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Industrial Enzymes - Present status and future perspectives for India

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Enzyme technology is a well established branch of the biochemical science which is going through a phase of maturation as well as evolution. The maturation is shown by the development of the theory, their function and through the formation and configuration of their three-dimension structure, in this era of global industrialization. A better understanding enzymes and their functional significance suggests many novel applications for these catalytic activities and for continual discovery with novel properties by R&D. They are being used on an industrial and research scale for the variety of reactions catalyzed with environmental conditions. This is just the start of the industrial enzyme era which is preparing itself to put known enzymes to novel uses and novel enzymes, discovered or tailored, to catalyze unexploited reactions. As the demand is for cleaner and greener technology to preserve our mother earth for our descendant, the use of enzymes that can replace harmful chemical reactions are extremely importance and most of the current R&D on enzymes is directed towards this issue. Similarly use of enzymes in extreme harsh conditions such as high and low temperatures and pH are also more prevalent. Hence the evaluation of present R&D directions on industrial enzymes seems to be important and this paper assesses the status of industrial enzyme research globally as well as in Indian context with use in various industries, it application, the present status of R&D and commercialization. Since enzymes are now widely used in biotransformation and considering the importance of enzymes in the synthesis of chiral molecules of pharmaceutical importance, a separate section on enzymes involved in biotransformation are also reviewed in detail. This study is based on the search from scientific databases such as SciVerse Scopus, Google, other web sources, etc.

Keywords: enzymes, micoorganism, biotechnology, cellulases, keratinase, phytase

Introduction

Enzymes are used in many environmental-friendly industrial purposes, as they are efficient, selective, accelerate and speed up reactions by forming transition state complexes with their substrate which reduces the activation energy of the reaction. With the advancement in biotechnology, especially in the area of genetics and protein engineering, have opened a new era of enzyme applications in many industrial processes and experiencing major R&D initiatives, resulting not only in the development of a number of new products but improvement in the process and performance of several existing processes also. Enzymes are useful in various areas of applications like manufacturing of food and feedstuff, cosmetics, medicinal products and as a tool for research and development. Technical enzymes are applied in detergents, for pulp and paper applications, in textile manufacturing, leather industry, for fuel production and for the production of pharmaceuticals and chiral substances in the chemical industry. The use of enzymes in animal nutrition is important and growing, especially for pig and poultry nutrition. Feed enzymes offer the benefit of degrading specific feed components otherwise harmful or of no value to the livestock. In cosmetic products, it is used for is skin peeling and future applications may be skin protection. Notable medications of enzymes are as digestive aids, for wound cleaning, lysis of vein thromboses, acute therapy of myocardial infarction and as support in the therapy of certain types of leukaemia. Enzymes can be used in chemical analysis and as a research tool in the life sciences.

History of enzyme technology

The history of enzyme technology began in 1874 when the Danish chemist Christian Hansen produced the first specimen of rennet by extracting dried calves' stomachs with saline solution, which was the first enzyme used for industrial purposes. This significant

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event had been preceded by a lengthy evolution. The digestion of meat by stomach secretions and the conversion of starch to sugars by plant extracts and saliva were known at that time itself. The fermentative activity of microorganisms was discovered in 18th century by the French scientist Louis Pasteur. In 1878 German physiologist Wilhelm Kühne (1837–1900) coined the term *enzyme* from Latin words, which literally mean “in yeast”. In 1897 Eduard Buchner began to study the ability of yeast extracts that lacked any living yeast cells to ferment sugar. In a series of experiments at the University of Berlin, he found that the sugar was fermented even when there were no living yeast cells in the mixture. He named the enzyme that brought about the fermentation of sucrose "zymase".

The first application of cell free enzymes was the use of rennin aspartic protease isolated from calf or lamb stomach in cheese making. The first commercial enzyme (trypsin) was prepared by Röhm in Germany in 1914; isolated from animals and used in the detergent to degrade proteins. Introduction of microbial proteases into washing powders have made a real breakthrough in detergent industries. The first commercial bacterial *Bacillus* protease was marketed in 1959 and became big business when Novozymes in Denmark started to manufacture it and major detergent manufactures started to use it around 1965[1]. Enzymes were used in 1930 in fruit juice manufacturing for clarification of juices. The major usage started in 1960s in starch industry. The traditional acid hydrolysis of starch was completely replaced by alpha-amylases and glucoamylases, which could convert starch with over 95%, yield to glucose. Starch industry became the second largest user of enzymes after detergent industry[2-5]. The use of enzymes results in many benefits like higher product quality and lower manufacturing cost, and less waste and reduced energy consumption. More traditional chemical treatments produce undesirable side effects and/or waste disposal problems. The degree to which a desired technical effect is achieved by an enzyme can be controlled through various means, such as dose, temperature, and time. Because enzymes are catalysts, the amount added to accomplish a reaction is relatively small. Enzymes used in food processing are generally destroyed during subsequent processing steps and not present in the final food product.

Industrial enzymes

Biotechnological innovations

Biotechnology has influenced almost every sector of industrial activity–chemical feedstock, food, feed, environment, energy and health care, which are directly driven by social and environmental needs, besides economic forces. Also, it requires an understanding and application of a range of basic scientific and engineering disciplines, including microbiology, biochemistry, physics, chemistry, chemical and bioprocess engineering, besides molecular biology and genetics. Other features include their dependence on renewable feedstock, low energy consumption and environmentally favourable processing that can potentially lead to sustainable development. Although biological processes involving living cells and their constituents have been used by mankind, real break though has emerged with developments in both biological and engineering sciences, during the last fifty years. All chemical reactions occurring in living cells (for breakdown of nutrients and synthesis of cellular constituents) are catalysed by their group of molecules – enzymes. These are naturally evolved biocatalysts that are designed to perform their function in an efficient manner, and still providing a precise and suitable control mechanism to the cell for survival under range of environmental conditions with numerous applications in every sector. There exist more than 3,000 different known enzymes of which only 150 to 170 are used commercially. Currently only 5% of chemical products are produced using biotechnological methods. Enzymatic processes are fast becoming better financial and ecological alternatives to chemical-physical and mechanical processes and applications by virtue of being cost effective and more environment friendly[6]. Enzymatic processing of various application areas for industrial enzymes is classified as i) Enzymes as final products; ii) Enzymes as processing aids; iii) Enzymes in food and beverage production iv) Enzymes as industrial biocatalysts and v) Enzyme in genetic engineering.

