lipids was accumulated at 24 h by yeast cells, then a decrease in lipidic content at 48 h and 72 h of cultivation occurred owing to intracellular lipids mobilization by cells, which coincide with the total depletion of hydrocarbons from the medium (Figure 1). By contrast, at DOC of 30 %, no decrease in lipids content was observed from 24 h to 48 h, considering that the total depletion of hydrocarbons from the medium was only reached at 48 h of cultivation time (Figure 1). In addition to lipids accumulation, the synthesis of extracellular lipase and protease was also observed (Table 1). Lipase activity was enhanced by the increase of DOC, and an 8-fold improvement in lipase activity was attained by increasing DOC in the culture medium from 30 % to 90 %. By contrast, no statistical differences were observed for proteolytic activity at different DOC conditions (Table 1).

Table 1. Values of maximum lipase (Lip_{max}) and protease ($Prot_{max}$) activities obtained in *Y. lipolytica* CBS 2075 batch cultures carried out in STR bioreactor. Data are average ± standard deviation for two independent replicates. Statistical analysis was performed by columns and values with the same letter are not significantly different ($p \geq 0.05$).

Dissolved oxygen concentration (% saturation)	Lip_{max} ($U \cdot L^{-1}$)	$Prot_{max}$ ($U \cdot L^{-1}$)
90	149 ± 2^{a}	841 ± 42^{a}
70	87 ± 0.3^{b}	585 ± 71^{a}
50	22 ± 3^{c}	628 ± 77^{a}
30	19 ± 0.2^{c}	648 ± 70^{a}

Conclusions

This work demonstrates the ability of *Y. lipolytica* CBS 2075 to grow in a mixture of hydrocarbons (dodecane, hexadecane, hexadecene) without lag phase or inhibition of biomass proliferation. Regardless of DOC in the culture medium, *Y. lipolytica* CBS 2075 growth profiles were similar and no differences were found in specific growth rate, biomass concentration, and biomass yield. By contrast, faster assimilation of hydrocarbons was achieved at DOC conditions of 90 %, 70 % and 50 % when compared to the lowest DOC conditions. Simultaneous with biomass production, the synthesis of intracellular lipids and extracellular enzymes was observed. Whereas lipase activity was positively affected by increasing DOC from 30 % to 90 %, no differences were found for protease activity at different DOC conditions. Although *Y. lipolytica* is known for being a strictly aerobic yeast, in the present study, the decrease of dissolved oxygen in the culture medium was not a limiting factor, thus representing a cost-saving in operating costs (power consumption) owing to the lower stirring rates needed to achieve these dissolved oxygen concentrations. The biotechnological approach presented here opens a new perspective on the application of *Y. lipolytica*-based cultures for the valorization of hydrocarbons, which intend to be helpful in the further development of sustainable strategies from the economic and environmental point of view, fulfilling the guidelines proposed in the circular economy concept.

Acknowledgements

This study was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UIDB/04469/2020 unit and doctoral grant (SFRH/BD/144188/2019), and by LABBELS – Associate Laboratory in Biotechnology, Bioengineering and Microelectromechanical Systems, LA/P/0029/2020.

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