ABSTRACT

Three different strains of *Bacillus licheniformis* were investigated for the production of commercially important extracellular proteins. The strain *Bacillus licheniformis*-GCU-2012 was found to be the most potent producer of these proteins. The Two Dimensional Gel Electrophoresis of extracellular proteins of *Bacillus licheniformis* GCU-2012 showed more than two hundred protein spots on the gel. Out of these, 82 more prominent spots were subjected to mass spectrometric analysis. About 46 proteins were identified on the basis of the annotated *Bacillus licheniformis* ATCC 14580 genome sequence. The analysis of the fermented broth of *Bacillus licheniformis*-GCU-2012, grown on nutrient broth, showed high expression of six commercially important proteins namely Amylase, Protease, Cellulase, Xylanase, Lipase and Bacitracin. The production of these proteins was investigated by solid substrate and submerged fermentations.

In submerged fermentation, the fermentation media were screened and fermentation parameters were optimized for the most suitable medium for each protein. The selection of the most potent fermentation medium and optimization of fermentation parameters were carried out in shake flasks. However, the fermentation behavior and kinetics were studied both in shake flasks and stirred fermenter. The mass balance investigations of different fermentations were carried out in the lab scale stirred fermenter. The produced enzymes were purified to single band level using ammonium sulphate precipitation, ion exchange chromatography and gel permeation chromatography. The specific activities of purified enzymes ranged from 226 U/mg in case of lipase to 628 U/mg in case of alpha amylase.

In case of alpha amylase, 32 units/ g were obtained in solid substrate fermentation while in the shake flask experiments, the alpha amylase produced up to 27 units/ cm^3. In case of stirred fermenter, the specific growth rate during the first 12 hours of fermentation was 0.30 h^{-1} which afterwards decreased to a 0.11 h^{-1}. On the other hand, in shake flasks the specific growth rate in log phase was 0.19 h^{-1}. Generation time and number of generations in the log phase during α-amylase production were 2.31 h and 5.21 in stirred fermenter and 3.48 h and 5.16 in the shake flasks, respectively. In the

stirred fermenter, the substrate consumption rate was 2.52 g/dm^3/hour while specific uptake rate remained 0.28 g.g^{-1}.h^{-1} during the log phase where as in shake flasks these values were 2.05 g.dm^{-3}.h^{-1} and 0.23 g.g^{-1}.h^{-1}, respectively. The overall amylase yield on substrate basis ($Y_{P/S}$), in the stirred fermenter was 902 units/g.

The production of alkaline protease was studied both by submerged and solid substrate fermentation. In shake flasks the maximum alkaline protease level was 56 units /cm^3 along with 38 units /cm^{3}of neutral protease. During the protease production in the stirred fermenter, the specific growth rate of 0.25 h^{-1} was achieved where as in shake flasks it was 0.183 h^{-1}. During protease production in stirred fermenter, the generation time and number of generations were 2.8 h and 4.33 whereas these values during shake flask studies were 3.79 h and 4.8, respectively. During fermentation in glucose based medium for protease production, the substrate consumption rates were 2.9 g/dm^3/hour and 1.8 g/dm^3/hour in stirred fermenter and shake flasks, respectively. During cellulase production by solid substrate fermentation, 92 U/g of the enzyme were produced. In stirred fermenter during cellulase production, the observed specific growth rate and substrate consumption rate were 0.242 h^{-1}and 1.78 g/dm^3/hour while in shake flasks the specific growth rate and substrate uptake rate were 0.180 h^{-1}and 1.5 g/dm^3/hour. The calculated number of generations and doubling time during the logarithmic phase of cellulase fermentation in stirred fermenter were 4.47 and 2.88 h while the values of these parameters in shake flasks were 4.92 and 3.85 h, respectively.

Xylanase production was studied by solid state fermentation and submerged fermentation in shake flasks. In shake flasks, maximum xylanase production of 82 units /cm^3 was obtained in peptone, yeast extract and wheat bran medium using 4% vegetative inoculum after 48 hours fermentation.

For lipase production, significantly high lipase activity (194 U/g) was achieved in solid substrate fermentation compared to submerged fermentation. In solid substrate fermentation maximum lipase activity was obtained at 40°C using 80:20 mixture of wheat bran and rice bran at 50% moisture level.

Bacitracin production was studied by both submerged and solid substrate fermentations. After 36 hours fermentation in stirred fermenter, the activity of 39

I.U/cm^3 was achieved at pH 8.0 and 40°C. Specific growth rate and specific substrate uptake rate were 0.247 h^{-1} and 0.3 $g.g^{-1}.h^{-1}$ during the 12 hours log phase of bacitracin production in stirred fermenter. The calculated number of generation and generation time during the log phase were 4.2 and 2.8 h, respectively.