PHYSIOLOGICAL BEHAVIOR OF YEAST *YARROWIA LIPOLYTICA* ON CRUDE GLYCEROL MEDIA IN BATCH AND FED-BATCH CULTURES

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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 yeast 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. The newly isolated strain (FMCC Y-74) was used, while trials were performed in different initial glycerol concentrations with and without addition of sodium chloride at concentration of 10 g L^1 and a fed-batch fermentation in shake flasks.

Methods and Materials

In the experiments conducted, all cultures contained glycerol as nomimal source by carbon and nitrogen-limiting conditions prevailed. In all cases the nitrogen source used were yeast extract (1 g L^{-1}) and peptone (2 g L^{-1}). The composition of mineral salts in the media (in g L^{-1}) 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 Erlenmeyer flasks 250mL, of 50mL liquid medium were inoculated with 1mL of exponential pre-culture. Flasks were incubated in an orbital shaker (180±5 rpm, 30±1ºC).

Biomass was harvested by centrifugation (9000 rpm, 4ºC, 10 min), washed twice with distilled water and centrifuged again. Dry biomass weight was determined, biomass was measured by drying and then extraction of the lipids for quantitive determination. The production of intra-cellular polysaccharides, determined by DNS, was also found.

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).

Table 1. Production of polyols with and without addition of sodium chloride.

$Glol_0$ (g L^{-1})	Time (h)	X (g L^{-1})	$Glol_{cons}$ (g L^{-1})	Mannitol (g L^{-1})	Erythritol (g L^{-1})	Arabitol (g L^{-1})	$\Sigma_{polyols}$ (g L^{-1})	$Y_{polyols/Glolcons}$ (g g^{-1})
with	96	7.58	38.62	6.13	4.02	3.21	13.36	0.35
	144	4.12	45.66	11.58	5.90	5.53	23.01	0.50
	264	6.01	55.67	24.34	3.79	3.63	21.76	0.39
without	96	7.00	37.83	9.17	6.49	4.11	19.77	0.52
	168	3.78	65.62	15.62	15.77	7.19	38.58	0.59
	216	3.05	73.36	18.45	13.07	9.73	39.25	0.54
	240	2.54	76.18	22.14	14.59	6.91	40.64	0.53

Results

At initial substrate concentration of 40 g L^{-1} with the addition of sodium chloride, the total quantity of polyols was 24 g L^{-1} in comparison without addition was 22 g L^{-1}. The main polyol which producted was mannitol, but the other polyos such as, erythritol and arabitol were also produced in appreciate quantities. In fed-batch fermentation, the polyols production were increased, when a sterile substrate was added again (38 g L^{-1} mannitol). Cellular lipids in restricted quantities (8-12% DCW) were produced, while cellular polysaccharides increased with the time reaching to values of 36-47% w/w in DCW at the stationary phase of growth. It was also produced biomass which the value reached up to 14 g L^{-1}.

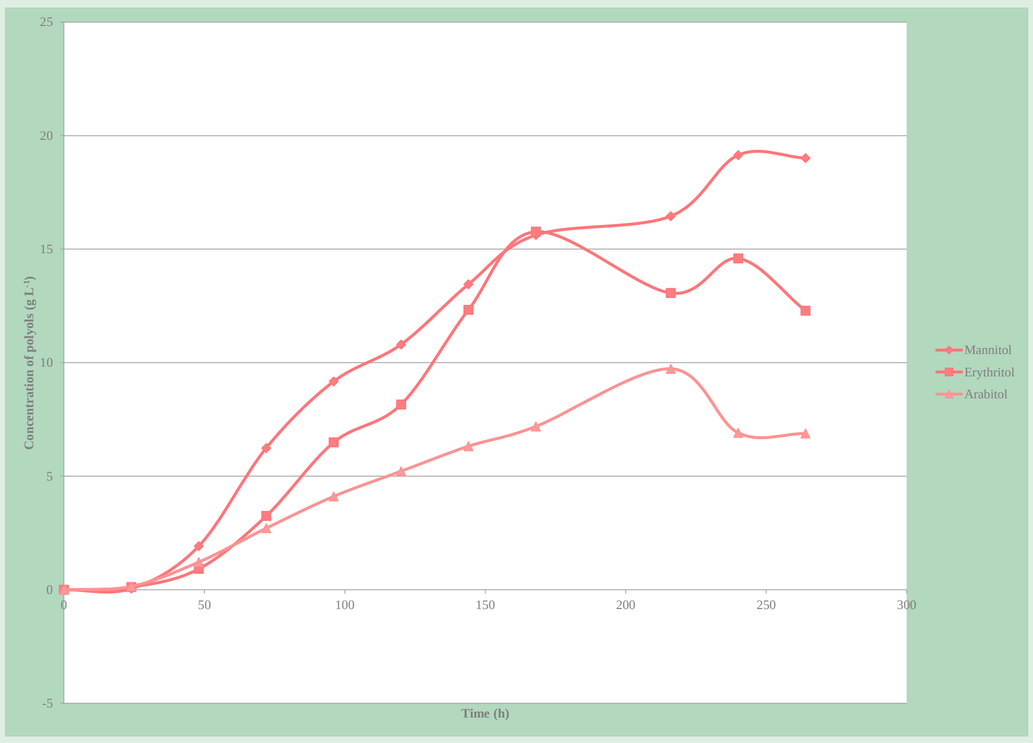

Figure 1. Production of polyols with addition of sodium chloride.

Conclusions

The findings in this study had been showed that the yeast could produce high amount of polyols . The use of glycerol in the science of Biotechnology leads to the production of metabolic products (mainly polyols) that can be used in a number of industrial applications, in pharmaceutical industry, in cosmetics and personal care products, in the production of resins, detergents, plastics, tobacco and as humectants in food.

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