Commercial processes

Many chemical transformation processes used in various industries have inherent drawbacks. High temperatures/pressures needed to drive reactions lead to high energy costs and may require large volumes of cooling water downstream. Harsh and hazardous processes involving high temperatures, pressures, acidity, or alkalinity need high capital investment, and specially designed equipment and control systems. Unwanted by-products may prove difficult or costly to dispose of. High chemicals and energy consumption as well as harmful by-products have a negative impact on the environment. These drawbacks can be virtually eliminated by using enzymes. The enzymatic reactions may often be carried out under mild conditions, they are highly specific, and involve high reaction rates. Industrial enzymes originate from biological systems; they contribute to sustainable development. Small amounts of enzymes are needed to carry out chemical reactions even on an industrial scale, both solid and liquid enzyme preparations with little storage space. Mild operating conditions enable uncomplicated and widely available equipment to be used, and enzyme reactions are generally easily controlled. Enzymes also reduce the negative impact on the environment by reducing the consumption of chemicals, water and energy, and the subsequent generation of waste. Developments in genetic and protein engineering have led to improvements in the stability, economy, specificity, and overall application potential of industrial enzymes. Carbonic anhydrase, which catalyzes the hydration of carbon dioxide to speed up its transfer in aqueous environments like the blood, is one of the fastest enzymes known. Because enzymes are highly specific in their actions they catalyze, an abundant supply of enzymes must be present in cells to carry out all the different chemical transformations. Scientists have identified over 10,000 different enzymes, for which a logical method of nomenclature has been developed. The major applications of industrial enzymes are discussed below.

Advantages

The use of biotechnology in industry does not simply remove pollutants but also will prevent pollution at the source. Efforts to achieve clean industrial products and processes will also bring great benefits to industry over the next ten or twenty years. Industrial biotechnology, using microorganisms and biological catalysts (enzymes) to produce goods and services, has come of age. The benefits of enzymes include higher product quality and lower manufacturing cost, and less waste and reduced energy consumption. More traditional chemical treatments are generally non-specific not always easily controlled, and may create harsh conditions. Often they produce undesirable side effects and/or waste disposal problems. The degree to which a desired technical effect is achieved by an enzyme can be controlled through various means, such as dose, temperature, and time. The use of enzymes in chemical processes saves the vast amounts of water like in the textile industry, where enzymatic treatment of textiles can save 70,000–90,000 litres of water for every ton of knitwear produced. With approximately 9 million tons of knitwear being produced annually, the world could save 630 billion litres of water every year if all knitwear were produced using enzymes[7].

Enzymes can often replace chemicals or processes that present safety or environmental issues. For example, enzymes can:

- Replace acids in the starch processing industry and alkalis or oxidizing agents in fabric desizing;
- Reduce the use of sulphide in tanneries;
- Replace pumice stones for "stonewashing" jeans;
- Allow for more complete digestion of animal feed leading to less animal waste; and
- Remove stains from fabrics. Clothes can be washed at lower temperatures, thus saving energy. Enzymes can be used instead of chlorine bleach for removing stains on cloth. The use of enzymes also allows the level of surfactants to be reduced and permits the cleaning of clothes in the absence of phosphates.

Enzymes also contribute to safer working conditions through elimination of chemical treatments during production processes.

Global market

Biotechnology is gaining rapid ground as it offers several advantages over conventional technologies. The global market for industrial enzymes is estimated at $3.3 billion in 2010 and expected to reach $4.4 billion by 2015. Technical enzymes are valued at just over $1 billion in 2010. This sector will increase at a 6.6% compound annual growth rate to reach $1.5 billion in 2015. The highest sales of technical enzymes occurred in the leather market, followed by the bioethanol market. The food and beverage enzymes segment is expected to reach about $1.3 billion by 2015, from a value of $975 million in 2010, rising at a CAGR of 5.1%. Within the food and beverage enzymes segment, the milk and dairy market had the highest sales, with $401.8 million in 2009. The global industrial enzyme market till the present date and the projected market are shown in Figure 1. The market segmentation for various areas of application shows that 34% of market is for food and animal feed followed by detergent and cleaners (29%). Pulp and paper share 11% market while 17%

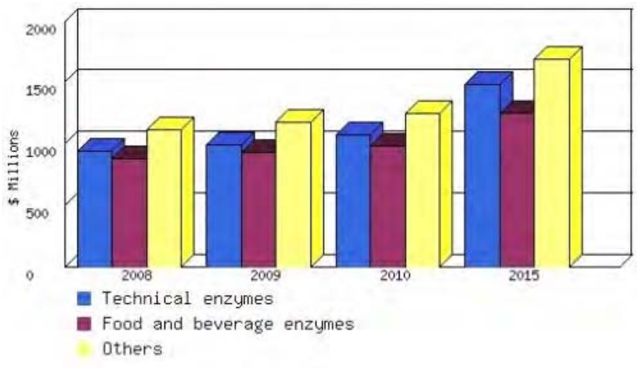

Fig. 1—Global industrial enzymes market, 2008-2015 (Source BCC Research)

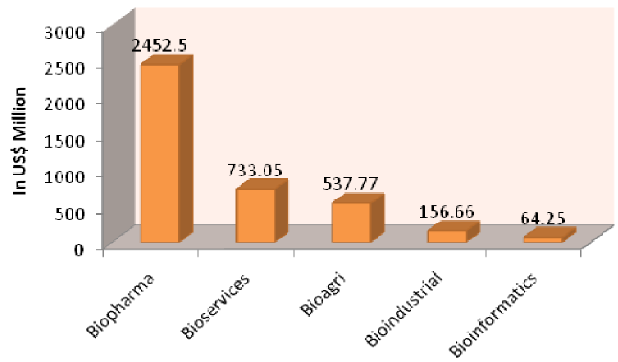

Fig. 2—Indian Biotech Market (Source: Biospectum 2011[9])

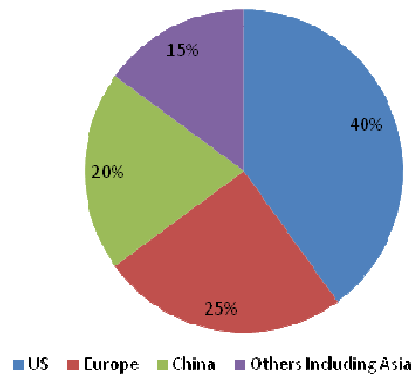

Fig. 3—Industrial Enzymes - Export Scenario

of the market is captured by textile, leather and fur industries.

Indian enzyme market

The biotech industry in India accounts for just 2% of global biotech markets. But it is gaining global visibility because of the investment opportunities. It is a powerful enabling technology that can transform agriculture and healthcare, use renewable resources to bring greater efficiency into industrial processes, check environmental degradation and deliver a more bio-based economy[8]. The Indian industrial enzymes market is making the most of the demand for easier formulations that help offer increased functional benefits and multi-application profiles. The influx of international companies has upped the quality requirements of local suppliers and importers, thereby providing consumers with a wider variety of inventive products to choose from. In the wake of increased environment consciousness and regulations, manufacturers have realized that eco-friendly chemical alternatives are the way forward, especially if they wish to penetrate untapped and niche areas such as the laundry bars market. Industrial enzyme manufacturers need to realize that consumers are likely to purchase ecological products only if they are cost effective and easy to use. Reacting to the need for huge niche market in the rural sectors and diversification, manufacturers launched a slew of new enzyme solutions for the Indian detergent market. However, the lack of government legislation and controls to ensure enzyme use will continue to hinder this market, which is associated with high capital and operational costs. Investors are apprehensive about funding R&D activities, as the developed products' market performance cannot be pre-evaluated. Enzyme companies are also required to invest heavily in customer service and technical support, which puts a heavy strain on their budgets.

