Optimization for trehalose production in Escherichia coli utilizing pretreated waste glycerol

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Waste glycerol contains high amount fatty acids (~20%) as well as glycerol (~80%). Fatty acids are separated from waste glycerol and can be used for cosmetics or fine chemicals after treatment. The solution remaining is pretreated waste glycerol, which is fatty-acid free waste glycerol. This solution is toxic to microorganisms because it contains peroxide, methanol, and salts. This work explored the potential of using this pretreated waste glycerol to produce trehalose in *E. coli* by expressing trehalose biosynthetic genes (*otsBA*). Emerging areas of applications of trehalose are in pharmaceuticals as stabilizer of vaccines during storage, in cosmetics as a liposome stabilizer and in food industry as stable sweetener. In preliminary experiment, it was found that 37°C was better than 27°C for cell growth and trehalose production, and IPTG induction was essential. The Box–Behnken design (*BBD*) was applied for the optimizing concentrations of IPTG, validamycin A, and NaCl for trehalose production by engineered E. coli. Cells produced maximum trehalose (304 mg/L) at 0.1 mM IPTG, 10 μ M validamycin A, and 298 mM NaCl. This result showed that pretreated waste glycerol can be used for the production of valuable products in engineered *E. coli*.