1. INTRODUCTION

1.1 PREFACE

Starch plays an important role as a renewable raw material in many industries. A native starch can be modified or chemically derivatised by simple process technology. In addition starch is very susceptible to total or partial hydrolytic degradation by acid or enzymes yielding oligomeric or monomeric products which can be additionally modified or derivatised. Further potential arises from separation of starch into amylose and amylopectin (Rexen, 1984; Munk, 1987).

Starch is used either as such as a staple food or as a derivative after hydrolysis for other useful purposes. It is used in the food industries and non-food industries.

In the food industries starch is used for the production of starch syrups like glucose syrups, maltose syrups and fructose syrups; for the production of alcoholic beverages and in baking industries.

In the non-food industries starch is widely used in the paper and textile industries as a thickener; as adhesive,
and in chemical and pharmaceutical industries. Even starch is found to be used in the mining and oil exploration.

The well-known biotechnological use of starch is in the production of amylases, viz., α-amylase, β-amylase and amyloglucosidases, which are widely used in the preparation of beverages, confectioneries, digestive aids and in the paper and textile industries. The starch hydrolyzed products maltose, glucose and maltodextrin are further used as starting materials in the production of alcohol, organic acids, aminoacids, single cell protein etc.

Fermentation is one of the oldest methods of food preparation and originated centuries ago. Generally traditional methods of preparing fermented foods are simple and inexpensive. However, the ancient methods of making such foods are changing rapidly through modern microbial technology.

Various fermentation industries are based on starch resources. Most of starch resources are obtained from cassava, potato, corn, wheat, maize etc. Corn starch is used extensively in the commercial preparation of sugar syrups. Cassava promises to be a potential raw material for microbial
fermentation and is widely used for the production of ethanol, single cell protein, sugar etc. However, this is awaiting for yet to be used in commercial preparations (Reade & Gregory, 1975). Among these resources rice is minimally exploited as a raw material in the fermentation industry.

Fermentation processes have been highly developed in the recent years for the production of alcohol, biomass, enzymes, sugar syrups, secondary metabolites like aminoacids, vitamins and pharmaceutical compounds etc., employing both aerobic, and anaerobic microorganisms in an extensive manner all over the world. It is believed by many that the bioprocess technology may even substitute the chemical processes in future. Consequently an intensive search for cheaper as well as renewable raw materials have drawn the attention of many scientists towards economic production of products.

Recently recognition of immobilization technology for the rapid conversion of several substrates into metabolites and repeated reuse of the biocatalysts have drawn the attention of the fermentation scientists and technologists to try these new technologies for the rapid production of product and enhancement of the efficiencies of the systems.
Hence in the present study rice was selected as a substrate since it is a rich source of starch, available and cultivated throughout the year almost in all part of our country. Rice although known for its use as a staple food in many forms as rice, idli, dosai etc., has not been used in industry extensively. However, it is a potential resource for the production of alcohol, high protein food and for sugar and sugar syrups as it is evidenced by the few reports mentioned in the review of literature.

Of the several microorganisms available, Bacillus sp. is a known candidate for the production of amylases. Hence in the present study Bacillus sp. was desired for its known efficiencies in starch conversion.

1.2 REVIEW OF LITERATURE

1.2.1 Starch—a substrate for fermentation

The first major technological breakthrough was achieved in 1940, when Langois & Dale patented the use of commercially available enzymes and hydrolyzed starch.

The first significant step in the production of dextrose from starch was the introduction of enzymes produced
by *Aspergillus niger* capable of complete hydrolysis of starch and dextrins to glucose (Denault, 1963).

In the later years production of simple sugars and sugar syrups using starch as raw material employing microbial amylases found their applications in food industry especially in confectioneries, bakeries, brewing and soft drinks. Glucose syrups, dextrose syrups, high fructose syrups and high maltose syrups have been produced from starch (Martensson, 1974; Palmer, 1975; Saha & Zeikus, 1987, 1989; Hebeda *et al.*, 1988).

