ABSTRACT

Pseudomonas saccharophila was grown in sucrose phosphate medium. When the age of the inoculum was increased from 10 hrs to 100 hrs, the time taken to reach late log phase decreased, attained a minimal value and again increased. The minimal value of late log phase was 20 hrs when the inoculum was 30 hrs old. Tween 20 and tween 80 were tested for any stimulatory effect on growth and enzyme activity. Tween 20 reduced the time taken to reach late log phase ie, half that of normal, while tween 80 was without effect. As regards sucrose phosphorylase activity, the results indicate both tween 20 and tween 80 had no inductive effect.

Using ammonium sulphate fractionation the precipitate obtained between 50 - 60% saturation showed the highest specific activity (30.4 units/mg) and percentage recovery was 41. The purification achieved was 61 fold. Further purification was done by hydrophobic interaction

chromatography where sucrose phosphorylase was eluted with 0.1M, ClO_4^- ions. The percentage recovery after hydrophobic chromatography was 34 and enzyme was 103 folds pure. The specific activity was 48.4 units/mg. At this stage of purity, sucrose phosphorylase had no invertase activity.

The sucrose phophorylase obtained by 50 - 60% $(NH_4)_2SO_4$ saturation was tested for stability at -2° C, 4°C and 29°C over a month. The enzyme was not affected at 4°C while at 29°C and -2°C the loss of activity were 28% and 99% respectively after 15 days. The optimum temperature for sucrose phosphorylase activity was found to be 30°C.

The $(NH_4)_2SO_4$ fractionated sucrose phosphorylase was used in sucrose synthesis, Production of sucrose was confirmed by paper chromatography. When the equilibrium was

not disturbed the sucrose synthesis acheived was 55% of the initial glucose concentration present in glucose-1-phosphate. When the equilibrium was disturbed the sucrose synthesized increased to 98%. There was a significant increase in the synthesis of sucrose in the presence of phosphatase inhibitors.