During the fiscal year 2010-11 the Indian biotech sector grew at 21.5% to reach Rs 17,400 crores in revenues. The biopharma segment continues to maintain dominance, followed by the bioindustrial segment and then by bioagri segment (Figure 2). India has a marginal share in the global market for industrial enzymes, which is estimated to be at about US$ 3387.30 million. The segment is forecasted to grow at a CAGR of 15 percent till 2015. Even though there is a prevailing domestic demand, the segment is largely export oriented. Major export markets include the US (global share 40%), Europe (global share 25%) China (Global share: 20%). Others include Rest of Asia (Global share of 15%). Realizing the potential of the opportunities outside India, many Indian companies are expanding their base outside the country even into difficult markets such as China. Following figure (Figure 3) shows the export scenario for industrial enzymes from India. It is difficult to strategize the Indian industrial enzymes market because it is at various stages of growth, ranging from penetration to product development. For instance, the pharmaceutical enzymes segment is relatively nascent and is the focus of a small group of specialized manufacturers. The textile and leather enzymes segments are mature, while the detergent enzymes segment is in the growth stage. Almost 50% of total enzyme demand covers pharma sector. The detergent manufacturing and textile processing sector cover almost 20% each. Food and feed industries as well as leather and paper industries demand 5% each. The following figure (Figure 4) shows the demand for enzymes in various sectors.

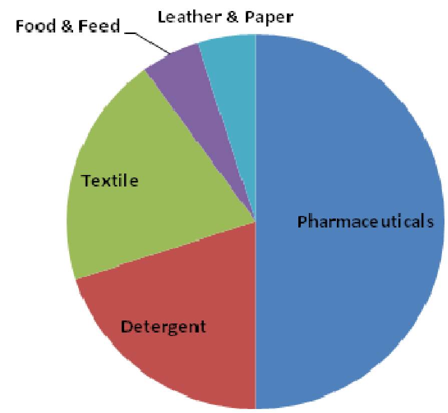

Fig 4—Sector wise demand for enzymes

R&D in India

India's rich human capital is believed to be the strongest asset for this knowledge-based industry having a large English speaking skill base, 3 million graduates, 700,000 post-graduates & 1500 PhDs qualified in biosciences and engineering. It is estimated that 10% of researchers and 15% of scientists in Pharma/Biotech R&D in USA are of Indian origin. Indian Government is embracing biotechnology as the next driver of innovation and economic growth. Unlike the biotech sectors worldwide, the global recession has left the Indian biotech industry untouched due to its less reliance on investors' capital. India's biotech industry has blossomed in recent years as domestic companies have grown aggressively in a liberalized intellectual property regime and as companies worldwide have sought to seize opportunities from India's large, skilled workforce, lower manufacturing and research costs. The Indian Economy has witnessed a sharp growth in deals involving outsourcing, technology transfer and entry of foreign players to tap the burgeoning Indian biotechnology market. The Government of India has introduced a number of important legislations in order to support and promote the growth of biotechnology industry in the country. However, several organisations are involved in regulating the development of biotechnology in the country which has often led to an overlap of functions. In order to streamline the regulatory process, the Government has proposed the establishment of the National Biotechnology Regulatory Authority (NBRA) to provide a consistent mechanism for regulatory approval.

The Indian companies are experiencing a substantial growth, and are thus focusing more on R&D due to which new applications are being discovered. The public-funded successful R&D institutions can also be encouraged to set up 'not-for-profit' companies for facilitating the collaborative work with industry. There needs to be an alliance between industry and academia wherein the industry could actively participate in planning the curriculum and evaluation methods. Meanwhile, the private firms may also step in and share the responsibility of encouraging academic excellence by creating Chairs in Universities and also by strengthening the industry–academia relationship. The public institutions can play critical role in the provision of a working space along with the chances for the incubated company to use some of the equipment from the incubator institution. The public-private partnerships and collaborations are increasing in the biotech industry. The initiatives taken by Bill & Melinda Gates Foundation and DBT Small Business Innovation Research Initiative (SBIRI) are examples of collaborations between the industry and the government. DBT has also tied up with UK-based Wellcome Trust to augment the cutting-edge biomedical research in the country. The trust will allocate around EUR 33.9 million for five years to fund R&D research in India.

SWOT analysis

India possesses a skilled man power with network of research laboratories such as CSIR, IITs, IISER and universities makes the country a potential knowledge hub. It is blessed with rich biodiversity western Ghats and Eastern Himalayas which harbour rich microbial diversity which can be exploited for new enzymes with diverse properties. The cost of manufacturing on the Indian soil is approximately 35-40% less than that in USA. Also, the overall cost of manpower and low installation charges further make

India a preferred destination. India presents a wide scope for generic manufacturing with world-class facilities, which comply with internationally acclaimed standards like Good Clinical Practices (GCP), Good Laboratory Practices (GLP), and current Good Manufacturing Practice (cGMP). Department of Biotechnology (DBT) has opened several top notch Centres of Excellence (COE) in India to generate a higher number of skilled manpower and also to support the corporate in their R&D efforts. But India still lack financial support for doing innovations and there is a shortage of the state-of-the art facility for R&D. The slow regulatory approval procedure and political issues makes a hurdle for industrial growth. There is a lack of awareness and understanding of biotechnology–derived medicines and products among physicians and public. Still Indian enzyme market is optimistic and development of new business model such as generating revenues from patent licensing and litigation can redefine existing business models completely, and shift them to a higher value-generation platform. India has a lot of opportunities for accelerated growth of industries by proper IP protection. There is an increased opportunity for entrepreneurial activities. The missing link between the research and commercialization is the major weakness in India. Doubts about Indian products to meet international quality are another problem. Danger of anti-biotech propaganda, brain drain and enhanced level of politics in the system are the major threat to Indian market.

The major industrial enzyme manufacturers are – Novozymes India, Bangalore, Advanced Enzymes, Ahmedabad, Rossari Biotech, Zytex and Maps India, Ahmedabad. Novozymes was the market leader in this segment during 2010–2011, with total revenue of Rs. 2,420 million. Advanced enzymes and Rossari Biotech were the other major players with sales of Rs. 1540 and 720 million respectively. Advanced Enzymes Technologies and Rossari Biotech have registered nearly 30 per cent growth in 2010 – 2011 against the previous year. Zytex registered 16 per cent growth while Maps (India) registered 2.6 per cent growth over the previous year's sales revenue[9].