Corn starch is widely exploited as a source of glucose and glucose syrups. Corn starch when treated with $\alpha$-amylase at $80-90^\circ$C, brought about $15-25\%$ of conversion into dextrose. When this was further subjected to glucoamylase treatment, at $60^\circ$C, $98.5\%$ of dextrose was obtained (Sinclair, 1965). A pilot plant production of crystalline dextrose from corn starch, initially liquified by acid followed by saccharification by glucoamylase at $60^\circ$C, yielded $96\%$ DE value is also reported (Kingma, 1969).
Raw corn starch was also used to obtain an initial high dextrose syrup and high fructose syrup followed by further isomerization with glucose isomerse in commercial scale (Harden, 1972, 1973). Continuous production of high glucose syrups and high fructose syrups from corn starch by immobilization (Oesterguard & Knudson, 1976; Hupkes & Van Telburg, 1976; Venkatasubramanian, 1978), and on the production of high fructose syrups from corn starch by liquification followed by a saccharification and isomerization using bacterial α-amylases, fungal glucoamylase and glucose isomerase (Kalevy, 1987) is also reported.

Cassava starch is a widely employed substrate for the production of glucose, glucose syrups and ethanol on a commercial basis. Available reports mainly dealt with preparation of high glucose syrup from cassava starch by a dual enzyme process (Park & Papini, 1970), production of 100 gm glucose/100 gm cassava starch by liquifaction of cassava starch by a thermostable bacterial amylase followed by a saccharification using fungal amylglucosidase (Ana et al., 1978).
Ethanol was produced from cassava starch by saccharification with amylolytic enzymes or by employing *Aspergillus usamii* which produced glucoamylase followed by an ethanol fermentation using *Saccharomyces cerevisiae* (Reade & Gregory, 1975; Nakamura et al., 1978; Thammarutwasi, 1978). A one step process of fermentation which combined the conventional process of liquifaction, saccharification and alcohol fermentation using *Aspergillus niger* amylase and yeast is also reported (Ueda, 1981). Different aspects of ethanol production from cassava starch were also studied by many authors (Kunhi et al., 1981; Queiroz et al., 1982; Prema et al., 1986; Srikanta et al., 1987).

Solid state fermentation studies were carried out to bring about a simultaneous solid phase saccharification and fermentation of cassava fibrous residue employing gluco-amyrase and *Saccharomyces cerevisiae* (Jaleel et al., 1988), to produce 6780 u/ml glucoamylase by *Aspergillus* sp. after 6 days (Kinoshita, 1979) and a highly thermostable α-amyrase by *Bacillus* sp. (Lonsane & Ramesh, 1990) through solid state fermentation with cassava starch as a substrate. An acidic food called 'gari' was attained by fermenting cassava starch employing a *Lactobacillus* sp. and *Streptomyces* sp. (Ngaba et al., 1979).
Starch syrups derived from potato starch are known years back (Heisler et al., 1952), production of a sweet glucose syrup from potato and maize starch by enzymatic hydrolysis (Dondero et al., 1978), and a starch syrup with a DE of 9.20 from potato starch by α-amylase liquifaction (Vance et al., 1972) are reported.

Reports on the production of high maltose syrup from waxy maize starch by enzymatic (β-amylase) hydrolysis (Hawling, 1973), and from potato starch by a thermostable maltogenic amylase of B. stearothermophilus (Slominska & Starogardska, 1986) are also available in the literature.

Ethanol production from potato starch using glucoamylase of Aspergillus niger followed by alcoholic fermentation by baker's yeast (Ueda et al., 1981), or by alcoholic fermentation of raw sweet potato starch in a one step process employing glucoamylase of Rhizopus sp. followed by alcoholic fermentation of baker's yeast which yielded 94% alcohol (Hiroshi, 1982, Chua et al., 1984) was studied.

Whole ground barley was employed for the production of ethanol by liquifaction and saccharification followed by an alcoholic fermentation (Wayman, 1988).
1.2.2 Rice starch as a substrate

Rice (Oryza sativa) is known as a substrate for the preparation of traditional foods. In Japan starch is used for the preparation of sake—a fermented beverage containing 20-30% alcohol, Mirin—a sweet liquid containing about 35% glucose and 12% alcohol, Amasake etc. Further it is used to prepare Ia-chao in China and Tape in Indonesia (Ko, 1972; Cronk et al., 1977).

