Production of erythritol by *Yarrowia* species from crude glycerol

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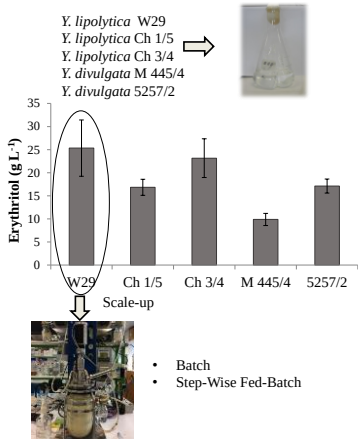

Erythritol is a four-carbon sugar alcohol that has about 70 % of the sweetness of sucrose. Erythritol is mostly produced by fermentation from glucose or glycerol using osmophilic fungi. In this work different strains and species of *Yarrowia* genus were evaluated for their ability to produce erythritol from crude glycerol, a byproduct of biodiesel industry. The performance of strains from *Y. lipolytica* and *Y. divulgata* species was compared in flasks batch culture. All strains were able to produce erythritol in the tested conditions. *Y. lipolytica* W29 strain presented the highest concentration, yield and productivity of erythritol. A scale-up to a lab-scale stirred tank bioreactor using two different operation modes, batch and step-wise fed-batch was performed. With the scale-up 3-fold increase on productivity was observed. In the step-wise fed-batch the addition of glycerol allowed to reach almost the double of erythritol concentrations (64 g L^{-1}).

Introduction

In the last century, the added sugar products consumption has increased and also the number of metabolic diseases [1]. Since these events seem to be related, the necessity of finding healthier options to added sugar, such as low-calorie sweeteners, became a priority [2]. Sugar alcohols, natural sweeteners, are used as sugar substitutes [1]. Erythritol, a four-carbon polyol, is a biological sweetener widely found in nature [1,3]. This sweetener has around 70 % of sweetness of sucrose [1,3]. Erythritol is a small molecule, with low calorific value and it is easily absorbed by the human body, so it not cause the common intestinal problems related with the use of other sweeteners [1]. Erythritol can be used as food sweetener by people with diabetes and obesity because this sweetener is not metabolized by the human body and is excreted unchanged in the urine without changing blood glucose and insulin levels. It is a not toxic sweetener and noncariogenic, since it cannot be fermented by the bacteria that cause dental caries [1]. Erythritol is industrially produced by fermentation of glucose, sucrose or glucose from chemically and enzymatically hydrolyzed wheat and corn starches by yeast-like fungi such as *Torula* sp. and *Moniliella pollinis* [3,4]. Erythritol can also be produced by some osmophilic yeasts and some bacteria [3-5].

Yarrowia lipolytica has the ability to grow in several carbon sources, including agro-industrial wastes, and produced a variety of value-added products such as organic acids, microbial lipids, lipases, biosurfactants and polyols like mannitol and erythritol [6]. *Yarrowia divugata* was recently described. This species can be found in animal related and marine sources [7]. This species can use several carbon sources like sugars, alcohols, organic acid and n-hexane. The production of erythritol, mannitol, citric acid and accumulate lipids was described [7,8].

The main goal of this work was to compare the production of erythritol by strains from two species of *Yarrowia* genus, *Y. lipolytica* and *Y. divulgata*. Using selected strain, the bioprocess was scaled-up from flasks to lab-scale bioreactor at different operating modes (batch and step-wised fed-batch).

Material and Methods

In this work, the production of erythritol was studied for 5 strains of two species of *Yarrowia*. The three strains from *Y. lipolytica* were *Y. lipolytica* W29 (ATCC 204600), *Y. lipolytica* Ch 1/5 and *Y. lipolytica* Ch 3/4, the last two were isolated from cheese, and *Y. divulgata* M 445/4 and *Y. divulgata* 5257/2, were isolated from grounded raw meat. Batch cultures to select the best strain were carried out in Erlenmeyer flasks with 200 mL of production medium.

After pre-growth in YPG medium (in % (w/v): glycerol 2, yeast extract 1 and peptone 2), 1 g L^{-1} of cells were added to the production medium, that was composed by (g $L^{-1)}$: crude glycerol 100; yeast extract 1; NH_4Cl 3; NaCl 25, KH_2PO_4 0.2; $MgSO_4 \cdot 7H_2O$ 1; dissolved in phosphate buffer 0.72 M, pH 3. The experiments were performed at 27 °C and 200 rpm. Experiments in bioreactor were performed in a Bioengineering fermenter (RALF PLUS SOLO, Biengineering, Switzerland) of 1.7 L Batch cultures were carried out at 27 °C, 3 vvm of aeration rate, 900 rpm of stirring rate and pH 3, using the same inoculum preparation as in flasks. In the step-wise fed-batch, after 72 h, 100 g L^{-1} of glycerol was added.

For all experiences, culture samples were collected for analysis of cell concentration (optical density measured at 600 nm and then converted to dry cell mass per liter), glycerol consumption and erythritol production. Glycerol and erythritol concentrations were determined by HPLC using an ion-exchange column (Aminex HPX-87 H) attached to a RI detector. The column, at 60 °C, was eluted with H_2SO_4 5 mM at 0.7 mL min^{-1}.

