Optimization for Biopolymer Production by *Enterobacter cloacae* WD7

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Abstract

Factors affecting the production of exopolysaccharide (EPS) from *Enterobacter cloacae* WD7 cultivated in basal medium (pH 7.0) using 1% glucose as carbon source for 5 days at 30 °C were investigated. The maximum EPS yield of 2.14 g/l was obtained after 3 days cultivation. Among the carbon sources (1%) tested, galactose and sucrose gave higher EPS yields (2.50 and 2.45 g/l, respectively) compared to maltose, fructose and glucose. Sucrose was selected due to its lower cost. The optimum concentration of sucrose was found to be 3% giving the EPS yield of 2.63 g/l. The addition of either inorganic nitrogen [$(NH_4)_2SO_4$, NH_4Cl and NH_4NO_3] or organic nitrogen (polypeptone) sources had no effect on the EPS yield. The optimum concentration of yeast extract was found to be 0.05% which was the same concentration as in the basal medium. The optimum initial pH was 7.0 at 30 °C. A comparison on growth and EPS production in the basal and optimized media with the initial pH of 7.0 at 30 °C revealed that their specific growth rates (μ) were 0.14 and 0.15 h^{-1} with a maximal productivity (R_m) of 0.03 and 0.04 g EPS/l.h, respectively and generation time (g) of 4.95 and 4.62 h. Control of pH at 7.0 during batch cultivation gave higher EPS yield than under uncontrolled pH condition. An increase in the aeration rate from 0.5 to 2.0 vvm at agitation speed of 200 rpm could elevate the EPS yield to 4.80 g/l while further increase the agitation speed reduced the production of EPS. In addition, 0.05% of yeast extract could be replaced by tuna condensate based on equal nitrogen concentration which gave higher EPS yield than using 10% tuna

condensate medium (4.66 and 3.24 g/l, respectively) as culture medium. In addition, replacement of analytical sucrose by the commercial sucrose gave lower EPS yield (4.90 and 4.18 g/l, respectively). The EPS produced was found to be a water absorbent with the water absorption capacity of 80.3 g/g dried EPS. Comparison on the kinetic parameters of the shake-flask and batch fermentor culture gave the following values; μ = 0.15 and 0.29 h^{-1}, g = 4.62 and 2.39 h, the cellular yield coefficient ($Y_{x/s}$) = 0.03 and 0.04 g cell/g sucrose, the conversion yield ($Y_{p/s}$) = 0.25 and 0.52 g EPS/g sucrose, the specific rate of product formation (q_p) = 0.60 and 0.56 h^{-1}, the specific rate of substrate utilization (q_s) = 5.0 and 7.25 h^{-1}, R_m = 0.04 and 0.07 g EPS/l.h, and saturation constant (K_s) = 1.30×10^{-5} and 2.60×10^{-5} g sucrose/l. For fed-batch culture, addition of 10% sucrose solution to maintain the final concentration in the fermentor at 3% sucrose every 3 days gave the highest EPS yield of 6.19 g/l at 5 days cultivation. Optimal dilution rate (D) of continuous culture was found to be 0.05 h^{-1} which gave the highest EPS yield of 7.28 g/l. The kinetic parameters of fed-batch culture were as follow; $Y_{x/s}$ = 0.05 g cell/g sucrose and R_m = 0.05 g crude EPS/l.h. The kinetic parameters of continuous culture (D = 0.05 h^{-1}) were as follow; the critical dilution rate (D_c) = 0.49 h^{-1}, the maximum dilution rate (D_m) = 0.485 h^{-1}, $Y_{x/s}$ = 0.03 g cell/g sucrose and R_m = 0.06 g crude EPS/l.h.