Present status of R&D–Global and Indian perspectives

Recent developments in biotechnology, in the areas like protein engineering and directed evolution, have provided important tools for the efficient development of new enzymes. This has resulted in the development of enzymes with improved properties for established process and technical applications and in the production of new enzymes tailor-made for entirely new areas of application where enzymes have not previously been used. The various application sectors are analysed below.

Energy

Ethanol fuels can be derived from renewable resources - dedicated agricultural crops such as corn, sugar cane, and sugar beet or from agricultural by products such as whey from cheese making and potato processing waste streams. It can also be utilized in petroleum fuels as a replacement for the toxic oxygenates Methyl t-Butyl Ether (MTBE). The sugars are fermented with yeast to produce ethanol. The current best available technology for conversion employs an acid hydrolysis of the biomass into sugars. The enzymatic alternative, using cellulase and hemicellulase, avoids the use of strong acids and results in a cleaner stream of sugars for fermentation and fewer by-products[10]. The environmental benefit is utilization of natural, renewable resources, safer factory working conditions, reduced harmful auto emissions.

Publication analysis: Cellulases (3rd largest enzyme) are the most extensively studied multiple enzyme comprising of endo-glucanases (EG), cellobiohydrolases (CBH) and β-glucosidases (BGL) and widely used in many applications with an increasing demand. The Scopus literature search during the last ten years on key word "cellulases" (2002-2011) was 4549 and out of this 44% was during the last three years. Among these 4549 publications in SCI journals, 978 are from USA followed by 742 from China, 438 from Japan and 407 from India(4th place). From India, there were 97 publications in 2011 and 67 in 2010. In this 22 are from CSIR-National Chemical Laboratory, Pune followed by CSIR-National Institute for Interdisciplinary Science and Technology, Trivandrum. There is a tremendous increase in R&D on biofuels and enzymatic hydrolysis of lignocellulosic biomass and are looking for new efficient cellulase producers. Studies are being carried out on the improvement of existing microbial strains to increase the production of various cellulases and reduce their cost[11, 12]. In 2002, there was only four publications specifically for the key word "cellulases AND bioethanol OR biofuels" but in 2011 there was 233 publications pertaining to this particular key word. This shows the importance of the research on lignocellulosic biofuels through enzymatic hydrolysis. Among the 233 (2011), US (1st with 98), China (24), India (16) in 2011 among them 4 are from CSIR-NIIST, two each from IIT-Kharagpur and Osmania University, Hyderabad.

Textiles

Textile processing has benefited greatly on both environmental and product quality aspects through the use of enzymes. Before the discovery of amylase enzymes, the only alternative to remove the starch-based sizing was extended treatment with caustic soda at high temperature. The chemical treatment was not totally effective in removing the starch (which leads to imperfections in dyeing) and also results in a degradation of the cotton fibre resulting in destruction of the natural, soft feel, or hand, of the cotton. The use of amylases to remove starch-based sizing agents has decreased the use of harsh chemicals in the textile industry, resulting in a lower discharge of waste chemicals to the environment, improved the safety of working conditions for textile workers and has raised the quality of the fabric. New enzymatic processes are being developed (cellulase, hemicellulase, pectinase and lipase), which offer the potential to totally replace the use of other chemicals in textile preparation processes.

Stonewashed Jeans Without Stones

Traditionally, to get the look and feel of stonewashed jeans, pumice stones were used. A big driver for the jeans industry is fashion. Enzymes give the manufacturer a newer, easier set of tools to create new looks of jeans. The pumice stones used to "stonewash" the denim clothes can also over abrade or damage the garment. By using enzymes, the manufacturer can give consumers the look they want, without damaging the garment.

Yarn Treatment

In the preparation of cotton yarn for dyeing and garment manufacture, hydrogen peroxide is used to bleach the yarn. An enzyme catalase can be used to breakdown the hydrogen peroxide to water and oxygen. With the use of catalase, the reducing agent can be eliminated or the amount of rinse water can be dramatically reduced, resulting in less polluted wastewater or lower water consumption.

Leather processing

Hides and skins have hair attached to them that must be removed for their use as leather. The conventional way to remove hair from hides is to use harsh chemicals such as lime and sodium sulfide. With enzyme-assisted dehairing, it is possible to reduce the chemical requirements and obtain a cleaner product and a higher area yield with fewer chemicals in the wastewater. To make the leather pliable bating is done using dog or pigeon dung. Since "dung bates" owed their softening effect to the action of a protease enzyme, during the 20th century, the Leather Industry has switched over to using bacterial proteases and pancreatic trypsin.

Dehairing of Leather

Enzymatic dehairing in tanneries has been envisaged as an alternative to sulphides. Tanneries are constantly concerned about the intolerable odour and pollution caused by the extremely toxic sodium sulphide used in the dehairing process step. A large number of cases of deaths due to this toxic chemical process have even been reported.

Publication analysis: There were totally 240 publications on keratinase during the last 10 years and India (1^{st} rank) with 55 publications, Brazil(2^{nd}) with 38 and US (3^{rd}) with 26 publications. During 2002 there were only 15 publications and it increased to 44 in 2011. There were no SCI journal publication for India on keratinase in 2002 and 2003 and there was one publication from University of Delhi South Campus in 2004. In 2005 and 2006 there were two publications each and in 2011 there were 20 publications. University of Delhi, University of Madras, Gulbarga University, Tezpur University and Anna University are some the research laboratories involved in keratinase research. CLRI is the major CSIR laboratory involved in keratinase research with 3 publications during the last ten years.

Degreasing of Leather

Traditionally, the degreasing of sheepskins is done by solvent-extraction using paraffin solvent systems. A new process based on the enzymatic breakdown of fats by a lipase enzyme has been introduced which replaces the solvent-based process[13].

Publication analysis: There are more than 17,000 publications regarding lipases during 2002 to 2011 among them more than 6,000 are during 2009-2011 (Figure 5). Even though the trends in increase in the number of publications is not so huge, there are still lot of new publications are emerging due to the importance of this enzyme. Majority of the publications are from US (nearly 3500) and India (995) with 5^{th}rank, mostly from University of Delhi and CFTRI, Mysore.

