5. SUMMARY

5.1 Amylolytic Bacillus sp, isolated from various samples were initially subjected to a primary and secondary screening and later selected four potent strains of rice fermenting Bacillus sp, which belonged to soil.

5.2 The isolated strains were identified as B. coagulans ACMN 1, B. coagulans ACMN 42, B. polymyxa ACMN 25, and B. cereus ACMN 33.

5.3 All the four strains were initially characterized for their optimal requirements of pH, temperature, NaCl, substrate concentration, incubation time, and inoculum concentration and carbon and nitrogen sources for maximal growth and enzyme production.

5.4 All the four strains could grow and produce enzyme at a wide range of pH (6-10) especially at alkaline range of pH. An optimum pH 9 was recorded for B. coagulans ACMN 1 and in B. polymyxa ACMN 25 and pH 7 and 8 for B. cereus ACMN 33 and B. coagulans ACMN 42 respectively.
5.5  All the strains showed maximal growth and enzyme production between 30-45°C.

5.6  All the strains preferred NaCl concentration up to 0-5% for maximal growth and enzyme production. While both B. coagulans strains opted for absence of NaCl, the other two strains B. polymyxa and B. cereus required 0.5% NaCl for maximal growth and enzyme production.

5.7  All the four strains could record maximal levels of growth and enzyme production at 1% substrate concentration.

5.8  An incubation period of 12-24 hrs was found sufficient for maximal growth and enzyme production by all the four strains.

5.9  Although inoculum concentrations 1-3% promoted the four strains to grow to a maximum, even 1% inoculum level led to maximal growth and enzyme production by all the four strains.

5.10 All the carbon sources tested were found to favour both growth and enzyme production for all the four strains.
Starch, maltose and dextrin encouraged higher enzyme production when compared to glucose, lactose and glycerol.

5.11 Among the nitrogen sources tested organic nitrogen sources favoured more growth and enzyme production than inorganic nitrogen sources. Peptone favoured maximal amylase production for all the strains. Yeast extract did not enhance amylase production.

5.12 All the four strains were found to grow fast and spend about 3-4 hrs in logarithmic phase and 6-12 hrs in logarithmic phase before entering stationary phase. The growth curve patterns for the strains did show much differences among the strains. Generation time for the strains were 57.75, 37.8, 48.0 and 39.0 minutes for *B. coagulans* ACMN 1, *B. coagulans* ACMN 42, *B. polymyxa* ACMN 42 and *B. cereus* respectively.

5.13 The amylolytic enzymes from the four species of *Bacillus* were partially purified by (NH₄)₂SO₄ fractionation followed by dialysis. An overall recovery of 54.5, 62.2, 45.9, 71.4% and a specific activities of 52.7, 41.2, 62.5 and 60.9 were obtained for *B. coagulans* ACMN 1, *B. coagulans* ACMN 42, *B. polymyxa* ACMN 25 and *B. cereus* ACMN 33 respectively.
5.14 The partially purified amylase recorded higher dextrinizing activity and a meagre level of saccharolytic activity within 10 min. They were identified as α-amyrase.

5.15 The partially purified amylase from all the four strains recorded activities and stability at wide range of pH and temperature. They preferred alkaline range of pH for maximal activity and stability. An optimum pH of 9 was recorded for all the four strains except for B. cereus which preferred pH 7 for maximal activity and stability. Amylase from B. coagulans ACMN 1 and B. polymyxa ACMN 25 showed an optimum temperature of 50°C while the other two strains preferred 40°C for maximal activity and stability. Interestingly B. coagulans ACMN 1 and B. polymyxa ACMN 26 recorded activity and stability even at 80-90°C.

5.16 Substrate concentration of 1 and 3% were found to be preferred for maximal activity of the enzymes while enzymes of B. coagulans ACMN 1 recorded maximal activity at 1% substrate concentration, the enzymes of all the other strains preferred 3%.

5.17 Heavy metals like Fe, Hg, Ag, Cu, Mn were found to inhibit activity of the α-amyrase especially Fe which effected 100% inhibition.
5.18 Fermentation in the case of B. coagulans ACMN 1, at pH 7, 30°C along with a rate of aeration of 2 vvm influenced rapid and maximal accumulation of sugar and enzymes in the broth within a short duration, when compared to other conditions. Similarly enhanced rate of aeration at 2 vvm enhanced the yield of DE for this strains. Fermentation of rice decanted water also found to enhance enzyme production, reducing sugar accumulation with significant reduction of starch.

5.19 This species, B. coagulans ACMN 42 unlike that of B. coagulans ACMN 1 was influenced by a rise in the incubation temperature and rate of aeration. Hence when these parameters were rised, they effected an increase in the amount and rate of production in the reducing sugar, total sugar, DE, cell protein and enzyme activity. Similarly arresting of aeration midway during fermentation also changed the course of fermentation in that, there was a rapid fall in the values from the levels achieved during aeration. This organism did not indicate acid production in the media unlike other species. This species required 16 hr incubation and 2 vvm aeration to produce a maximal DE while under normal conditions, it could produce maximal enzyme. However, rice decanted water could
enhance enzyme production, DE and enhance the growth. Like _B. coagulans_ ACMN 1, this species was also recorded significant level of enzyme production, reducing sugar and DE during the fermentation on this substrate.

5.20 _B. polymyxa_ ACMN 25 also showed some response to changes in the fermentation conditions. Rise in the temperature from 30° to 35°C enhanced the level of production of reducing sugar, total sugar, cell protein, enzyme activity and DE. However, the increase was only marginal. When rise in pH effected a decrease in the levels of all variables except enzyme activity which was comparatively more at pH 9 than at pH 7 at 30°C. Rate of aeration when increased, also effective rapid conversion and enhanced production of sugars, DE and enzymes at short period of incubation. Arresting of aeration after 12 hrs not only resulted in the decrease in the enzyme production but also indicates a rapid acid production by the organisms. Fermentation of rice decanted was also found to favour significant level of enzyme production, growth, reducing sugar, accumulation and DE.

5.21 _B. cereus_ ACMN 33, similar to _B. coagulans_ ACMN 1 showed poor response to change in fermentation conditions.
However, it responded to enhanced aeration than the rise in pH and temperature. Enhanced aeration could in fact double the level of DE in the medium when compared to all other conditions. Like other strains, rice decanted water found to favour significant level of amylase production, reducing sugar, growth, and DE during fermentation by this species of Bacillus also.

5.22 $\alpha$-amylase obtained from all the four strains brought about a significant level of hydrolysis of starch and production of reducing sugar and DE at 50-60°C. Interestingly enzymes of B. polymyxa ACMN 25 could bring about the rice starch hydrolysis even at 90°C.

5.23 Whole cells of all the strains on ca-alginate beads showed no marked differences in the rate of conversion, reducing sugar production, DE and enzyme activity when compared to that of free cells, at different pH and temperatures. However, the cells, upon immobilization required higher incubation time than that of free cells for maximal conversion and enzyme production.
5.24 The enzymes in the immobilized conditions were less active than that of free enzyme. However, they were not influenced by the immobilization process, in terms of their optimal conditions for activity.