Biotechnological production of polyols through conversions of crude glycerol by newly isolated strains of the yeast *Yarrowia lipolytica*

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Abstract
The purpose of this study is to investigate the ability of newly isolated *Yarrowia lipolytica* strains to grow on crude glycerol, the main by-product of the industrial production of biodiesel. In particular, the ability of the yeasts to metabolize glycerol and produce dry cell weight (DCW) and secondary metabolites such as lipid, endopolysaccharides and polyols (e.g. mannitol, arabitol, erythritol) was assessed. Two newly isolated strains (LMBF Y-46 and LMBF Y-47) were used, while trials were performed in different initial glycerol concentrations ($Glol_0$=40-120 g/L) and various initial pH values (3.0-7.0) in shake-flasks. It has been seen that polyols production increased with decrease of pH value into the medium. At low $Glol_0$ concentrations (=40 g/L), almost exclusively mannitol was synthesized (i.e. the strain LMBF Y-46 produced ~20 g/L of mannitol at pH=3.0). When $Glol_0$ increased, other polyols (i.e. erythritol and arabitol) were also produced in appreciable quantities. At a pH=3.5 and for $Glol_0$~120 g/L, a total quantity of polyols ~57 g/L was synthesized for the strain LMBF Y-46. Cellular lipids in restricted quantities (8-14% in DCW) were produced, while cellular polysaccharides increased with the time reaching to values of *c*. 35-42% w/w in DCW at the stationary phase of growth.
Keywords: biodiesel-derived glycerol, erythritol, mannitol, polyols, *Yarrowia lipolytica*

1. Introduction

The worldwide decrease of hydrocarbon feedstocks, the continuously increasing CO_2 emissions due to the consumption of the several types of non-renewable fuels and the concomitant rise in the price of crude oil have rendered as a very important priority the application of "renewable" fuels in various types of engines and heating systems, with biodiesel being considered as one of the most important ones (Papanikolaou and Aggelis 2009; 2011). Thus, the forthcoming significant rise in the production of biodiesel and the concomitant glycerol over-production and disposal (glycerol is the principal residue deriving from biodiesel production facilities) is likely to cause very serious environmental problems in the near future. Therefore, conversion of concentrated glycerol-containing water (that is the so-called "crude", "waste" or "industrial" glycerol) to higher added-value products is currently a very important priority in the Industrial Microbiology (Chatzifragkou and Papanikolaou 2012; Papanikolaou and Aggelis 2019).
In the present study, newly isolated yeast isolates of the species *Y. lipolytica* were tested in relation to their potential to convert crude glycerol, waste deriving from biodiesel-producing facilities, under nitrogen-limited conditions for the production of yeast biomass, microbial lipid and polyols.

2. Materials and Methods

In the experiments conducted, two strains of *Y. lipolytica*, namely LMBF Y-46 and LMBF Y-47, were used. All cultures contained glycerol as the sole carbon under nitrogen-limited conditions. In all cases the nitrogen source used were yeast extract (1.0 g/L) and peptone (2.0 g/L). Glycerol was employed as substrate in various initial concentrations ($Glol_0$ ranging between 40 and 120 g/L). The composition of mineral salts in the media (in g/L) was: KH_2PO_4 7.0, Na_2HPO_4 2.5, $MgSO_4*7H_2O$ 1.5, $FeCl_3*6H_2O$ 0.15, $CaCl_2*2H_2O$ 0.15, $MnSO_4*H_2O$ 0.06 $ZnSO_4*7H_2O$ 0.02. Submerged fermentations were conducted in 250-mL Erlenmeyer flasks filled with 50±1 and inoculated with 1 mL of exponential preculture. Flasks were incubated in an orbital shaker (180±5 rpm, T=30±1ºC).
Biomass was harvested by centrifugation (9000 rpm, T=4 ºC, 10 min) and washed twice with distilled water. Dry cell weight (DCW) was determined and then extraction of the lipids for quantitative determination was carried out (Papanikolaou et al 2013). The production of intra-cellular polysaccharides, determined by DNS was also carried out. Production of mannitol, consumption of sugars as well as qualitative determination of the produced intra-cellular polysaccharides was determined by high performance liquid chromatography (HLPC) (Papanikolaou et al 2017).

3. Results and Discussion

In the set of experiments performed, the production of polyols (mannitol + arabitol + erythritol) increased with decrease of pH value into the medium for both strains tested. At low initial glycerol ($Glol_0$) concentrations imposed (=40 g/L), almost exclusively mannitol was synthesized (i.e. the strain LMBF Y-46 produced ~20

g/L of mannitol at pH=3.0, while the respective value for pH=7.0 was 6.8 g/L – in both instances almost all of the available glycerol quantity had been assimilated.). When $Glol_0$ increased, other polyols (i.e. erythritol and arabitol) were also produced in appreciable quantities, while the conversion yield of mannitol produced per unit of glycerol consumed decreased. Therefore, at a pH of 3.5 and for $Glol_0$~120 g/L, a total quantity of polyols ~57 g/L was synthesized for the strain LMBF Y-46 (21.7 g/L of mannitol, 24.6 g/L of erythritol and 10.3 g/L of arabitol). The kinetics of the assimilation of glycerol (Glol, g/L) as well as biomass (dry weight – X, g/L) and polyols (ΣPol, g/L) production by the above-mentioned strain cultivated in shake-flask trials is seen in Fig. 1. It is interesting to observe in this figure, that after the arrival of the stationary growth phase, non-negligible cell autolysis occurred at the late growth steps, which, in any case, did not provoke any cease in the consumption of glycerol and the secretion of polyols (Fig. 1).

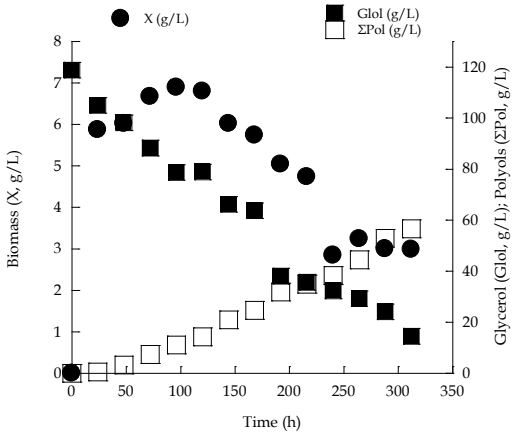

Fig1.
Kinetics of biomass (X, g/L) production, glycerol assimilation (Glol, g/L) and total polyols (ΣPol, g/L) accumulation into the medium by *Yarrowia lipolytica* LMBF Y-46 during growth on crude glycerol-based media, in shake-flask experiment under nitrogen-limited conditions. Culture conditions as in "Materials and Methods". Each point is the mean value of two independent measurements (SE<15%).

Cellular lipids in restricted quantities (8-14% in DCW) were produced. It has been seen that somehow elevated lipid quantities were detected at the early growth phases, decreasing afterwards. Total cellular polysaccharides increased with the time reaching to values of *c*. 35-42% w/w in DCW at the stationary phase of growth. Lipids produced by both strains, consisted of mainly palmitic acid (C16:0), stearic acid (C18:0), oleic acid (Δ9C18:1), linoleic acid (Δ9,12C18:2) and *α*-linolenic (Δ9,12,15C18:3) acids, with the dominant fatty acid in all the fermentations being oleic acid (Δ9C18:1).

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