1. INTRODUCTION

Biotechnology is professed to catalyse positively the socio-economic development of mankind in the new millennium and among the various biotechnologies, enzyme biotechnology has a major role to play. Enzymes are the central dogma of life and catalyse thousands of biochemical reactions both inside the cell and outside the cell in the environment and hence there is no life without enzyme. From the role of an inevitable biocatalyst in any living cell mediating life, they have assumed greater significance in the day to day activities of mankind. Consequently enzymes have become one of the major commodity today and their manufacture and applications, in the recent past, has emerged as one of the major industry with a global market exceeding US$ 500 million and is expected to record further growth in the coming years.

Enzymes are frequently used, as biocatalyst for process improvement, to enable utilisation of new types of raw materials, production of metabolites through biotransformations, texture improvement in textiles, leather processing, detergents, flavour improvement of food, food processing, waste treatment and several other applications. Enzymes also have tremendous potential as therapeutic agents. Among the industries, food industry is by far the largest consumer of commercial enzymes (Lonsane & Ramakrishna, 1989, Bhotmange and Shastri, 1994) followed by textile, detergent and leather industries.

Enzyme based analytical protocols like ELISA have revolutionised the biomedical field and highly sensitive biosensors based on immobilized enzymes (Karube, 1988, Brueckel et al, 1990) have replaced conventional analytical procedures used in the health care industry. Enzyme based biosensors have also been employed in food technology (Wagner & Schmid, 1990), fermentation control (Bradley et al, 1989), and environmental monitoring (Rawson et al, 1989). With the overwhelming increase in biotechnology-based industries and research world wide, the demand for speciality enzymes like nuclease and polymerases has increased, and enzyme based analyses and diagnostic protocols are rapidly developing. This is expected to cause a boost in the enzyme industry especially in the production of high-cost analytical grade enzymes. Klein & Langer (1986) had reviewed the
application of immobilized enzymes in clinical medicine and proposed a bright future for enzyme based drug therapies.

Developments in enzyme-based processes and their applications in several fields have resulted in an increase in the quest for novel enzymes with improved activities in the recent years and a wide variety of sources are being explored for enzymes with commercial applications. Although animal, plant and microbial enzymes have been used in industries in the past, current trend is replacement of animal and plant enzymes with microbial enzymes. This is mainly due to the ease with which microbial enzymes can be obtained, and as they are generally more stable. Furthermore, the economic production, consistency, ease of process optimisation, and modification, along with the possibility of enzyme production using genetically engineered microorganisms with enhanced yield have qualified microorganisms as the apt sources of industrial enzymes. Often extracellular enzymes are preferred over intracellular or cell-bound enzymes, as they are easier to isolate (Harrison, 1987). About 80% of the industrial enzymes are produced from microorganisms, owing to the fact that a wide spectrum of enzymes are elaborated by them, which offer an opportunity to select specific enzymes for specific purposes (Bhotmange & Shastri, 1994). In fact, intensive screening is perused all over the world for enzymes with novel properties and functions from various microorganisms inhabiting different environments (Fogarty & Kelly, 1990).

Interest in amidohydrolases such as L-asparaginase and L-glutaminase started with the discovery of their antitumour properties (Broome, 1961., Roberts et al, 1970., Bauer et al, 1971). L-glutaminase apart from its use as a therapeutic enzyme, also earns its importance in food industry as a flavour enhancing agent (Yokotsuka, 1985., Koibuchi et al, PT-WO 9960104, 1999). Recently, several other applications has been proposed for L-glutaminase and the enzyme is used in biosensors for determination of glutamine and glutamate in pharmaceutical formulations (Botre et al, 1993), mammalian cell cultures (White et al, 1995., Mulchandani & Bassi, 1996), liquid samples (Luong et al, PT-CA 2109896, 1994), and hybridoma cultures (Meyerhoff et al, 1993). Glutaminase is also used in the industry for manufacture of speciality chemicals like threonine (PT-JP5068578, 1993., JP11225789, 1999) and other γ-glutamyl alkylamides (PT-JP05284983, 1993., Tachiki et al, 1998). Immobilized glutaminase is also used in conversion of glutamine to glutamate.
specifically employed in flavour enhancement of liquid seasonings (Koseko et al., 1994). Since the industrial sources of L-glutaminase are limited and mainly confined to few species of Bacilli, Yeast, and Apsergillus oryzae, the search for potential microorganisms that hyper produce the enzyme with novel properties, and economically viable bioprocesses for their industrial production is perused all over the world (Nagendraprabhu & Chandrasekaran, 1995).