However, in India rice is used as a staple food and as a substrate along with other legumes for the preparation of traditionally fermented foods such as idli, dosai, appam etc., by natural fermentation. In rural areas, rice is used to prepare country arracks, which contains alcohol (Sakunthala & Shadeksharaswami, 1987).

Rice and black grams (Phaseolus mungulus) mixture is fermented in the proportion of 3:1 and 1:1 for the preparation of two South Indian foods—idli and dosai. Several reports are available on the methods of preparation, microbiology and nutritional value of idli (Desikacher, 1960; Rajalakshmi, 1967; Ramakrishna, 1979; Batra, 1981).

Among bacteria species of *Bacilli*, like *B. subtilis*, *B. amyloliquificiens*, *B. polymyxa*, *Lactobacillus delbrueckii*, *Streptococcus faecalis* and yeast species including *Oosporidium margaritiferum*, *Kluyveromyces maximonus*, *Candida kefyr*, *C. krusei* were reported to ferment the batter produced for dosai preparation.

In general rice starch is minimally exploited in industries based on fermentation. Few reports are available on the use of rice starch in the fermentation production of alcoholic beverages and sugar syrups.

Available reports on alcoholic beverages mainly centered around the production of alcoholic chinese food
Ia-chao from rice with 2% alcohol after 45 hrs of incubation using on amylolytic filamentous fungi and an Endomycopsis sp. (Wang & Herseltine, 1970). Preparation of Tape—an indonesian delicacy with a sweet acid taste and mild alcoholic flavour by the fermentation of glutaneous rice using fungi like Chlamydomucor oryzae and Endomycopsis fibuliger for 5 days (Ko, 1972) and using fungi Amylomyces rouexii and Endomycopsis burotonii (Cronk et al., 1977), production of sake—a Japanese alcoholic beverage with an alcoholic content of 160 g/l which is the highest among the naturally fermented beverages not distilled, made from rice and water by the action of two organisms Aspergillus oryzae and Saccharomyces cerevisiae (Miyoshi et al., 1973; Fugita et al., 1984; Kondo, 1984; Sugimoto et al., 1986), on the development of sake fermentation processes (Miyoshi, 1973; Fugita et al., 1983; Sugimoto, 1984; Taneka et al., 1984), and on the single step ethanol fermentation from finely powdered rice employing glucoamylase preparations, Kojis, or Koji extract of Rhizopus strains with compressed baker's yeast (Elegado et al., 1986).

Reports on rice starch as a substrate for sugar production included the production of high dextrose syrups
from rice and other starches by amylolytic enzymes (Leach et al., 1975; Muller, 1978), high maltose syrups from rice, corn, potato, wheat, tapioca by an α-amylase of Streptomyces sp, and β-amylase of plant origin (Yamoto et al., 1976) and high fructose syrup (Chen & Chang 1984).

Rice starch is also reported to be a substrate for the production of high protein rice flour, childrens feed, from broken rice by partial enzyme digestion with α-amylase (Hansen et al., 1981; Chen & Chang, 1984).

1.2.3 Bacillus and their amylases

Amylolytic enzymes are widely distributed in plants, animals and in microorganisms (Boyer & Ingle, 1972). Among them microbial amylases has drawn the attention of many investigators in the recent years (Robyt & Ackerman, 1971; Shinke, 1975).

Among the many candidates of bacteria, Bacillus is an acknowledged source of amylase for various applications. (Coleman & Elliot, 1962; Welker & Campbell, 1963; Shinke, 1975; Anderson, 1985).
Bacillus sp. is known to produce both $\alpha$ and $\beta$ amylase. Species of Bacillus subtilis, B. coagulans, B. stearothermophilus, B. licheniformis and B. amyloliquefaciens have been reported to produce $\alpha$-amylase (Welker & Campbell, 1963; Saito, 1973; Medda & Chandra, 1980; Pinches et al., 1985; Shah, 1989; Kochhar & Dua, 1990). While Bacillus cereus, B. polymyxa, B. megaterium and B. circulans were reported to produce $\beta$-amylase (Marshall, 1974; Shinke, 1975; Takasaki, 1976; Taniguchi, 1983; Kawasu, 1987). Recently these strains are reported to produce $\alpha$-amylases besides $\beta$-amylases (Yoshigi et al., 1988; Uozumi, 1989).

