# INHIBITION KINETICS OF GLYCOLIC ACID PRODUCTION BY THE YEAST *Yarrowia lipolytica*

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## ABSTRACT

*Yarrowia lipolytica* is a yeast with multiple industrial and biotechnological applications, as it can be used to obtain different products, such as organic acids, lipases, proteases, lipids, among others. More recently, it was discovered that *Y. lipolytica* is capable of bioconverting ethylene glycol into glycolic acid. The production of glycolic acid by microorganisms is an alternative route that has attracted attention because it is a clean synthesis route. Therefore, the objective of this work is to explore the ability of *Y. lipolytica* to produce glycolic acid from ethylene glycol, evaluating critical effects such as substrate and/or product inhibition. *Y. lipolytica* tolerated high levels of glycolic acid and was able to consume it slowly and demonstrated a preference for ethylene glycol as a substrate compared to glycolic acid. The critical concentration of ethylene glycol was 150 g/L, which inhibited cell growth. In this work it was possible to produce 528.4 mM of glycolic acid, which is the highest concentration of glycolic acid produced by *Y. lipolytica* reported in this moment.

**Keywords:** Glycolic acid 1. Ethylene glycol 2. *Yarrowia lipolytica* 3. Bioprocess 4. Bioconversion 5.

## 1 INTRODUCTION

Glycolic acid is a molecule that has diverse industrial applicability from metal cleaning and coating, to the production of detergents and adhesives, being a constituent of biodegradable polymers and personal care products.[1-2] The global glycolic acid market was valued at US$ 325 million in 2022 and is estimated to have a compound annual growth rate (CAGR) of 6.58 % between 2023-2030.[3] Among all the applications mentioned, the cosmetics industry has gained prominence due to its representativeness in the global consumption of this organic acid, which corresponded to a 43.2% share of demand in 2021.[4]

The majority of glycolic acid is produced by the catalytic reaction of formaldehyde with synthesis gas.[5] However, new economically viable and environmentally sustainable glycolic acid production routes have been developed. Among these is the biological route, which has mild temperature and pressure conditions that favor less intensive energy expenditure compared to traditional chemical processes.

The glycolic acid production route by wild microorganisms reported in the literature is related to the oxidation of ethylene glycol[6-7] and the hydrolysis of hydroxy acetonitrile.[8] The ethylene glycol oxidation pathway is considered an attractive alternative to make the glycolic acid production route renewable, clean and completely independent of the usual petrochemical routes.

The yeast *Yarrowia lipolytica* is among the microorganisms capable of producing glycolic acid from ethylene glycol, being recently reported in the literature as capable of consuming ethylene glycol.[9] In this study, the inhibition of cell growth and glycolic acid production by *Y. lipolytica* were quantitatively examined for the first time.

## 2 MATERIAL & METHODS

Brazilian wild strain of Y. lipolytica IMUFRJ 50682, isolated from Guanabara Bay estuary in Rio de Janeiro[10] was used in all cultivation assays.

Maximum non-inhibitory concentration (MNIC) of *Y. lipolytica* to glycolic acid was tested based on M27-A2 reference method for broth dilution antifungal susceptibility testing according to Clinical and Laboratory Standards Institute.[11] Different concentrations of these compounds were tested through two-fold serial dilutions in 96-well microplates containing 100 µL of YP medium (1% yeast extract and 2% bacteriological peptone; both in w/v). 3.4 M of glycolic acid was used as stock solution (25%; w/v). MNIC of glycolic acid was determined by visual appearance of medium turbidity after cell incubation at 28°C, for 24 h.

Initially, *Y. lipolytica* was cultivated in YPD medium (1% yeast extract, 2% bacteriological peptone and 2% dextrose; all in w/v) at 28°C, 160 rpm, for 72 h. Then, 1 $g.L^{-1}$ of cells were inoculated in 200 mL YP medium supplemented with different concentrations of ethylene glycol (1%, 5%, 10% and 15% w/v), glycolic acid (1% w/v), ethylene glycol and glycolic acid (both 0.6% w/v), and cultivated at 28°C, 250 rpm, for 72 or 168 h. In some cases, 200 mM phosphate buffer (pH 7) was used in cultures with glycolic acid. Cultures were carried out in duplicate.

