Improvement of silk degumming protease production from Bacillus subtilis C4 by using statistical designs

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Sericin is a protein coated on the raw silk yarn and must be removed during degumming process by either protease or chemical agents. However, enzymatic method is more suitable. The bacterial strain, C4, was isolated from wastewater of a silk factory and was identified as Bacillus subtilis. It grew very fast and showed the highest activity of silk degumming protease in BMSM medium at 24 h cultivation. The crude enzyme removed sericin very well i.e. about 24% of total dry weight of the raw silk yarn or 94% of sericin removal after incubation at pH 8 and 37 degree celsius for 2 hours. The preliminary study for an optimal medium of the enzyme production was investigated in shake flasks. The result showed that medium containing 2% (w/v) hydrolyzed cassava starch, 0.1% (w/v) skim milk and 2% (w/v) soy flour gave the highest protease activity. In this study, the interaction between hydrolyzed cassava starch and shaker speed was carried out in the optimal medium by using 3² factorial designs. The best combination of hydrolyzed cassava starch and shaker speed for maximum protease production (1,724 units/ml) was 0.6% (w/v) and 280 rpm, respectively which was satisfactory as the coefficient of determination (R²) of 0.975. Since the oxygen concentration was limited in shake flasks, the suitable hydrolyzed cassava starch concentration in the medium was lower. Hence the effect of aeration rate on protease production was further investigated in a well controlled batch fermenter using central composite design and cultivated in the optimal medium mentioned above. The efficiency of agitation and aeration was evaluated through the volumetric mass transfer coefficient (kL,a). The results revealed that both agitation and aeration rate significantly affected protease production, specific growth rate, specific protease production rate (qp) and kL,a. The optimal agitation rate and aeration rate were 400 rpm and 2 vvm, respectively, which was the corresponding kL,a of 183.24 h⁻¹. Under these conditions, the maximum protease production obtained was 1,890 Units/ml at 27 h cultivation. This result was approximately 3 fold higher than that obtained in unoptimized medium (BMSM medium) by shake flask. Protease production obtained experimentally coincident with the predicted value and model was proved to be adequate. This optimized value also gives maximum qp, reached approximately 270.75 Units/g/h, with the R² of 0.940.
keywords: Optimization, silk degumming, protease production, agitation rate, aeration rate