Anyloglucosidase (glucosamylase) is one of the amylolytic enzymes. It has assumed much importance during the last decade, because it hydrolyzes starch almost quantitatively to glucose, and because this process has been industrially exploited for sometime in the USA, Japan and Germany and only recently in India. The investigations on the microbiological aspects of the process described in this thesis were initiated some years back when the process had not been commercialised in India. Subsequently, the scope of the investigations has been expanded to include studies on the physico-chemical properties of the amyloglucosidases formed by *Aspergillus niger* NRRL 330:

1. The first (introductory) chapter surveys the existing literature on the starch-hydrolysing enzymes, especially amyloglucosidase and processes for production of glucose, utilizing the enzyme. A survey of literature shows that many species and strains of *Aspergillus* and *Rhizopus* form this enzyme. The scope of the present work is also described at the end of this chapter.

2. The second chapter enumerates and describes the methods and materials used in the studies.

3. The third chapter deals with the experiments conducted and
is broadly divided into two sections. The first section deals with the microbiological and production aspects and second section is devoted to the physico-chemical studies on the amylglucosidases of A. niger NRRL 330.

Several species and strains of Aspergillus and Rhizopus were screened first to select the most suitable strain for submerged cultivation yielding highest amylglucosidase activity. A. niger NRRL 330 was found to be the best and was used for subsequent studies. Numerous experiments have been conducted in order to study the enzyme yields under a variety of cultural conditions, viz., pH, temperature, inoculum type and amount, aeration, etc. Study of the nutritional requirements of this strain showed that a starch-peptone-medium with carbon: nitrogen ratio of 20:1 supplemented with 0.3 per cent potassium dihydrogen phosphate resulted in maximum enzyme production. Maltose, starch, dextrin are the best inducers whereas glucose does not induce the enzyme formation at all. Mashed whole grains with or without prior liquefaction with acid or malt amylases and supplemented with minerals and vitamins have been used. Jowar was found to be the best, followed by ragi, and then corn. Twenty-litre fermenter experiments with jowar gave low yields possibly due to inefficient aeration of the thick slurry. Results of these experiments have been described. A comparative study of the different commercial amylolytic enzymes and the
mixed amyloglucosidases produced here using _A. niger_ NRRL 330 has been made and it has been found that a 40 per cent starch slurry, is converted almost quantitatively (97 per cent) to glucose in 24 to 48 hours at 60°C.

Three amyloglucosidases in contrast to only two reported in literature (Pazur _et al._ and Lineback _et al._) were detected in the culture broth with the growth conditions used: amylases and trans-glucosidases could not be detected in the culture medium. These have been separated from one another and purified to homogeneity. The homogeneity of the enzymes has been checked by ultracentrifugation and by disc gel electrophoresis. Perhaps the production of three in place of two amyloglucosidases by _A. niger_ NRRL 330 is influenced by the medium.

A number of properties of the enzymes have been studied. It has been found that these enzymes differ from those described by Pazur and Lineback in a number of aspects. For example, treatment with periodate inactivates them; the N-terminal amino acids are different, the carbohydrate content is different in the case of two isoenzymes, etc. The molecular weights however are comparable and so also conditions of pH, temperature, etc. under which the enzymes are active. The molecular weights have been determined by the SDS-polyacrylamide
electrophoresis procedure and by gel filtration. There is apparently no sub-unit structure.

The effect of a number of group-specific reagents have been tested on the enzymes with a view to determine the amino acids in the active site region. Calcium ions, SH groups and serine are not involved in the active site as ethylene diamine tetraacetate, p-chloromercuribenzoate and di-isopropylfluorophosphate do not diminish the activity. Probably histidine tryptophane, etc. may be involved as there is lowering of activity due to photooxidation with rose bengal or methylene blue.

4. In the fourth chapter, the results described in the previous chapter have been discussed. The economic possibilities of utilizing the enzyme have been indicated. The properties of the enzymes have been compared with similar enzymes from other sources.

5. A summary of the results obtained and the conclusions have been given.

6. The references cited in the text have been given at the end.