Results and discussion

All strains tested were able to produce erythritol (Figure 1). Only the results between W29 and M 445/4 were statistically different. However, the higher concentration was obtained by W29 strain (25 g L^{-1}). The strain with lower concentration of erythritol was *Y. divulgata* M 445/4 that produced only 9.8 g L^{-1}.

Rakicka et al. [8] studied the production of erythritol by several species from *Yarrowia* clade, including a strain of *Y. lipolytica* and one of *Y. divulgata*. In that studied *Y. divulgata* presented a better result than *Y. lipolytica*, although the production medium composition was very different. The optimum culture

conditions to produce erythritol by *Y. divulgata* can be different, and are not described.

No statistically differences were observed between *Y. lipolytica* strains, the strain W29 was selected to further studies in bioreactor since it presented the highest erythritol concentration.

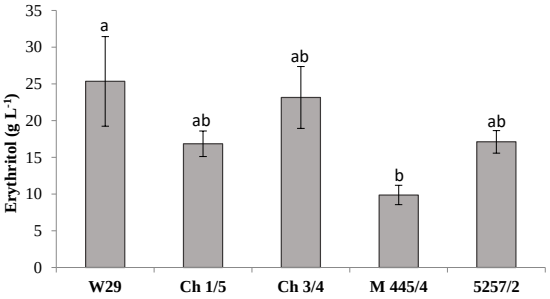

Figure 1 – Maximum concentration of erythritol produced by the strains *Y. lipolytica* W29, *Y. lipolytica* Ch 1/5, *Y. lipolytica* Ch 3/4, Y. *divulgata* M 445/4 and *Y. divulgata* 5257/2 from crude glycerol in flask batch cultures. The values are presented as average and standard deviation of two experiments. The letters represent the statistical analysis (one-way ANOVA, $p \leq 0.05$) equal letters correspond to equal results.

With the scale-up to a lab-scale bioreactor the concentration of erythritol obtained was almost 10 g L^{-1} higher than in flask (Figure 2). Moreover, a 3-fold increase in productivity (0.45 g L^{-1} h^{-1}) was observed.

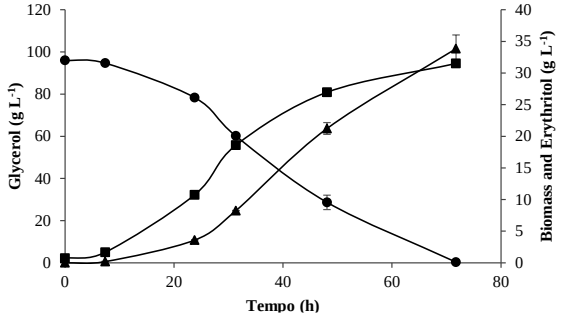

Figure 2 - Cellular growth (■), glycerol consumption (●) and erythritol production (▲) profile in batch cultures of *Y. lipolytica* W29.

During the batch mode, the erythritol production was increasing without showing a decrease or a slowing down until the glycerol is completely consumed (Figure 2). This profile suggests that if the carbon source had not been exhausted, the cells would continue to produce erythritol. Thus a step-wise fed-batch was planned (Figure 3). In this experiment, after 72 h of batch culture more glycerol was added to the bioreactor. As expected the production of erythritol continued and no cellular growth as observed. In this experiment was possible to double the final concentration of erythritol (64 g L^{-1}). The productivity was the same though all experiment. After adding glycerol to the bioreactor, the yield (0.56 g g^{-1}) in erythritol increased 1.65-fold comparing to batch phase. With this step-wise fed-batch strategy the yield achieved was closed to the theoretical value (0.66 g g^{-1}) [9].

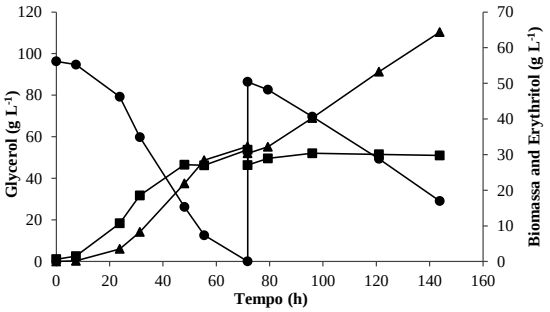

Figure 3 - Cellular growth (■), glycerol consumption (●) and erythritol production (▲) profile in step-wise fed-batch cultures of *Y. lipolytica* W29.

Conclusions

Besides the well-studied *Y. lipolytica* also the new species *Y. divulgata* can produce erythritol, although at lower titers than the ones reached by *Y. lipolytica* for the experimental conditions used in this work.

Y. lipolytica W29 prove to be a great producer of erythritol, from crude glycerol, a low cost carbon source and a byproduct from biodiesel industry.

Acknowledgments

This study was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UID/BIO/04469 unit and COMPETE 2020 (POCI-01-0145-FEDER-006684) and BioTecNorte operation (NORTE-01-0145-FEDER-000004) funded by the European Regional Development Fund under the scope of Norte2020 - Programa Operacional Regional do Norte and through the bilateral cooperation project FCT/NKFIH 2017/2018

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