Marine biosphere is one of the richest of earth's innumerable habitats, and yet is one of the least characterised. Because of the diversity and scale, it offers enormous opportunities for non-destructive exploitation within many facets of modern biotechnology (Sabu, 1999). Although the oceans cover more than two thirds of the world's surface, the knowledge on marine microbes is still very limited, and they remain as untapped sources of many metabolites with novel properties (Faulkner, 1986, Chandrasekaran, 1996). Marine bacteria and their enzyme systems are useful in several industrial applications due to their increased tolerance to salinity, and consequently are being intensively screened for products of economic importance (Moriguchi et al., 1994).

In view of the tremendous potential of marine microbes, as sources of useful metabolites, there exist an urgent need to develop viable bioprocess technologies for efficient utilisation of them, besides appropriate selective isolation methods and screening programs for novel metabolites. Efforts have recently been made in this direction and there are reports on marine microorganisms, which are potent producers of adhesives (Abu et al., 1992) roslipins (Tomoda et al., 1999), pyrostatins (Aoyama et al., 1995), siderophores (Martinez et al., 2000), Hydrogen (Matsunaga et al., 2000) anti-HIV compounds (Schaeffer & Krylov, 2000) and several enzymes (Farrell & Crosa, 1991, Moriguchi et al., 1994, Kim et al., 1999, Araki et al., 1999, Sabu et al., 2000). With the emerging interest in marine bioresources, researchers world-wide have started to look upon marine microorganisms as potent sources of enzymes with improved and novel properties important for the industry, pharmaceuticals and research. Reports are available on the production of industrially important enzymes like protease (Michels & Clark, 1997), amylase (Brown & Kelly, 1993), lipase (Ando et al., 1991, 1992), cellulase (Bronnenmeier et al., 1995), agarase (Vera et al., 1998), DNA polymerase (Lundberg et al., 1991), chitinase (Suresh & Chandrasekaran, 1999), glutaminase (Nagendraprabhu & Chandrasekaran, 1995, 1996).
Sabu et al., 2000), and several other enzymes. Niehaus & Antranikian (1997) had recently reviewed the production and application of heat stable enzymes from marine microorganisms. Nevertheless, much of these efforts were focused on basic studies and as such very little information is available on the large-scale production of marine microbial enzymes.

Marine bacteria are recognised for their ability to colonise immersed surfaces (Austin, 1988) and most of them are able to obtain sufficient nutrients, only when they grow as aufwuchs, a condition during which the bacteria remain adsorbed onto solid particles (Chandrasekaran, 1996). The unique property of marine bacteria to adsorb on solid supports make them ideal candidates for immobilized cell processes and indeed a Marinobacter sp. adsorbed on porous glass beads was tested for degradation of a hydrophobic C-18 isoprenoid ketone (Bonin et al 2001). In spite of the fact that marine bacteria possess the ability to colonise immersed surfaces and consequently become ideal candidates for immobilized whole cell processes, such processes has rarely been attempted in the production of extracellular enzymes by marine microorganisms.

Conventionally, commercial scale production of microbial metabolites are carried out by submerged fermentation (SmF) which allows a better process control and automation together with reduced risk of contamination. There is resurgence in interest, in the use of solid state fermentation (SSF) for large-scale manufacture of microbial metabolites (Lonsane, 1994). The application of immobilized living microbial cells represents a new, fascinating and rapidly growing trend in microbial technology. Immobilization of microbial cells represents the transfer of the cells from a free state to a state of confinement or localisation in certain defined region of space with retention of catalytic activity and often with retention of viability so that the cells can be used repeatedly or continuously (Klein & Wagner, 1983). Natural biofilms also qualify as immobilized cultures, and adsorbed cells are increasingly being used in bioprocesses for metabolite production (Truck et al, 1990a,b, Linko et al, 1996) and waste treatment (Wong et al, 1993., Fuji et al, 2000). Compartmentalisation cells implies that the cell density in solid phase is very high, with cell loading greater than $10^{10}$ cells per cm$^3$ of support matrix not an uncommon occurrence (Chibata, 1979., Klein & Wagner, 1983); a very attractive feature in increasing the productivity per unit reactor volume.
In contrast with the batch or continuous submerged fermentations, where free cells are utilized for metabolite production, immobilized cells offer several advantages (Kolot, 1981), which include the acceleration in reaction rate due to increased cell density per unit reactor volume and feasibility for using high dilution rates, as wash out of cells is not a problem with immobilized systems. Cell metabolism (Galazzo & Bailey, 1989) and cell wall permeability (Fletcher & Marshall, 1982) is increased upon immobilization, and as the cells are able to multiply on or inside the support matrix, they can be activated on site if needed. Also, use of immobilized cells eliminate the need of costly steel fermenters, and the production plants can be designed to be smaller in size, comprising of columns packed with immobilized cells. As a result, better process control could be achieved (Kolot, 1981).

