



Selection of yeast species for hydrocarbons and phenolic compounds degradation

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Highlights

- *Yarrowia lipolytica* W29 was able to grow on hydrocarbons (hexadecane and hexadecene).
- *Candida tropicalis* demonstrated an extraordinary ability to grow on phenolic compounds.
- Lipase and microbial lipids were produced from hydrocarbons and olive mill wastewater.

1. Introduction

Large amounts of pollutant compounds are present in industrial effluents and, often, they are not totally degraded by physical and/or chemical methods before the discharge into the environment. Hydrocarbons and phenolic compounds are two examples of pollutants present in agro-industrial effluents, respectively in petroleum refinery effluents and olive mill wastewater (OMW). Biodegradation strategies involving microorganisms to simultaneously degrade these wastes and obtain high added-value products become an interesting approach, since the abundance of these compounds ensures the economic viability of bioprocesses while prevents major environmental problems. This work address the study of the ability of yeast species to grow on hydrocarbons and phenolic compounds as sole carbon and energy source. Moreover, the production of valuable compounds from these wastes was also assessed.

2. Methods

96-well microplate experiments were used to evaluate the ability of: (a) 6 yeast species (*Candida tropicalis* ATCC 250, *Candida utilis* CBS 621, *Candida cylindracea* CBS 7869, *Yarrowia lipolytica* CBS 2075, *Yarrowia lipolytica* W29 and *Pichia pastoris* CBS 2612) to grow on 1 g·L⁻¹ hydrocarbons (hexadecane) and 1 g·L⁻¹ phenolic compounds (catechol, tyrosol and phenol) as sole carbon source; (b) *Yarrowia lipolytica* W29 to grow on different concentrations of hexadecane or hexadecene (1 g·L⁻¹ - 10 g·L⁻¹); and (c) *Candida tropicalis* to grow on different diluted OMW-based media (5 % - 50 %). Batch experiments in 250-mL Erlenmeyer flasks were carried out to confirm the ability of: (a) *Y. lipolytica* W29 to grow on hexadecane (10 g·L⁻¹), hexadecene (7.5 g·L⁻¹) and a mixture of both hydrocarbons (5 g·L⁻¹ of each hydrocarbon); and (b) *C. tropicalis* to grow on undiluted OMW. The effect of oxygen mass transfer was studied in 500-mL Erlenmeyer flasks, by raising the volume of flask headspace, for both yeast strains. Biomass concentration, lipase activity, microbial lipids content and long chain fatty acids were quantified as described by Lopes et al. [1]. Reducing sugars and total phenols were measured as described by Gonçalves et al. [2].

3. Results and discussion

The first screening carried out in 96-well microplates demonstrated: (a) the extraordinary capacity of *C. tropicalis* to grow in all phenolic compounds tested; and (b) the ability of *Y. lipolytica* W29 to grow on hexadecane. A considerable *Y. lipolytica* W29 growth was obtained in media with 10 g·L⁻¹ of hexadecane or hexadecene comparatively to the control (without carbon source). It was also observed that *C. tropicalis* was able to grow on diluted OMW up to 50 % (v/v) medium. In fact, in this condition, a 3-fold improvement on cellular growth was obtained compared to the control (without carbon source). In Erlenmeyer flasks experiments, it was confirmed that *Y. lipolytica* W29 was able to grow on hydrocarbons-based media and using a mixture of hexadecane and hexadecene as carbon source led to an enhancement of final biomass concentration. In these substrates, the yeast had the capability to produce valuable compounds, namely lipase and microbial lipids. The increase of oxygen mass transfer, by raising the volume of Erlenmeyer headspace, had a clearly positive effect on cellular growth, intracellular lipids accumulation and lipase production, especially with hexadecane as carbon source (Table 1).

Table 1. Values of maximum biomass (X_{max}), microbial lipids content, microbial lipids concentration and maximum lipase activity obtained from hydrocarbons by *Y. lipolytica* W29 batch cultures in flask experiments.

Flask	Hydrocarbon	X _{max} (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	15.1 ± 0.1	0.7 ± 0.001	2730 ± 304
	Mixture	7.3 ± 0.05	6.6 ± 0.1	0.5 ± 0.01	868 ± 270

Candida tropicalis was able to grow in undiluted OMW and consume almost all of phenolic compounds in Erlenmeyer flask experiments. A considerable amount of microbial lipids was also produced. The increase of oxygen mass transfer led to an improvement on cellular growth and reducing sugars consumption. However, total phenols consumption and intracellular lipids accumulation were not significantly affected by the raise of Erlenmeyer headspace (Table 2).

Table 2. Values of maximum biomass (X_{max}), reducing sugars consumption, total phenols consumption, microbial lipids content and microbial lipids concentration obtained from OMW by *C. tropicalis* batch cultures in flask experiments.

Flask	X _{max} (g·L ⁻¹)	Reducing sugars consumption (%)	Total phenols consumption (%)	Microbial lipids (%, w/w)	Lipids concentration (g·L ⁻¹)
250 mL	16.3 ± 0.03	38.2 ± 1.1	84.2 ± 0.9	18.3 ± 1.5	3.0 ± 0.8
500 mL	32.0 ± 0.4	55.7 ± 1.3	85.2 ± 0.6	19.8 ± 1.9	6.3 ± 0.8

4. Conclusions

This work demonstrated the potential of: (a) *Y. lipolytica* W29 to degrade hydrocarbons-contaminated effluents; and (b) *C. tropicalis* to degrade phenolic-rich effluents. Both yeast strains were able to produce valuable compounds, lipase and microbial lipids, from these pollutants. Microbial lipids, due to its composition (rich in oleic and linoleic acids) can be used to obtain biodiesel, a renewable fuel.

References

- [1] M. Lopes, S.M. Miranda, J.M. Alves, A.S. Pereira, I. Belo, Eur. J. Lipid Sci. Technol. 121 (2019) 1800188–1800196.
- [2] C. Gonçalves, M. Lopes, J.P. Ferreira, I. Belo, Bioresource Technol 100 (2009) 3759-3763.