Of the two types of amylases, $\alpha$-amylases of Bacillus is used in numerous commercial processes including brewing, starch degradation and textile manufacturing (Rose, 1980; Wiseman, 1985).

Several aspects of amylase production by Bacillus has been studied in detail for various organisms. It was reported that in B. stearothermophilus there was an inverse relationship between growth and $\alpha$-amylase synthesis and the enzyme was found to be induced by maltose and maltodextrin while inhibited by fructose and chloramphenicol (Welker & Campbell, 1963 a, b).
B. amyloliquifaciens showed increased rate of production of α-amylase in the post logarithmic phase when most of the enzymes were secreted, independent of carbon sources tested, fructose, maltose, starch which were present in the production medium (Coleman et al., 1962). But Boyer and Ingle (1972) and Tsuchiya (1975) have observed that glucose repressed α-amylase synthesis. Whereas during another study on the α-amylase synthesis by B. amyloliquifaciens it was observed that a four fold increase in α-amylase activity in the culture medium could be obtained with maltose than with glucose and changes in concentration of yeast extract in the medium influenced the formation of amylase in different phases of growth, Yoo et al., (1988) and Magee & Kosaric (1987) suggested that carbon and nitrogen sources in the medium influenced the metabolite formation in B. amyloliquifaciens which in turn had a modulating effect on enzyme synthesis by changing the pH of the system. When the enzyme production medium contained a higher concentration of yeast extract, a drastic change in pH from neutral to acidic range and a complete repression of α-amylase activity was observed (Alam et al., 1989). In another study, while higher cell growth with reduced amylase production was obtained with
glucose as the carbon source, higher levels of cell mass along with total and specific enzyme activities and enzyme production were obtained with maltose as carbon source (Siddhartha et al., 1989).

At the same time, while a high concentration of phosphate promoted maltose uptake and growth of microorganism, high maltose uptake rates repressed enzyme biosynthesis (Yoon et al., 1989).

*B. subtilis* was observed to synthesize a high amount of \(\alpha\)-amylase in the presence of adenine and there was no repression by adenine (Tsuchiya et al., 1975). Inducive effect of starch and maltose and repressive effect of glucose on \(\alpha\)-amylase synthesis was reported by Saito and Yamamoto (1975). Caesinate as a nitrogen source and hydrolyzed products of starch were found to repress the enzyme synthesis during growth in batch cultivation while promoting a two fold increase in enzyme production in fed batch cultivation (Pazlarova et al., 1984).

*B. acidocaldreus* was found to secrete a thermostable, thermoacidophilic \(\alpha\)-amylase at maximum levels during the
stationary phase of growth and was induced by carbon sources such as glycogen, starch, maltose and maltotriose with an optimum pH of 3.5 and a temperature of 75°C (Buonocore et al., 1976).

β-amylases production by B. cereus was repressed by polysaccharides, glucose and maltose in the culture media. However, a high β-amylase activity was observed even in the absence of a carbon source for this species (Shinke et al., 1977).

β-amylase production by B. megaterium was not observed in the absence or in the presence of carbon sources other than starch suggesting that β-amylase production by this species is only by induction (Yamane & Tsukano, 1977).

B. coagulans is also known to secrete a α-amylase when induced by starch than by other carbon sources tested (Babu & Satyanarayana, 1990).

Thermostable α-amylases have had many commercial applications for several decades. These enzymes are used in
textile and paper industries, starch liquifaction, food, adhesive and sugar production (Bajpai & Bajpai 1989).

Buchanan & Gibbons (1974) described 48 species of Bacillus of which 32 are reported to produce \( \alpha \)-amylase but only a few among them are capable of secreting thermostable enzymes.

