



range of 7-9. PAI was stable at pH range from 6.5-11.5 whereas PAII was stable at pH range from 6.5-10. In studying heat stability, PAI and PAII were stable upto 45 °C, both peaks rapidly lost activities at temperature above 55 °C. The Michaelis-Menten constant ( $K_m$ ) of PAI and PAII for penicillin G were found to be 7.232 and 0.69 mM, respectively. The  $K_m$  value for 6-nitro-3-phenylacetamido benzoic acid (NIPAB) of PAI and PAII were 0.268 and 0.308 mM, respectively. When the effect of ammonium sulfate on enzyme activity was studied, it was found that 0.2-5 mM ammonium sulfate had no significant effect on activities of PAI and PAII, both with and without preincubation of the enzyme with ammonium sulfate prior to the assay. However, activities of PAI and PAII increased about 1.6 time when They were preincubated with 260 mM ammonium sulfate prior to the assay. About 1.2 fold increase in activities of PAI and PAII were observed when 260 mM ammonium sulfate were added to the assay mixture without preincubation. It was found that 0.2-5 mM potassium bromide had no significant effect on enzyme activity, both with and without preincubation of potassium bromide with the enzyme prior to the assay.

The results from time course study showed that only one peak of penicillin G acylase was obtained when *B.subtilis* was cultured for 24 h and 36 h while two peaks of enzyme were obtained from 48 h culture. However, enzyme at 72 h-culture showed only peak corresponded to PAI.