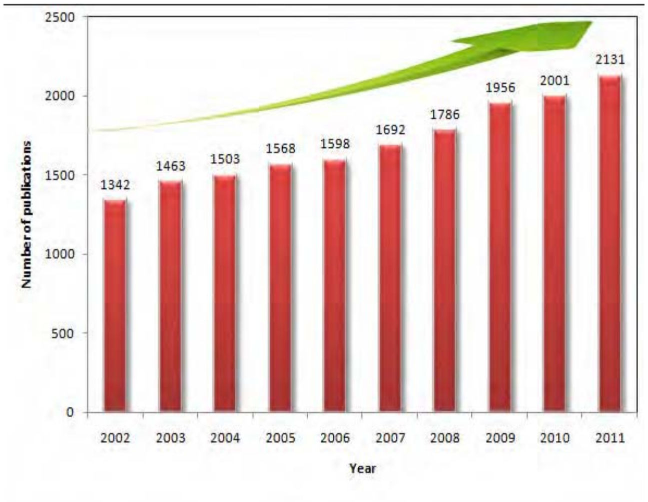

Fig. 5—Number of publications in SCI journals for the key word "lipases" (Scopus search)

Paper

The pulp and paper industry employs chlorine oxidants to bleach pulp. As a result, chlorine-containing organics, a class of compounds with toxicity concerns, are produced as by-products. The classic problem with chlorine bleaching is that in whitening the paper. Enzymes help papermakers to reduce the use of harsh chemicals such as chlorine bleach. Hemicellulase enzymes such as Xylanase can enhance the bleaching efficacy allowing a dramatic reduction in the consumption of chlorine.

Publication analysis: There were only 36 publications on keyword "xylanases" in 2002 and in 2011 it is 267. In 2002, the only publication on purification of xylanases is from IIT-Delhi. During the last 10 years, the number of publications increased to 121 and India at third place. Kurukshetra University, Guru Nanak Dev University, IIT-Roorkee, IIT-Delhi and NCL Pune are some of the laboratories which published research articles on xylanases.

Deinking of Waste Paper

During the recycle of waste paper the cellulosic fibres can readily be separated by repulping and cleaning, and made into new paper. The residual printing inks and adhesives are the most difficult of the components to remove. Caustic surfactants and large quantities of wash water are used to separate the ink from the cellulosic fibres. They can be dramatically reduced by the use of the enzymes cellulase and hemicellulase.

Household & personal care applications

Lower Temperature & No Phosphate Clothes Washing

The global trend has been to reduce wash temperatures and ban phosphates for which detergent manufacturers have turned to enzymes into their products. A lower wash temperature significantly reduces the energy needed to do a load of laundry. Also, the reduction in phosphate load to rivers and lakes is believed to lower the human-induced decline of these systems.

Milder Dishwashing Detergents

Automatic dishwashing detergents are formulated to be very alkaline in some countries. Enzymes have replaced harsh chemicals while maintaining the cleaning by reduced chemical load.

Contact Lens Cleaner

When you wear contacts proteinaceous and lipid materials from the eye gradually accumulate on the contact lens for which lysozyme helps. When a contact lens, a foreign object, is introduced onto the eye surface it interferes with the normal cleansing process. Incorporating protease and lipase enzymes in the lens cleaning system can dramatically enhance removal of this soil that accumulates on the contact lens.

Food and feed – digestive aids

Alpha-galactosidase for improved nutritional value of legume- and soy-based foods

Enzymes can be used to improve the nutritional quality of food for humans and animals. The full utilization of the potential nutritive value in legume- and soy-based foods is limited by the presence of non-digestible sugars such as raffinose and stachyose. These sugars contain chemical linkages that cannot be broken by the natural enzymes produced by the body. Consequently, the sugars proceed through the digestive tract until reaching the large intestine where they are hydrolyzed by the natural microflora in the intestine. These organisms utilize the sugars that are converted to gas during this metabolism causing discomfort and flatulence. The enzyme, alpha-galactosidase, is used to convert stachyose and raffinose to simple sugars that are adsorbed by the human digestive tract, thereby preventing the flatulence often caused by legumes such as beans and soy-based foods. This enzyme can be used to hydrolyze raffinose and stachyose during soy processing, during the food preparation process or by addition to the food itself immediately before ingestion.

Publication analysis: There are more than 1800 publications on alpha-galactosidases during (2002-11) and constant with an average of 180 per year. From India (11th position) there were nearly 48 publications, US (802), Japan (270), UK (174) and 120 from France.

Reduced Phosphorous Animal Feed

Poultry and hog feed grains contain phosphorous which is bound to phytic acid. Animals need phosphorous for bone growth and other biochemical processes, the feed suppliers normally add extra phosphorous to the diet. A specific enzyme, phytase releases the bound phosphorous, making it digestible to the chicken or hog. Phytase added to the feed eliminates the need for compensating levels of phosphorous and thus dramatically reduces the phosphorous content of the animal waste[14, 15].

Publication analysis: There were nearly 1400 publications on "phytase" and India (3rd rank) with 123 after US (318) and China (171). CAZRI, Rajasthan, NIPER, Mohali, CFTRI, IMTECH, NIIST, NCL and University of Delhi are some of the major organisations publishing research output on phytases, which shows there are large opportunities for R&D.

Sugar syrups from starch

During the 19th century, boiling starch with strong acids like sulfuric acid produced sugar syrups and in the 20th century, enzymes were rapidly supplanting the use of strong acids which has many advantages. In the 1970's, table sugar was developed known as High Fructose Corn Syrup (HFCS). The use of enzymes with greater specificity and mild use-conditions emerged as the production method of choice. Today large quantities of corn (maize) and other botanical starches to this and other useful sweeteners are used in soft drinks, candies, baking, jams and jellies and many other foods.

Dairy applications

Rennet, an enzyme mixture from the stomach of calves and other ruminant mammals, is a critical element in cheese making, which facilitates the separation of the curd (cheese) from the whey. Chymosin, is produced through microbial route from genetically modified microorganisms contain the gene for calf chymosin and commercially available today.

Publication analysis: There were 408 publications with key word chymosin or rennin during 2002-2011. The maximum number of publications were reported in 2010 (64) and in 2011 (53) only. US (1st) with 58 articles, Italy (36) and India (15th) position with 11 publications from National Diary Institute and Kurukshetra University.

Cheese flavours

The lipases contribute to the distinctive flavour development during the ripening stage of production, that acts on the butterfat in cheese to produce flavours that are characteristic of different types of cheese. Specific lipases are responsible for the flavours in cheeses ranging from the piquant flavour typical of Romano and provolone cheeses to the distinct flavours of blue and Roquefort cheeses.

Lactose-free dairy products

Lactase, an enzyme that occurs naturally in the intestinal tract of children and many adults (mostly absent) in sufficient quantity, that converts the milk sugar found in dairy products and glucose and galactose. People now enjoy these nutritious foods due to the digestive enzyme, lactase or labelled as "lactose-free".

Publication analysis: There were only 63 articles listed by Scopus search during 2002-2011 and in 2002 (12). India had 4 articles, each 2 in 2006 and 2008 are from Punjab University, Chandigarh.

Baking applications

Modern bread production uses chemical oxidants such as bromates, azodicarbonamide and ascorbic acid to strengthen gluten. Potassium bromate is used for improving flour quality. Bromate has been used to bake bread of a consistently high quality with a high consumer acceptance, but however it has been abandoned in many countries now. Glucose oxidase have been used to replace the unique effect of bromate.