Among the many Bacillus sp. that have been tried as the sources of thermostable \( \alpha \)-amylase, *B. stearothermophilus* is shown to produce enzymes that are active at 90°C even after 12 hrs (Hartman, 1955). Pfueller & Elliott (1969) and Ogasahara (1970) purified and characterized this enzyme and found that addition of polyols and dimethyl formacide in the media increased thermostability and half life.

*B. licheniformis* is also known for the production of thermostable enzyme. Saito (1973) observed that the \( \alpha \)-amylase of this species after purification exhibited an optimum temperature of 76°C and a pH of 9. In another study, activity of the enzyme was observed at 110°C and at narrow pH range of 6-7 (Madson et al., 1973; Chiang et al., 1979).
Whereas, Morgan & Priest (1981) observed optimum temperature of 70-90°C at pH 7 and maximal activities at pH 7 and 10 for the α-amylase. Kinetic studies of starch hydrolysis by α-amylase from *B. licheniformis* revealed that maximum conversion rate was observed at 100°C, with pH 7 and a substrate concentration of 300 g l⁻¹. This enzyme gave a higher rate of hydrolysis and a higher dextrose equivalent within a shorter period of time (Yankov et al., 1986). Bajpaiand Bajpai (1989) tried to liquify corn starch (30%) with a α-amylase from *B. licheniformis* which is active up to 100°C with an optimum at 90°C and a pH range of 5.5-10. They found that presence of Ca⁺⁺ and Na⁺⁺ in the reaction mixture, while repressing starch hydrolysis, enhanced reducing sugar production.

*B. subtilis* was found to produce a calcium requiring thermostable liquifying α-amylase at an optimum temperature of 70°C and with a broad pH range of 5.9-9.5 (Mosely & Keay, 1970). Lin and Kang,(1988) found that when the starch substrate concentration was increased up to 50% there was a decrease in thermostability of *B. subtilis* amylase. Whereas, presence of calcium along with the substrate effected only a secondary effect on thermostability.
An $\alpha$-amylase with pH optima at two pH levels of 6.5 and 7.0 and an optimum temperature of 90$^\circ$C was produced by *B. amyloliquifaciens* by means of solid state fermentation (Ramesh & Lonsane, 1989). Another $\alpha$-amylase with a molecular weight of 68,000 with temperature optimum of 65$^\circ$C and a pH of 6 was isolated from this species by Kochhar & Dua (1990).

A facultative, thermophilic *B. coagulans* was reported to produce $\alpha$-amylase which maintained its thermostability even after crystallization and retained 90% of its activity after 1 hr at 90$^\circ$C (Campbell, 1954).

Medda & Chandra (1980) described two species of *Bacillus* *B. coagulans* and *B. licheniformis* producing thermostable $\alpha$-amylase active at alkaline pH. The enzymes from *B. licheniformis* showed a wide range of temperature for activity with optimum at 91$^\circ$C and was stable for 1 hr at this temperature. It was active over a wide range of pH of 4-10 with optimum at 9.5. Enzymes of *B. coagulans* showed activity upto 90$^\circ$C with optimum at 85$^\circ$C and had a wide pH range with optimum at 7.5. A thermostable alkaline $\alpha$-amylase active at a wide range of pH and temperature from a strain of *B. coagulans* ACMN 1 is reported by Nandakumar & Chandrasekaran (1989).
Pure cultures of microorganisms are inherently variable in their growth characteristics and metabolic activities. Therefore, the initial activity of the microbiologist is to minimize the genetic variability of the microorganisms by selecting out stable and genetically uniform isolates which produces a minimum number of unwanted metabolites and copious amounts of the desired component. Mutagenesis followed by the subsequent selection, and purification of superior strains represents the most important activity in improving the yield of a fermentation product. This led to several mutation studies in *Bacillus* sp. towards strain improvement to get enhanced enzyme yield.