Cell immobilization technology is particularly suited for production of extracellular enzymes and there is a growing interest in applying cell immobilization techniques for continuous production of enzymes (Ramakrishna & Prakasham, 1999). This offers several advantages over the conventional processes, which include the easy separation and reuse of cells, high cell concentrations, flexibility in reactor design, and operation as well as stabilisation of several cell functions (Dervakos and Webb, 1991).

Immobilized whole cell systems have been successfully employed in the production of several extracellular enzymes by terrestrial microbes e.g. α-amylase (Duran-Paramo et al, 2000), glucoamylase (Fiedurek & Seczodark, 1995), Lipase (Ferrer & Sola, 1992), Protease (el-Aassar et al, 1990), Cellulase (Xin and Kumakura, 1993), Xylanase (Mamo & Gessesse, 2000), and Ribonuclease (Manolov, 1992).

Several methods of immobilization have been attempted and by far the commonest methods used in immobilization of whole cells is gel entrapment in alginate or carrageenan and physical adsorption on solid supports, probably because these methods are less harsh on the living cells. The supports used for cell adsorption are wide and varied, which includes ceramics (Shiraishi et al, 1989), porous glass (Jager & Wandrey, 1990), polyurethane foam (Haapala et al, 1994), Nylon web (Linko et al, 1996) stainless steel biomass support particles (Webb et al, 1986), and Luffa sponge (Ogbonna et al, 1994). However, the industrial use of immobilized
viable microbial cells is limited to few processes, the most important of which is ethanol production.

Though the immobilized cell processes offer several advantages over the conventional fermentation technologies, a complete exploitation of these potential advantages in industrial applications will strongly depend on the wise selection of a set of processing parameters allowing for high productivity combined with extended operational stability. This set includes; immobilization method, mode of operation (repeated batch vs continuous), aeration and mixing, bioreactor configuration, medium composition (including feeding of substrates, precursors or additional nutrients), temperature, pH, and whenever required, in situ product and/or excess biomass removal (Freeman and Lilly, 1998). A proper understanding of the influence and interactions of these parameters, cell physiology, and behaviour under immobilized conditions, productivity, and operational stability is required for a rational and systematic design and evaluation of new processes.

With the pressing need and demand for information on immobilization of marine bacteria and processes for metabolite production by them, in the present study an effort was made to study the production of extracellular L-glutaminase by marine Pseudomonas sp. BTMS-51 under immobilized conditions.
OBJECTIVES OF THE PRESENT STUDY

From the ongoing review of literature it is understood that there exists a dearth of knowledge on use of immobilized cell processes for metabolite production and production of exoenzymes employing marine bacteria despite the fact that they are very much amenable to immobilization due to their adhesive properties. Hence, it was proposed to study in detail the process for the immobilization of marine *Pseudomonas* sp, BTMS 51, and extracellular L- glutaminase production by the immobilized cells.

The primary objective of the study was to evaluate the commonest whole cell immobilization protocols towards extracellular L-glutaminase production by the marine *Pseudomonas* sp. under immobilized condition and to develop a viable bioprocess technology employing the immobilized cells. The specific objectives of the present study include

1. Process optimisation for immobilization of whole cells of *Pseudomonas* sp. in Ca-alginate beads and L-glutaminase production by immobilized bacteria under batch mode operation
2. Evaluation of the reusability of immobilized cell beads under repeated batch operation
3. Process development for continuous production of the enzyme by Ca-alginate immobilized bacteria in a Packed bed reactor (PBR) and a Circulating bed reactor (CBR)
4. Process optimisation for immobilization of *Pseudomonas* sp. BTMS -51 on preformed carriers and evaluation of enzyme production by cells immobilized on three different carrier systems
5. Optimisation of the enzyme production by adsorbed cells using response surface methodology and generating information on the interaction between process variables
6. Evaluation of the reusability of the cell adsorbed carrier particles in L-glutaminase production under repeated batch operation
7. Continuous production of the enzyme in packed bed reactor with cells adsorbed onto a bed of polystyrene, PUF or Nylon web.