Quantification of cell concentration ($g.L^{-1}$) was done using a linear equation derived from the correlation between cell dry weight and optical density at 570 nm measured by an UV-1800 spectrophotometer (Molecular Devices, SpectraMax M2e). pH was determined in a digital pH meter (Tecnal), at room temperature (27 °C). Target compounds present in culture samples were analyzed by a HPLC instrument (Shimadzu, Japan), equipped with Aminex® HPX-87H column (300 × 7.8 mm) and pre-column with cation-exchange resin (both from Bio-Rad Laboratories Ltd, United States). Detection was done in a refractive index cell at 55°C, column temperature was at 60°C, injection volume was 20 µL, and mobile phase was made of 5 mM $H_2SO_4$ (flow rate of 0.6 $mL.min^{-1}$).

## 3 RESULTS & DISCUSSION

Based on the glycolic acid MNIC assay, determined by turbidity, *Y. lipolytica* proved to be tolerant to high concentrations of GA (below 1.7 M; 129.3 $g.L^{-1}$). This indicates that the product of interest in the study has no inhibitory effect on the yeast in the range of concentrations studied.

In order to verify if the yeast would be able to use glycolic acid as a carbon source, as well as tolerate its presence in high concentrations, the growth kinetics of *Y. lipolytica* in the presence of glycolic acid in shaken flasks was evaluated (Fig. 1). Buffered medium was used to neutralize the effect of glycolic acid on the pH of the medium. However, the 200 mM potassium phosphate buffer used was not enough to bring the medium close to neutral pH. Even so, the buffered medium favored cell growth compared to the cultivation with non-buffered medium, in which there was a longer period of adaptation of the cells to the medium (lasted up to 48 h). After this period, cell growth occurred. In addition, *Y. lipolytica* consumed 28% of the glycolic acid in the buffered medium, while in the non-buffered medium, a slight consumption was observed after 72 h of cultivation. The yeast showed preferential consumption of ethylene glycol compared to glycolic acid, while glycolic acid was accumulated and consumed only after the preferential substrate had been completely exhausted.

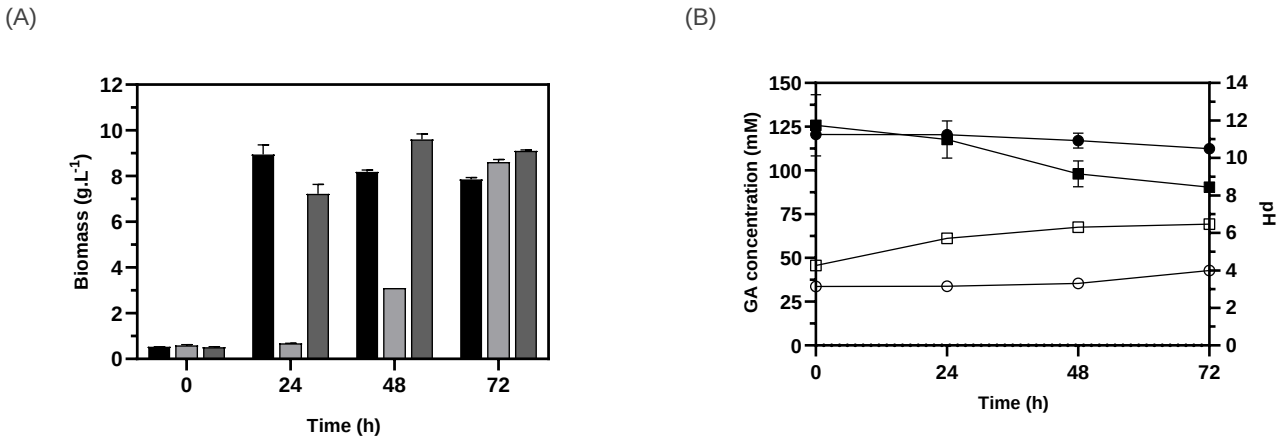


**Figure 1.** Growth kinetics of *Y. lipolytica* in YP medium supplemented with 1% glycolic acid and/without 200 mM potassium phosphate buffer (pH 7), and control (without glycolic acid). Cultures were carried in shaken flasks at 250 rpm, 28 °C. (A) Biomass growth variations over time in control (black), glycolic acid without potassium phosphate buffer (light gray), glycolic acid with potassium phosphate buffer (dark gray) conditions. (B) Consumption of glycolic acid (closed symbols) and pH (open symbols) in absence potassium phosphate buffer (circles) and in presence potassium phosphate buffer (square) over time.