*B. subtilis* is used widely as a model system for studies on regulation and synthesis of \(\alpha\)-amylases. There are many reports on the expression of cloned \(\alpha\)-amylase genes on plasmids on *B. subtilis* (Palva, 1982; Aiba et al., 1983); Ortlepp et al., 1983; Joyet et al., 1984; Corfield, 1984). Yoneda (1982) reviewed the reports on the regulatory genes of amylase which demonstrated synergistic effect on the production of \(\alpha\)-amylase by *B. subtilis*. Absence of glucose catabolite repression of \(\alpha\)-amylase genes in the recombinant
B. subtilis was reported (Pinches et al., 1985). A high yielding stable mutant of B. subtilis which secretes 5 fold more α-amylase activity was isolated by successive exposures to N-methyl-N-nitro-N-Nitrosoguanidine and UV radiation (Shah et al., 1989).

B. cereus NY-14 mutants were found capable of producing α-amylase in the presence of glucose (Yoshigi et al., 1988).

B. polymyxa amylase was shown to be synthesized as a precursor protein with β and α-amylases activities and that give rise to β and α-amylase (Uozumi et al., 1989).

1.2.4 Application of Bacillus amylases in industry

A substantial percentage of bacterial enzymes produced in the industry is accounted by the amylases and proteases of Bacillus sp. The main amylolytic enzymes used for the production of glucose, maltose, and maltosaccharides namely α-amylase, β-amylase and glucoamylase, are now commercially derived from Bacillus sp., fungal sp., and used in various other starch based industries (Shinke, 1975; Anderson, 1985).
α-amyloses of Bacillus sp. and fungal amyl-glucosidases are used for the production of high dextrose syrups from starch substrates (Leach et al., 1975; Takasaki & Takahara, 1976). A liquifying α-amylase from Bacillus sp. and glucoamylase from Aspergillus sp. were employed to produce glucose from cassava starch (Ana et al., 1978). An alkalo-philic maltohexose forming Bacillus sp. H 167 from soil was found to be able to hydrolyze soluble starch to produce 25-30% maltohexose (Hayashi, 1988).

Thermostable amylases derived from B. amyloliqui-faciens have been in use for many years. However, a more heat stable (upto 110°C) α-amylase from B. licheniformis (Madson et al., 1973) was found and introduced to commercial application after its discovery. The α-amylase from B. licheniformis was highly active and sufficiently stable to allow gelatinization of the starch at 105°C without any significant loss of enzyme activity and are widely used for starch liquifaction (Anon, 1984), a preparation of starch liquifying α-amylase from B. amyloliquifaciens was able to degrade raw corn starch rapidly and produced maltooligo-saccharides and glucose from starch (Bergman, 1988).
\( \beta \)-amylase from *B. polymyxa* was used for the production of high maltose syrups with DE 58 and maltose content 60% (Armbruster & Jacaway, 1970). High maltose syrups with DE 50 and maltose content 75% and very high fermentables 90% was prepared by using pullulanase enzyme and *B. polymyxa* \( \beta \)-amylases (Heady & Armbruster, 1970).

Takasaki (1976) and Takasaki & Takahara (1976) patented a *B. cereus* var *mycoides* that produce an extracellular pullulanase and \( \beta \)-amylase simultaneously and employed for the production of high maltose syrups (80-90% maltose) from starch.

A thermostable, acid stable maltogenic amylase from *B. stearothermophilus* was used for the production of high maltose syrups containing 70-80% maltose from liquified starch and this together with pullulanase or fungal \( \alpha \)-amylase enabled the production of 85% maltose from the same substrate (Slominska & Sturogardzka, 1986).

Amylase from *B. megaterium* in combination with glucoamylase was suggested for the production of high dextrose syrups from starch (Hebeda & Styrlend, 1988).
Recently, dextrins and cyclodextrins were produced from potato starch by \( \alpha \)-amylase enzyme of \( \textit{B. macerans} \) (Koto, 1989).

1.2.5 Immobilization

Biocatalysts based on immobilized cell technology has attracted wide attention in recent years (Mattiason \textit{et al.}, 1983; Chibata \textit{et al.}, 1983; D'souza, 1989). Much of the reports available on immobilized biocatalysts including enzymes, microbial, plant and animal cells and cellular organells and their potentials in industry, medicine and analysis were reviewed extensively by D'souza (1989).

