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Effective Production of α-ketoglutarate from Crude Glycerol by Yarrowia lipolytica

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Abstract

Crude glycerol is a main by-product derived during biodiesel production. Although pure glycerol is a valuable product with thousand applications, the excessive cost of crude glycerol refining makes the latter a problematic waste. Considering market availability, the possibility of crude glycerol valorization to valuable products creates the possibility of additional income for biodiesel plant. It was already proved that raw glycerol might be successfully used as the substrate in microbiological production of different high-value compounds e.g. single cell biomass, lipids, erythritol, mannitol or citric and pyruvic acids with the use of *Yarrowia lipolytica* yeast. In turn, for the production of α-ketoglutaric acid substrates metabolized via beta-oxidation are usually applied, because the use of substrates utilized via glycolysis - including glycerol - results in a high by-production of pyruvate, which reduces the process selectivity. Moreover, the impurities present in crude glycerol may affect yeast metabolism and additionally lower the process yield. The aim of the work was to verify the possibility of glycerol application in the production of α-ketoglutaric acid by *Y. lipolytica*.
In the study pure glycerol and crude glycerol (86%) were used as the sole substrates for the production of α-ketoglutarate by *Y. lipolytica* A-8. In the batch-bioreactor cultures total substrate concentration was 300 g/L and the yeast growth was performed in a simple mineral medium supplemented with thiamine. The impact of thiamine concentration (18-40 µg/L) on the α-ketoglutarate biosynthesis was evaluated in the cultures in which pure glycerol was used at concentration of 100 g/L at the beginning and fed twice in equal portions of 100 g/L every 24 hours of the cultivation process. Biomass concentration was observed to increase (15.8 - 39.5 g/L) simultaneously with the increased thiamine concentration. Production of α-ketoglutaric acid increased from 72.2 to 90 g/L in the cultures with 18 and 30 µg/L of thiamine, respectively. In the same cultures pyruvic acid formation showed the opposite trend, and lowered from 65.3 to 2.8 g/L. In all the cultures comparatively high erythritol production (16.9-36.2 g/L) was observed. The change of substrate dosing strategy - 50 g/L of glycerol applied at the beginning of the process and the rest of the substrate (250 g/L) fed into the culture at a constant rate of 6.4 g/Lh - allowed to reduce erythritol concentration to 0.9-1.2 g/L. Application of this dosing method and crude glycerol resulted in α-ketoglutaric acid

production of 86.7 - 117.7 g/L, depending on the substrate dosing rate (6 - 9 g/Lh). The best results were achieved in the culture with feeding rate of 8 g/Lh in which 41.4 g/L of biomass was determined and α-ketoglutarate production reached 117.7 g/L, corresponding to volumetric productivity and selectivity of 0.58 g/g and 91%, respectively. The obtained results showed that biosynthesis of α-ketoglutaric acid by *Y. lipolytica* was possible in the media with both pure and crude glycerol. However, the effective process of α-ketoglutarate production required an appropriate biomass level, which was controlled by supplementation of thiamine and selected substrate dosing conditions.

Keywords: α-ketoglutaric acid, crude glycerol, Yarrowia lipolytica

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