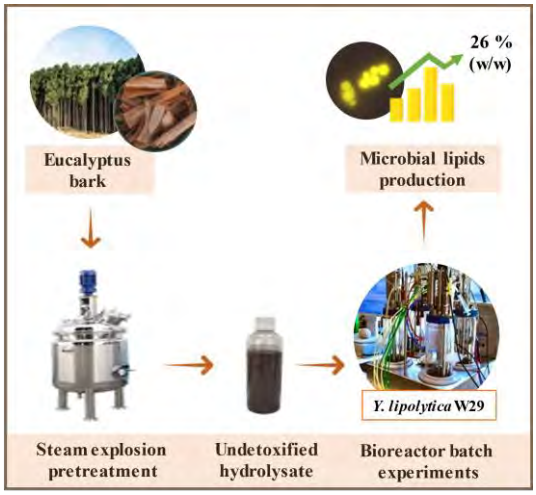
Microbial lipids production by *Yarrowia lipolytica* W29 from eucalyptus bark hydrolysate

B. Dias[1,2], H. Fernandes[1,2], S. Marques[3], F. Gírio[3], M. Lopes[1,2], I. Belo[1,2]*

[1]Centre of Biological Engineering, University of Minho, Campus de Gualtar, Braga, Portugal; [2]LABBELS – Associate Laboratory, Braga/Guimarães, Portugal; [3]Unit of Bioenergy and Biorefineries, LNEG, Estrada do Paço do Lumiar, 22, 1649-038 Lisboa.

*ibelo@deb.uminho.pt



Eucalyptus bark hydrolysate (EBH) is an abundant and renewable source of fermentable sugars for microbial lipids production. However, microorganisms need to be tolerant to antimicrobial compounds present in these type-hydrolysates and formed during lignocellulosic biomass processing. The ability of *Y. lipolytica* W29 to grow and produce lipids in undetoxified and undiluted EBH was evaluated in stirred tank bioreactor. The effect of medium composition and volumetric oxygen transfer coefficient on biomass and lipids production was also studied. A final biomass concentration of 21.8 $g \cdot L^{-1}$ and 26 % (w/w) of lipids (5.6 $g \cdot L^{-1}$) were obtained at optimum conditions (2 $g \cdot L^{-1}$ corn steep liquor supplementation and volumetric oxygen transfer coefficient of 66 h^{-1}). *Yarrowia lipolytica W29* demonstrated robust characteristics to be used as a platform for lipids production into a lignocellulose-based biorefinery bioprocess.

Introduction

The increase in the world population drove researchers to search for alternative lipidic sources to vegetable oils used in biofuels, food, feeds, and pharmaceutical industries [1]. Agricultural waste and lignocellulosic biomass can guarantee a cost-effective and sustainable production pattern to meet the lipids market demand [2]. Eucalyptus bark is an example of abundant and renewable lignocellulosic biomass that can be used for microbial cultures after appropriate pretreatments [3]. In pretreatments, the cleavage of main biomass constituents' chemical bonds (lignin, cellulose, and hemicellulose) results in the release of monomeric assimilable sugars and some compounds (e.g., organic acids, furans, phenolic compounds) with antimicrobial activity [3]. The microbial lipids production from eucalyptus bark hydrolysate (EBH) is highly dependent on the microorganism's suitability to consume hexoses and pentoses and its tolerance to EBH-derived compounds (EBH-C) [4]. This work evaluated the potential of using EBH as a low-cost substrate for microbial lipids production by *Y. lipolytica* W29, studying the effects of medium composition and volumetric oxygen transfer coefficient (k_La).

Methods

Eucalyptus bark hydrolysate preparation

EBH was obtained by steam explosion pre-treatment of eucalyptus bark, followed by enzymatic treatment with Cellic® CTec3 and was composed of 56 $g \cdot L^{-1}$ glucose, 9 $g \cdot L^{-1}$ xylose, 6 $g \cdot L^{-1}$ acetic acid, 0.6 $g \cdot L^{-1}$ formic acid, 0.2 $g \cdot L^{-1}$ 5-HMF, 0.7 $g \cdot L^{-1}$ total phenolic compounds, 0.4 $g \cdot L^{-1}$ total nitrogen and 0.01 $g \cdot L^{-1}$ total phosphorus.