In the past years, much interest has been expressed in the use of immobilized microorganisms in the production of urocanic acid, L-citrulline, L-aspartic acid, cortisol to prednisolone, L-malic acid, L-sorbose, glycerol to dihydroxyacetone, preparation of lactose hydrolysed milk, high fructose syrups, L-alanine, vitamin \( B_{12} \), butanol, lactic acid, acrylamide, L-glutamate, vinegar, acetic acid, proinsulin proteases amylases, \( H_2 \), ethanol employing bacterial systems; chlorotetracycline, nikkomycin, daunorubicin,
thienamycin, proteases and cholesterol degradation using actinomycetes; sucrose to fructose and gluconic acid, NADH regeneration, lactose free milk, ethanol and alcoholic beverages, meat, citric acid, glutathione, hydrolysis of inulin employing yeasts; citric acid, cellulose, itaconic acid, alkaloid, penicillin G, Alkaline proteases, chlorinated benzoates, degradation of n-alkanes, detoxification of cyanide and clarification of pectin using fungi and wastewater nutrient removal, ammonia photoproduction, ammonia production, glycollate, hydrogen, glycerol and polysaccharide production employing algae (D'souza, 1989). Both organic and inorganic supports have been used for immobilizing cells and enzymes. Some of the commonly used supports for whole cell immobilization include inorganic supports like kaoline, colloidal silica, glass particles, controlled pore glass, alumina, nickel oxides, ziconia, charcoal, hydroxyapatite iron oxide, ceramics, sand, hornblends, magnetite, steel and bentonite, organic supports like cellulose, agarose, agar, dextran, polyacrylamide, nylon, colloidon, polystyrene, polyacrylic acid, chitin, chitosan, ca-alginate, carrageenan, polymethane, prepolymers, polyvinyl alcohol and proteinic supports like collagen, fibrin, feather protein silk, elastin, albumin, gelatin casein and hen egg white (D'souza, 1989).
Immobilization of amylases is now considered as the most suitable techniques for starch hydrolysis rather than different fermentation processes (Sen & Chakraborthy, 1987). A reduction of production cost and a more precise reaction control could be achieved by recycling immobilized amylases in industrial glucose production (Linko et al., 1975).

Alpha amylase production was studied by immobilizing Bacillus subtilis on polyacrylamid gel (Kokobu et al., 1978), Bacillus amylobiduliciens in kappa carrageenan (Shinmyo 1982), Bacillus subtilis in alginate (Oriel, 1988), Bacillus amylobiduliciens on ion exchange resins Groom et al., 1988) and Bacillus coagulans on alginate (Nandakumar & Chandrasekaran, 1990). Alpha amylases are known to be immobilized on cyanogen bromide activated carboxymethyl cellulose for the production of glucose and maltose (Linko et al., 1975), on Duolite DS 73141 for heating paper mill effluent (Smiley et al., 1975) on millipore filter for continuous hydrolysis of soluble starch (Okada & Urabe, 1976) on magnetic iron oxide (Kennedy, 1976; Kennedy & White, 1979) on 2-hydroxy ethyl methacrylate (Kumakura et al., 1977; Kaetsu et al., 1979) by gamma irradiation on a polymer containing methyloacrylamide and methanol (Karube et al.,

1.3 OBJECTIVES OF THE PRESENT STUDY

In the present study, it was decided to use rice starch and related products like rice decanted water as a substrate for fermentation. From the review of literature it is clear that not much work has been done in India in the utilization of this substrate for the production of sugar and related products.

Main objectives of the present study included the following:

- to isolate amylolytic *Bacillus* sp. from natural environments.

- to select suitable strains that ferment rice starch efficiently.

- to identify the selected *Bacillus* sp. upto species level.
- to characterise the organism for their growth and enzyme production with respect to various physicochemical parameters.

- to prepare, purify and characterise the amylases from the selected strains of Bacillus.

- to study the conversion of rice starch to sugars by free enzyme.

- fermentation of rice starch and rice decanted water by whole cells of selected strains of Bacillus with respect to various physicochemical parameters towards optimisation of fermentation process.

- to immobilize the whole cell and free amylases and effect conversion of rice starch.