Concentrations above 1% ethylene glycol inhibited the cell growth of *Y. lipolytica*. Therefore, the condition of 1% ethylene glycol favored cell growth, reaching up to 12 $g.L^{-1}$ of cell biomass, while in the condition of 15% ethylene glycol only 3.8 $g.L^{-1}$ of biomass was reached (Table 1). There was a 66.6% decrease in biomass production in the 15% ethylene glycol condition compared to the 1% ethylene glycol condition. Only the 1% ethylene glycol concentration was completely consumed by *Y. lipolytica* after 72 h of cultivation, while the other concentrations were not completely consumed after 168 h of cultivation.

The highest concentration of glycolic acid produced was obtained in the 5 and 10% ethylene glycol conditions when compared to the other conditions, corresponding to concentrations of 410.5 mM and 528.4 mM, respectively (Table 1). These results show that the concentration of ethylene glycol is an important factor to be evaluated in the glycolic acid production process. High concentrations of ethylene glycol (i.e. 15%) not only inhibit cell growth, but also the production of glycolic acid by *Y. lipolytica*, indicating that the ideal initial ethylene glycol concentration is near 10%, or another operating approach, such as fed-batch strategy should be used.

**Table 1.** Results of glycolic acid production kinetics by *Y. lipolytica* at different ethylene glycol concentrations. Cultures were carried in shaken flasks at 250 rpm, 28 °C for 168 h.

| | Ethylene glycol concentration (w/v) | | | |
|---|---|---|---|---|
| | 1% | 5% | 10% | 15% |
| Biomass final ($g.L^{-1}$) | 12.0 | 10.4 | 7.6 | 3.8 |
| Glycolic acid (mM) | 136.4 | 410.5 | 528.4 | 260.6 |
| pH | 8.1 | 3.8 | 3.7 | 4.1 |

## 4 CONCLUSION

In this study, the ethylene glycol concentration was a key factor for glycolic acid production. High concentrations of ethylene glycol inhibited yeast cell growth, on the other hand, *Y. lipolytica* was able to tolerate high concentrations of glycolic acid (up to 1.7 M). The highest concentration of glycolic acid produced by *Y. lipolytica* (528.4 mM) was obtained in the presence of 10% ethylene glycol. This work provides important information on improving glycolic acid production.

## REFERENCES


[1] SHARAD, J. 2013. Clinical, Cosmetic and Investigational Dermatology, 281-288.
[2] VALDERRAMA, M. A. M., VAN PUTTEN, R. J., GRUTER, G. J. M. 2019. European Polym. J. 119. 445-468.
[3] RM, Research and Markets. 2023. Global Glycolic Acid Market Size, Tends, By Grade, By Application, and By Region: Global Opportunity Analysis and Industry Forecast, 2023-2030. Available in: <https://www.researchandmarkets.com/reports/5792453/global-glycolic-acid-market-size-trends-grade#tag-pos-4>.
[4] TMR, Transparency Market Research. 2023. Glycolic acid market. Available in: <https://www.transparencymarketresearch.com/glycolic-acid-market.html>
[5] DRENT, E., MUL, W. P., RUISCH, B. J. 2002. Process for the carbonylation of formaldehyde. https:/patents.google.com/patent/WO2001049644A1
[6] KATAOKA, M., SASAKI, M., HIDALGO, A. R. G., NAKANO, M., SHIMIZU, S. 2001. Bioscience, Biotech. and Biochem. 65(10). 2265-2270.
[7] WEI, G., YANG, X., ZHOU, W., LIN, J., WEI, D. 2009. Biochem. Engineering J. 47(1-3). 127-131.
[8] HE, Y. C., XU, J. H., SU, J. H., ZHOU, L. 2010. Applied Biochem. and Biotech. 160. 1428-1440.
[9] CARNIEL, A., SANTOS, A. G., CHINELATTO, L. S., CASTRO, A. M., COELHO, M. A. Z. 2023. Biotech. J. 2200521.
[10] HAGLER, A. N., MENDONÇA-HAGLER, L. C. 1981. Applied and Environmental Microbiology. 41(1). 173-178.
[11] National Committee for Clinical Laboratory Standards. 2002. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeast. Approved standard. 2nd ed. NCCLS document M27-A2. 22(15).


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