Bioreactor experiments

Yarrowia lipolytica W29 batch cultures were carried out in 2-L DASGIP Parallel Bioreactor System (Eppendorf, Hamburg, Germany) filled with 400 mL of undiluted EBH. To study the effect of EBH supplementation, corn steep liquor (CSL) (0.5 $g \cdot L^{-1}$ and 2 $g \cdot L^{-1}$) and KH_2PO_4 (0.3 $g \cdot L^{-1}$) was added to EBH with a C/N ratio of 75 by the addition of $(NH_4)_2SO_4$. The experiments were conducted at constant agitation of 600 rpm and an aeration rate of 2 vvm. The effect of k_La was studied in a medium composed of undiluted EBH, 2 $g \cdot L^{-1}$ CSL and 1.8 $g \cdot L^{-1}$ $(NH_4)_2SO_4$, by varying the agitation and aeration rates, obtaining values of k_La equal to 44 h^{-1} (600 rpm, 2 vvm), 54 h^{-1} (800 rpm, 2 vvm) and 66 h^{-1} (800 rpm, 3 vvm). All bioreactor experiments were conducted at 27 °C and a constant pH of 5.5.

Analytical methods

Biomass concentration was determined by cell counting using a Neubauer counting chamber and converted to cell dry weight ($g \cdot L^{-1}$) through a conversion factor. Glucose, xylose and EBH-C (acetic acid, formic acid and 5-HMF) were quantified by HPLC using an Aminex HPX-87H column at 60 °C, coupled with RI and UV detectors. Sulfuric acid 5 mM was used in the mobile phase at a flow rate of 0.7 $mL \cdot min^{-1}$. Microbial lipids were quantified using the phospho-vanillin colorimetric procedure according to Lopes et al. [5]. The fatty acid composition of microbial lipids was determined by analysis of fatty acid methyl esters, according to the protocol described by Lopes et al. [5].

Results

Yarrowia lipolytica W29 grew in undiluted EBH without the removal of EBH-C and no latent phase was observed (Figure 1). Increasing CSL concentration from 0.5 $g \cdot L^{-1}$ to 2 $g \cdot L^{-1}$ led to approximately 1.5-fold improvement of biomass concentration at the end of the exponential growth phase (Figure 1) and specific growth rate (from 0.06 h^{-1} to 0.09 h^{-1}). Furthermore, the addition of 2 $g \cdot L^{-1}$ of CSL to the EBH medium also led to faster consumption of glucose and xylose (Figure 1). CSL is a by-product of the corn steeping process, which can promote yeast growth by providing "unidentified growth factors" [6]. Although the specific growth rate and sugars and acetic acid consumption were higher in the medium containing 0.3 $g \cdot L^{-1}$ KH_2PO_4 compared to cultures without phosphorus supplementation, the final biomass (Figure 1) and biomass yield were statistically equal. It is important to highlight that both sugars (glucose and

xylose) and EBH-C (acetic acid, formic acid, and 5-HMF) were completely and simultaneously consumed, proving that *Y. lipolytica* W29 was able to assimilate all compounds of EBH without needing detoxifying methods, which agrees with previous *Y. lipolytica* studies in sugarcane bagasse hydrolysate [2]. Medium supplementation also influenced microbial lipids production. Although maximum lipids contents obtained were statistically equal regardless of CSL concentration (Table 1), lipids concentration at 48 h was 2.7-fold higher in 2 g·L^{-1} CSL compared to 0.5 g·L^{-1} CSL. Lipids accumulation was lower in KH_2PO_4-supplemented hydrolysate in comparison with non-phosphorus-supplemented medium (Table 1).