Publication analysis: A search in Scopus on glucose oxidase retrieved 768 publications in 2011 and 707 (2010) wherein 1188 (US 1st), 244 (India 6th) after China, Japan, France and Germany. Major institutions involved are National Physical Laboratory, University of Delhi, University of Madras, Jamia Hamdard University and Dr Babasaheb Ambedkar Marathwada University.

Softer Bread Products

Enzymes are used to modify the starch, keep the bread softer for a longer period. The staling of white bread is considered to be related to a change in the starch. Over time, the moisture in the starch becomes unbound when starch granules revert from a soluble to an insoluble form. When the starch can no longer "hold" water, it loses its flexibility and the bread becomes hard and brittle. This results in a subsequent reduction in taste appeal of the bread and it is termed "stale." By choosing the right enzyme, the starch can be modified during baking to retard staling. The bread stays soft and flavourful for a longer time: 3-6 days.

Low calorie beer

Calorie-conscious consumers can enjoy reduced calorie beer due to the use of special enzymes in the brewing process. The grains like rice, barley are essential components in the conversion of carbohydrates to alcohol during yeast fermentation. By using enzymes to transform the complex carbohydrates to simpler sugars, the desired alcohol content can be achieved with a smaller amount of added grain. This results in a beer with fewer carbohydrate calories and ultimately, a lower calorie beer.

Clear fruit juice

Juices extracted from ripe fruit contain a significant amount of pectin that imparts a cloudy appearance. Pectinases are naturally occurring enzymes that act on pectin yielding a crystal clear juice with the appearance, stability, mouth-feel, taste, and texture characteristics preferred by consumers.

Publication analysis: Total number of publications on pectinases during the last ten years was 366 and 65 from India (1st), 62 (China), 35 (US) and rest are from other countries. Kurukshetra University, IIT-Delhi, CFTRI, Mysore are the major laboratories involved in pectinase research.

Other food applications

Meat Tenderizing

Some cuts of meat are tenderer than others and responsible by the myofibrillar proteins and the connective tissue proteins. Protease enzymes are used like papain and bromelain to tenderize tougher cuts of meat for many years. This can be a difficult process to control since there is fine line between tender and mushy. To improve this process, more specific proteases have also been introduced to make the tenderizing process more robust[5, 16].

Publication analysis: There are more than 70,000 SCI journal publications on "protease" or "proteinase", wherein there is a gradual decrease after 2006 (7556), 2009 (7054) and in 2011 (6764). Mostly from US (nearly 25,000) followed by Japan (nearly 6,000) and India (10th) with nearly 2000 publications from NCL, Pune, University of Delhi, IISc Bangalore, Banaras Hindu University, Varanasi, CFTRI Mysore. There are nearly 700 publications for alkaline proteases, wherein India (1st) with 200, China (90 - 2nd) and US (65 – 3rd). Saurashtra University ranks first in the number of publication on alkaline protease with 16 publications in 10 years time where as IICT and Andhra University published 9 and 8 publications during this period.

Confectioneries

Soft candy, soft cookies and other treats made with sugar have short shelf life because the sugar sucrose contained in the product begins to crystallize soon after the confection is produced. An enzyme, invertase, converts the sucrose to two simple sugars, glucose and fructose and thus prevents the formation of sugar crystals that otherwise would severely shorten the shelf life of the product or make some product virtually unavailable at reasonable prices.

Enzymes for biotransformation

Biotransformation is referred to the enzymatic catalysis to produce chiral molecules which can be used as building blocks in synthesis of many of specialty molecules such as drug intermediates, agro chemicals, flavour and perfume ingredients and application chemicals. The enzymatic catalyst can thereby consist of whole cells, cellular extracts, or isolated enzymes. The biocatalytic process starts with catalyst manufacture by fermentation, then the biomass is used directly or is harvested and formulated as whole-cell catalyst. The enzyme is separated from other cell constituents in a down-stream process and formulated. The synthetic reaction is carried out using enzyme as a catalyst at optimized physical parameters (pH, temperature, time, etc.). At the end of the biocatalytic reaction, the product is isolated and removal of all biologic material is important[17]. Applications of enzymes in chiral synthesis have been growing rapidly[18]. Commercial development of biotransformation-based chiral synthesis typically starts with screening of microbial sources. A platform technology would provide a set of enzymes with improved properties such as broad substrate range, high enantio-selectivity, and tolerance toward high substrate concentrations[17]. The enzymes available can be modified and screened for

Table 1—Chemical synthesis vs biocatalysis

Chemical Synthesis Methods	Bio-catalytic Methods
High cost of chiral metal catalyst	Low cost
Lower optical purity	Higher optical purity
Often use hazardous chemicals and reaction conditions, with concomitant environmental issues	Less hazardous reaction conditions, Fewer environmental issues

biotransformation of structurally different molecules. A key challenge will be the development of a high to medium throughput screening technology to screen thousands of alleles for the desired properties[19]. The main deliverable of the work is a set of alleles of commercially important enzymes with improved properties[20]. Enzymes and whole-cell biocatalysts have several attractive properties, which make them privileged catalysts for organic synthesis (Table 1). These are as follows:

1. They have high chemo-, regio-, and stereo-selectivities.
2. They require mild reaction conditions. Therefore, biocatalysis offers great chances and advantages for Successful applications (also in cases where either the substrates or the products of the reaction are chemically labile).
3. Biocatalysis is normally performed in an aqueous environment and also be conducted in solvent mixtures, liquid–liquid two-phase systems, and even in pure organic solvents which has only limited use of protecting groups.

Commercial Significance

The global chemistry market is estimated at 2292 billion US$ and is expected to grow to 3235 US$ by 2015 and 4000 billion US$ by 2020, by Chem Manager 2008 study. Of the 2292 billion US$, the global industrial biotechnology market is believed to represent only about 50 billion US$ (not including biofuels). Currently approx. 550 Billion US$ while Chiral chemicals industry estimated to be around 25 billion US$ by 2012 in which biocatalysis to account for more than 35% by 2013. Pharmaceutical industry accounts for more than 80% of the total chiral chemicals industry, followed by agrochemicals and other sectors. In 2011, all top 5 highest selling drugs in pharmaceutical sector were chiral viz. Plavix (Clopidogrel), Lipitor (Atorvastatin), Nexium, Fluticasone, Zocor. Four techniques are majorly used to manufacture chiral products, such as 1) chiral pool, 2) chiral separation, 3) asymmetric synthesis and 4) biocatalysis. Chiral pool starts the organic synthesis of a complex enantiopure chemical compound from a stock of readily available enantiopure substances.

