Efficient Conversion of Inulin to Inulooligosaccharides through Endoinulinase

from Aspergillus niger

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MEDIUM FORMULAS

Minimal dextrose medium (MD) contains the following (w/v): 1.34% YNB, 4 x 10^{-5} % biotin, 2% dextrose and 1.5% (w/v) agar.

Yeast peptone dextrose medium (YPD) contains the following (w/v): 1% yeast extract, 2% peptone, 2% glucose and 2% agar.

Buffered glycerol complex medium (BMGY) contains the following (w/v): 1% yeast extract, 2% peptone,100 mM potassium phosphate (pH 6.0), 1.34% YNB, 4 x 10⁻⁵% biotin and 1% glycerol. For buffered methanol complex medium (BMMY), add 0.5% methanol instead of glycerol.

Fermentation basal salts medium (BSM) contains the following (per liter): 26.7 mL 85% phosphoric acid, 0.93 g calcium sulfate, 18.2 g potassium sulfate, 14.9 g, magnesium sulfate-7H₂O, 4.13 g potassium hydroxide and 40.0 g glycerol.

PTM1 trace salts solution contains the following (per liter): 6.0 g cupric sulfate-5H₂O, 0.08 g manganese sulfate-H₂O, 3.0 g sodium iodide, 0.2 g sodium molybdate-2H₂O, 0.02 g boric acid, 0.5 g cobalt chloride, 20.0 g zinc chloride, 65.0 g ferrous sulfate-7H₂O, 0.2 g biotin and 5.0 mL sulfuric acid.

NETHANOL FEEDING STRATEGY

A methanol feeding strategy was used during the fed-batch phase. After terminated glycerol feeding, the methanol fed-batch phase was initiated by starting a 100% methanol feed containing 12 mL PTM1 trace salts per liter of methanol. The induction temperature was 28 °C. During the first 2 hours, the feed rate was 0.43

mL/h per liter initial fermentation volume. When the strain was fully adapted to methanol utilization (2-4 hours) and was limited on methanol, a steady DO reading and a fast DO spike time were observed. Then, the feed rate was increased in 10% increments every 30 minutes. After 4 hours, the rate of 2.57 mL/h was maintained during the methanol fed-batch phase. The entire methanol fed-batch phase lasted approximately 120 h with a total of approximately 580 ml methanol fed in the 3 L fermentor. It was according to the instructions of *Pichia* Fermentation Process Guidelines (Invitrogen, USA).