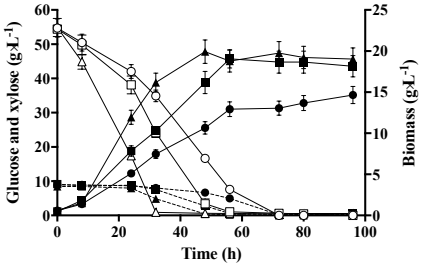


Figure 1. Time course of biomass (closed symbols), glucose (open symbols), and xylose (dashed line) concentration obtained in *Y. lipolytica* W29 batch cultures carried out with undiluted eucalyptus bark hydrolysate supplemented with 0.5 g·L^{-1} CSL (●, ○); 2 g·L^{-1} CSL (■,□); 2 g·L^{-1} CSL and 0.3 g·L^{-1} KH_2PO_4 (▲,△). The error bars represent the standard deviation of two independent replicates.

Table 1. Values of maximum lipids content and lipids concentration at 48 h obtained in experiments carried out with undiluted eucalyptus bark hydrolysate supplemented with CSL and KH_2PO_4. Data are present as average ± standard deviation of two independent replicates. Statistical analysis was performed by columns and values with the same letter are not significantly different ($p \geq 0.05$).

Medium (g·L^{-1})	Maximum lipids content (%)	Lipids conc. (g·L^{-1})
0.5 CSL	16.2 ± 2.0^{a}	1.0 ± 0.1^{a}
2 CSL	13.6 ± 2.2^{a}	2.6 ± 0.5^{b}
2 CSL + 0.3 KH_2PO_4	8.5 ± 1.4^{b}	1.2 ± 0.2^{a}

The effect of k_La on biomass and lipids production by *Y. lipolytica* was also studied. Although the increase of k_La did not affect the specific growth rate, with values varying between 0.095 h^{-1} and 0.103 h^{-1}, biomass yield, with values between 0.27 g·g^{-1} and 0.32 g·g^{-1}, final biomass and glucose and xylose consumption (Figure 2), the stationary growth phase was reached early at k_La of 66 h^{-1} condition than in lower k_La conditions. Lipids content was positively affected by k_La increase (Table 2). Furthermore, a 1.4- and 1.6-fold improvement in microbial lipids concentration were attained increasing k_La from 44 h^{-1} to 54 h^{-1} and to 66 h^{-1}, respectively. The main fatty acids of the lipids were oleic (48 % - 64 %), palmitoleic (20 % - 21 %), palmitic (10 % - 14 %) and stearic acids (6 % - 17 %), making these lipids suitable feedstock for biofuel industry.

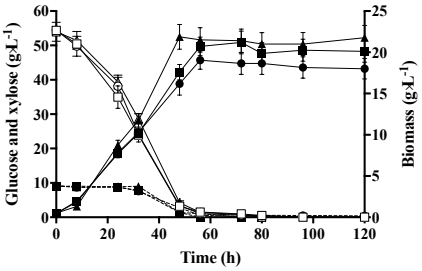


Figure 2. Time course of biomass (closed symbols), glucose (open symbols), and xylose (dashed line) concentration obtained in *Y. lipolytica* W29 batch cultures carried out at different k_La conditions: 44 h^{-1} (●, ○, black line); 54 h^{-1} (■,□, dark grey line); and 66 h^{-1} (▲,△, light grey line). The error bars represent the standard deviation of two independent replicates.

Table 2. Values of lipids content and concentration at 48 h obtained in *Y. lipolytica* W29 experiments carried out at different k_La conditions. Data are present as average ± standard deviation of two independent replicates. Statistical analysis was performed by columns and values with the same letter are not significantly different ($p \geq 0.05$).

k_La (h^{-1})	Lipids content (%)	Lipids conc. (g·L^{-1})
44	16.2 ± 2.0^{a}	2.6 ± 0.5^{a}
54	20.7 ± 3.8^{ab}	3.6 ± 0.5^{a}
66	25.6 ± 4.4^{b}	5.6 ± 0.9^{b}

Conclusions

This work demonstrates the possibility of using undetoxified EBH for *Y. lipolytica* W29 growth and microbial lipids accumulation, being media composition and k_La important factors in bioprocess optimization. The reuse and valorization of lignocellulosic biomass through *Y. lipolytica* W29 lipids production fulfill the assumptions of the biorefinery and circular bioeconomy.

Acknowledgements

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