Derivatization has been widely applied for manufacturing of many of the important chiral drug such as method of chiral resolution is used in the organic synthesis of the drug Duloxetine[21]. Asymmetric synthesis or stereo selective synthesis introduces chirality, which is important in the field of pharmaceuticals, expanding and emerging different possible reaction classes. Referenced examples include efforts to expand the available enzymes through recombinant technology and directed evolution[19]. There are complex drug compounds with multiple chiral centres and the biocatalytic step(s) associated with the development of each synthetic route, which are active compounds[22]. Biocatalysis increase in the production of high value specialty chemicals using either isolated enzymes which allows the ability to catalyze very selective reactions. For example Oxidoreductases contains 22 subclasses, out of which 5 to 6 subclasses show similar type of activity and all of them are used for production of single product. Each subclass demands different approach for reaction engineering[23]. Yeast alcohol dehydrogenase (YADH) was used for preparation of secondary alcohol from prochiral ketones.

Publication analysis

Alcohol Dehydrogenase

Alcohol dehydrogenases (ADH) are a group of dehydrogenase enzymes that occur in many organisms and facilitate the inter-conversion between alcohols and aldehydes or ketones with the reduction of nicotinamide adenine dinucleotide (NAD^+ to NADH) for which 6700 articles are published during the last ten years, where India accounts to 226 from University of Madras, Annamalai University, IISc and IIT-Bombay.

Laccase

The research publications on laccase are approx. 3000 articles in last ten years of which China is ahead with (407) and India (236). It shows a dramatic increase by China from 2004 onwards.

Mono-oxygenase

There are only 47 articles on mono-oxygenase during the last ten years of which India (1) from IIT-Madras in 2002. From US and UK there were 30 and 22 publications respectively during the last ten years.

Nitrilase

There were totally 315 articles published during 2002-2011 on nitrilase out of these US (61), Germany (45), China (40) and India (35). Out of these 35 articles from India, 14 are from NIPER, Mohali.

Hydantoinase

China (1st) with 24 publications on the topic hydantoinase during 2002-2011, while India (9th rank) with 5 publications after Spain, South Korea, US, Taiwan, Germany, South Africa and Brazil. After 2007 there is no publication with this keyword from India.

Platform technology

Due to the diversity of microorganisms, there is a need for establishment of enzyme bank that should work in harmony to convert the biocatalysis proof of concept to actual technology with the following:

1. Develop skills for high throughput screening technology to tap India's vast biodiversity
2. Characterization of genetic systems so as to enable directed enzyme evolution
3. Develop methods, catalysts and synthetic enzymes for screening of biocatalysts.
4. Use of whole cell biocatalysts and immobilization of cell surface.

Screening is carried out with the authentic reaction of interest under its operating conditions and reaction progress is monitored using analytical instruments such as high-performance liquid chromatography, gas chromatography, nuclear magnetic resonance, or mass spectrometry, or simply thin-layer chromatography.

Site directed evolution

Enzymes are increasingly being used as biocatalysts in the products of pharmaceutical and agrochemical building blocks to fine and bulk chemicals, chiral chemicals and, more recently, components of biofuels, subjected to improvement and optimization. Directed evolution involves repeated rounds of i) random gene library generation, ii) expression of genes in a suitable host and iii) screening of libraries of variant enzymes for the property of interest. Both *in vitro* screening–based methods and *in vivo* selection–based methods have been applied to the evolution of enzyme function and properties. Significant developments have occurred recently, particularly with respect to library design, screening methodology, applications in synthetic transformations and strategies for the generation of new enzyme function (Reetz *et al*., 2007).

Directed Evolution

Following experimental strategies and methodology can be applied during directed evolution.

1. To establish systems for cloning and controlled expression of recombinant proteins in *E. coli*.
2. To obtain a mutagenized library of a group of enzymes suggested in *E. coli*
   i. Determination of best conditions for Error Prone PCR for each enzyme sequence to be mutagenized.
   ii. Primer design, vector and *E. coli* strains selection.
   iii. Purification of template DNA from the best organism according to experimental design.
   iv. Error Prone PCR experiments and control testing.
   v. Cloning of mutagenized library into selected expression vector.
   vi. Transformation of mutagenized library into expression *E. coli* strain.
3. Screening of mutagenized libraries for novel or improved enzyme function.
   a. Optimization of expression conditions.
   b. Design of enzyme function high-throughtput screening methodology for library analysis.
4. To design and establish a system for cloning and controlled expression of recombinant proteins in *E. coli.*
5. Selection parameter for final biocatalyst depending on substrate range, stability in organic solvent, stability towards reaction conditions and thermo stability[24].
6. Cloning and over-expression
7. Formulation of enzyme such as CLEA, PCMC, immobilization.
8. Hurdles in scale up
9. Hurdles in Biotransformation and downstream processing

Strategy for biocatalysis hub

Rich microbial diversity of India will be explored for the bio prospecting of industrially important enzymes, (from wild isolates) with better catalytic efficiency, broader substrate profile and improved tolerance to higher temperature and organic solvents. It will also provide candidate strains from which one can construct recombinant strains for the industrially profitable production of enzymes and optically pure compounds, which will lead to the networking of the prominent academic institutions and industry.

Multidisciplinary approach

Biotransformation is a high risk activity and requires expertise in multidisciplinary areas of Science and Engineering. Networking between funding agencies, knowledge domains, SMEs and production houses is required. A program shall be developed based on the product market research and work shall be distributed to knowledge domains. For example, a program for molecules such as secondary and tertiary amines can be undertaken considering long term goals. No remarkable success has been achieved, even in abroad in this area. For molecules, produced in large quantities, independent program requires to be developed. Following schematic diagram shows multidisciplinary approach for development of biocatalysts.

Strategies and suggestions

There is a need for the complexity and potential of bio-transformation / biocatalysis by industry, public funded research institutions and funding agencies. Directed evolution of enzyme can be simplified by development of chemo-informatic tools. Self-sufficiency in the overall domain of biocatalysis & chiral molecules can be achieved by focused research in Interfacial areas that will make India a hub for high value, low volume products. A product based research program, including applications should be developed with the help of funding agency, knowledge domains, commercial companies and the collaborative effort with research groups to assist technology transfer for commercialization, research exchange program and developing strategic plan for India, where in National Institute can be a collaborator or partner. Identification of expertise in specialized knowledge domains and development of technology module for specific applications such as Universities, CROs, R &D Institutes will lead to expert domain for their application. These can be developed as cluster or centre of excellence.

Technological Contribution by CSIR-NIIST, Trivandrum

The NIIST Biotechnology has been engaged in industrial enzyme research since more than two decades and has evolved as a centre of excellence. It is considered as an outstanding centre in solid-state fermentation (SSF) by having more than 100 SCI papers. It is utilizing agro-industrial residues those available in the region as substrate for microbial cultivation[25-27], which brings socio-economic benefits like controlling the environmental pollution problems. Approx. 20 enzymes have been investigated by NIIST with the financial support from public (governmental agencies such as Department of Science and Technology, Department of Biotechnology, Council of Scientific and Industrial Research, etc) and from industries. These activities were also linked with several national (such as Madurai Kamaraj University- Madurai, Indian Institute of Technology-Kharagpur, Institute of Chemical Technology-Mumbai, etc) and international (such as Federal University of Parana- Brazil, Technical Universty Budapest – Hungary, Universite Blaise Pascal – France, University of Ulster – Northern Ireland UK, Institute of Biotechnology – La Plata, Argentina, etc) collaboration.

