CONTINUOUS PRODUCTION OF ACID PROTEASE BY SOLID STATE FERMENTATION

Vasanthe Senthuran, Vasanthy Arasaratnam and Balasubramaniam, K.
Department of Biochemistry, Faculty of Medicine, University of Jaffna.

Continuous acid protease production from *Aspergillus niger* N4 by solid state fermentation was studied in optimized medium containing (g kg^{-1}) KH_2PO_4 (0.5); $FeSO_4$ (0.01); $MgSO_4$ (0.5), KCl (0.5); NH_4NO_3 (3.0); soy flour (200.0); starch (5.0) gluten (10.0) and rice bran (903.0) at 30°C. Maximum clotting (437.6 SU g^{-1} DMB^{-1}) and proteolytic (9.4 IU g^{-1} DMB^{-1}) activities were obtained at 48h when the initial spore density was 5 x 10^6 spores g $medium^{-1}$. For scaling up of the process, preparation of large number of spore inoculum is difficult. Hence the use of mycelial inoculum and continuous recycling of the biomass were studied as an alternative. Mycelial inoculum of 12h old and 5 % (w/w) was the best for maximum production of acid protease clotting activity (471.6 SU g^{-1} DMB^{-1}). To study the continuous production of acid protease, for first flask, spores were used as inoculum. After 12h of incubation at 30°C, 2.5 g of mouldy bran was withdrawn from the first flask and inoculated to the second flask containing fresh optimized medium (47.5 g). Likewise from the second flask 2.5 g of mouldy bran was withdrawn and inoculated to the third flask containing fresh optimized medium (47.5 g). Similarly another eight sets of flasks containing optimized medium (47.5 g) were inoculated with 12h old mouldy bran (mycelial inoculum) from the previous batch. Maximum clotting activity obtained in the first and 2nd batches were 1998.5 SU g DMB^{-1} and 3473.4 SU g DMB^{-1} respectively. However from 3rd batch maximum clotting activity obtained started to decrease gradually and in the 11th batch only 654 SU g^{-1} DMB^{-1} clotting activity was obtained. Similar activities were obtained for proteolytic activity too. However up to 5th batch proteolytic and clotting activities obtained were more than or equal to that obtained with spore inoculum. Hence it is possible to use mycelial inoculum instead of spore inoculum and continuous operation of the process for at least five cycles is also possible.