The scope of the R&D has been essentially based on development of data-based knowledge, then to protect the intellectual property right (IPR) by patenting the critical data (Table 2) and then to exploit these for industrial application, wherein is has been able to transfer three technologies to industries. The sixteen granted projects on microbial enzymes handled successfully by the division are listed in Table 3.

Table 2—Patents filed/approved

S. No	Title	Investigators	Number
1	A process for the preparation of phytase from jackfruit seed powder using *Aspergillus ficcum*	K, Madhavan, Nampoothiri and Ashok Pandey	Indian Patent Number 248793, Application Number, 1891 /DEL/2004, Issue Date : 2011-08-26
2	An improved bioprocess for the production of extracellular L-methionine aminopeptidase (L-MAP) from *Streptomyces gedanensis* under solid-state fermentation	K Madhavan Nampoothiri, Raji Rahulan & Ashok Pandey	Indian Patent, 0155NF (2010)
3	A novel process for the production of fungal spores in solid-state fermentation for enzyme	Ashok Pandey, P Selvakumar, L Ashakumary & AD Damodaran	Indian, IPA No 379/DEL/96,dated 23.2.96
4	An improved process for the preparation of glucoamylase enzyme	Ashok Pandey	Indian Patent 178612;IPA No 1041/DEL/91, dated 29.10.91

Table 3—Granted projects on microbial enzymes

S. No	Project Title	Duration
1	Solid-state fermentation for the production of glucoamylase	1992-1995
2	Microbial synthesis of Inulinase	1997-2000
3	Production of Industrial enzymes in solid- state fermentation	2000-2003
4	Phytase: Environmentally friendly feed enzyme	2002-2005
5	Substrate specific enzymes complexes in solid-state fermentation	2003-2006
6	Exploration and exploitation of microbial wealth of India	2003-2007
7	Development and application of food enzymes- Part 1- alpha amylase	2004-2007
8	Molecular cloning, overexpression and biochemical characterization of beta lactamases of *Mycobacterium tuberculosis*	2004-2006
9	Molecular studies on peptide deformylase and methionine aminopeptidase involved in the peptide processing of *M. tuberculosis* H37 Rv	2004-2008
10	Production of chitinase enzyme and its evaluation as biocontrol agent again the insect pest *Helicoverpa armigera*	2005-2006
11	Isolation and evaluation of microbial strains for the production of nitrilase, reductase and hydantoinase	2007-2009
12	Construction and screening of environmental DNA libraries for novel beta- lactamase inhibitors and lipases	2007-2011
13	Development of thermostable and low pH tolerant phytase from *Aspergillus niger* using site-directed mutagenesis	2007-2011
14	Exploration of India's rich biodiversity	2007-2012
15	Bioethanol from lignocellulosic biomass	2007-2012
16	Recombinant L-asparginase	2012-2015

Currently R&D is focussed on gene cloning, over-expression and enzyme engineering to impart desirable properties, developing microbial systems and other heterologus proteins. Some of the important genes recently identified and submitted in the NCBI include alpha amylase gene of *Bacillus amyloliquefaciens* ATCC 23842 (NCBI Accession no GU591658), Myo-inositol hexaphosphate phosphohydrolyase (phyA) gene, complete cds from *Aspergillus niger* strain (JN1964), *Escherichia coli* strain K-12 acid phosphatase (appA) gene, complete cds (JF274478), L-asparaginase (iaaA) gene, complete cds from *Enterobacter* sp (JF280914) etc. Some of the ongoing programmes on enzymes include the cloning and over-expression of the therapeutically important asparginase, engineering a thermo-tolerant phytase[15], production of substrate specific aminopeptidases, production and application of chitinolytic enzymes[28-30], metagenomic approach for thermotolerant and solvent-stable lipases and esterases[13,31,27], etc. Cellulases and glucose tolerant betaglucosidases are of prime importance as part of the Centre for Biofuels program of the Division. Construction of a fungal expression system for heterologus expression of these enzymes is currently in progress. Other interest includes enzyme inhibitors of therapeutic value such as inhibitors of beta lactamases, aminopeptidases, etc., where a dozen doctoral students have worked on various enzymes such as amylase, lipase, chitinase, inulinase, aminopeptidases, xylanase, pectinase, galactosidases, etc and obtained Ph D.

Future perspectives

Enzymes contributed to more environmentally adapted clean and green technology due to their biodegradable nature and replace harsh chemicals. Future trend may be the development of more effective systems that use much smaller quantities of chemicals, less water, and less energy to attain maximum product yield and performance. New enzymes through modern biotechnology will lead to enzyme products with improved effects at diverse physiological condition such as low/high pH and temperatures, which may allow various industrial processes to carry out at low temperatures; less harm to the environment; greater efficiency; lower costs; lower energy consumption; and the enhancement of a product's properties. Enzyme immobilization is an area of active research where the recent advances in the design of immobilization support have enabled more precise control of enzyme immobilization[32].

Major observations

During the last four decades, enzyme applications has now become large and is continuously increasing with highly R&D oriented activity covering various scientific and technological issues. There are some enzymes which need rigorous R&D to explore commercially for which CSIR and universities are involved in basic research in enzymology applied R&D and process engineering activities have been lacking behind. Creation of multidisciplinary R&D groups involving industries to develop application oriented research on enzymes can lead to the desired goals. Networking and strengthening of capabilities available in existing institutions and universities should be given priority. Special efforts are needed to formulate favourable government policies to promote academic-industry interaction.

Suggestions

It is suggested that more focus on R & D expenditures and knowledge based innovation in industrial enzyme sector will give a boost to novel application of these enzymes. NIIST can collaborate to find out the vision of more techno-economic product for industries through the public-private partnerships in areas on product development and technology that are vital for national development—economically, scientifically or socially. It is necessary to make efforts for knowledge transfer by better coordination among the institutes, universities and corporates, which could have a preferential access to the intellectual property. It is suggested to:

- Consider industrial biotechnology as one of the preferred tools to achieving sustainable development
- Promote investment in more effective manufacturing systems
- Stimulate the demand for sustainable products from white biotechnology and encourage sustainable policies
- Provide incentives for a broad range of chemical industries (especially SMEs)
- Creation of a data bank covering information on enzyme production, application and R&D status in India and world.
- Strengthening of manpower training in bioprocess engineering and provide for equal career growth in scientific R&D, technology / engineering and management.
- Reorientation of scientific R&D with direct relevance to industrial activities or development potentials.

Conclusion

Industrial enzymes are playing a significant role in today's commercial status of biotechnology & will witness more and novel applications in India.

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