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Value-added enzymes: Production technologies and commercialization

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ABSTRACT

This review discuss about the industrial enzymes and their commercialization. Particular emphasis is placed on their novel applications apart from the conventional applications. Application of enzymes has always got an upper hand over to routine chemical conversion processes due to fewer loads of chemicals, faster conversion rates, and high reproducibility with safe and clean environment. Due to significant developments in modern genetic engineering and proteomics, an unprecedented growth has been seen in last three decades for the commercialization of industrial enzymes which in turn gave them the today's successful label of house hold commodities. However, there is still a phenomenal scope left for researchers in designing of new, cheap and efficient tailor-made enzymes having broad specificity and wide applications showing a good stability in stringent conditions. India has huge market of industrial enzymes and import 70% requirement from countries like USA, Canada and China. By increased indigenous production of enzymes, India can reduce the current rate of import, which would not only fetch the foreign exchange reserves savings but also will open new employment opportunities.

KEYWORDS

Industrial enzymes;
Fermentation;
Enzyme manufactures;
Enzyme applications;
Enzyme production;
Enzyme business.

INTRODUCTION

Enzymes are present in cells in minute amounts and capable of speeding up chemical reactions associated with life processes. Within a living cell, enzymes function in sequences of reactions necessary to perform normal cellular activities[1]. These enzymes can be isolated from cell in pure form by following a number of recovery steps. After isolation of enzymes, they can be used for different applications which depend upon the native property of the particular enzyme. They have been used since the dawn of mankind in cheese manufacturing and other applications such as alcoholic or acetic acid production via yeasts or bacteria. The first commercial application of isolated enzymes was in detergents in the year 1914. Their large-scale production started in 1960s after proven their proteinaceous nature in 1926[2,3].

Since then, industrial enzyme business is steadily growing due to regular improvisation in production technologies, engineering enzyme properties and their application in various domains related to human health, food and other applications[4-6]. Today, the increased demand of enzymes is being fulfilled by exploiting the microorganisms at large scale fermenters/reactors using submerged cultivations or tray reactors under solid state cultivation by exploiting the GRAS (Generally regarded as safe) microorganisms[7]. Usually the production microorganism or biocatalyst is genetically manipulated, mutated or adapted to enhance the desired metabolite yield, productivity while maintaining their native properties. The requirement of enzyme purification steps depends upon their applications. Enzymes produced at large scale are usually not purified and directly sold as concentrated liquids or granulated dry powder after their proper formulation. Enzymes used in special applications like diagnostics, health, and recombinant DNA technology or as a supplement in food/feed need to be highly purified prior to their application[1,4]. Isolated enzymes have found several applications in fine chemical industries for their bulk production. Recently enzymes got the limelight for their specific and effective applications in production of chirally pure amino acids, rare sugars, key antibiotics and other specialized applications[3,6].

They are also used in production of fructose or low calorific sweeteners and semi-synthetic penicillin(s)/cephalosporin(s) derivatives as well as several other important fine grade chemicals[1]. Enzymes now can be considered as a part of a rapidly growing biocatalyst industry which involves genetically optimized living cells and other chemical based recovery and purification steps. The rising interest in sustainable development and environment benign processes, implications of microbial enzymes in transformation processes has been preferred over the conventional chemical conversion process[8]. The former has multiple advantages in terms of less chemical load in environment, efficient, and diluting multiple downstream transformation attempts while maintaining the product yield and recovery[14,9].

Historical background of industrial enzymes

In 18th century, fermentative activity of microorganisms was discovered by the great French scientist Louis Pasteur. The term "enzyme" literally means "in yeast". The first clear-cut recognition of enzymes came in 1833 during alcohol precipitate of malt extract which contain a thermo labile substance, and starch into sugar and today known as-amylase. Finally, Sumner proved the protein nature of enzymes in 1926 after crystallization of urease enzyme from jack bean[3,10].

The use of rennin from calf stomach in cheese making is considered probably the first application of enzymes. Rennin is an aspartic protease which has the capability to coagulate milk protein and has been used for hundreds of years by cheese makers. In 1914, Rohm in Germany prepared the first commercial enzyme preparation. This trypsin enzyme isolated from animals degraded proteins and was used successfully as a detergent[8]. However, the real breakthrough of enzymes occurred with the introduction of microbial proteases into washing powders which revolutionized the commercialization of detergent based enzymes[1,11]. Danish Company, Novozyme firstly marketed the *Bacillus* protease in 1959. However, the major application of microbial enzymes was reported in food industry in 1960. Other enzymes which got full limelight at that time were -alpha amylases and glucoamylases, which convert starch with over 95%, into glucose. Starch industry became the second largest user of enzymes after detergent industry[8].

Presently the industrial enzyme companies sell enzymes for a wide variety of applications after formulating in a certain ratio of different enzymes making it a fine cocktail. The main application sectors include-detergents (37%), textiles (12%), starch (11%), baking (8%) and animal feed (6%)[1]. Even today, only a limited number of commercially enzymes are known. A lot of research attention is required to find out novel enzymes which have broad specificity with potent applications in harsh conditions. More than 75% of industrial enzymes, which have been commercialized by today, are hydrolases. Protein-degrading enzymes or so called proteases constitute about 40% of all enzyme sales alone. Proteases have shown new and different applications but their use in detergents still dominates the enzyme market. Today, more than 50 commercial industrial enzymes are available. This number is increasing steadily having the strong back of research developments and innovations towards the precisely application domains[3].

Enzyme production technologies: a brief assessment

Enzyme technology, a sub-field of bio-industrial technology, or white biotechnology, is the application of nature's toolset (microorganisms, lignocelluloses and raw nutrient commodities etc) for the production of enzymes. Due to continuous research efforts for improvement in fermentation technology and genetic engineering, microorganisms have opened new vistas of biotech products with enhanced productivity and substantial cost and time savings[4]. Enzyme production is mainly based on fermentation technology by growing microorganisms on cheaper carbon and nitrogen sources under different culture cultivation (solid surface or liquid medium) to get products of choice with a safe and clean environment[8]. Figure 1 shows the production methodologies for industrial enzymes. Microorganisms are generally considered as an asset to enzyme production industries and are cultivated in designed bioreactors under controlled feasible conditions to produce a variety of interesting compounds of importance[7]. In current vogue, the results of studying giant "microbial libraries" for microbial conversion of cheaper carbohydrates from the renewable plant biomass and other wastes into value-added products can serve as suitable and cost-effective raw materials for enzymes production[12]. Enzymes, derived from these (micro-) organisms, are applied to catalyze a conversion in order to generate the desired products. The desirable characteristics of industrial microorganisms depends upon their ability to ferment

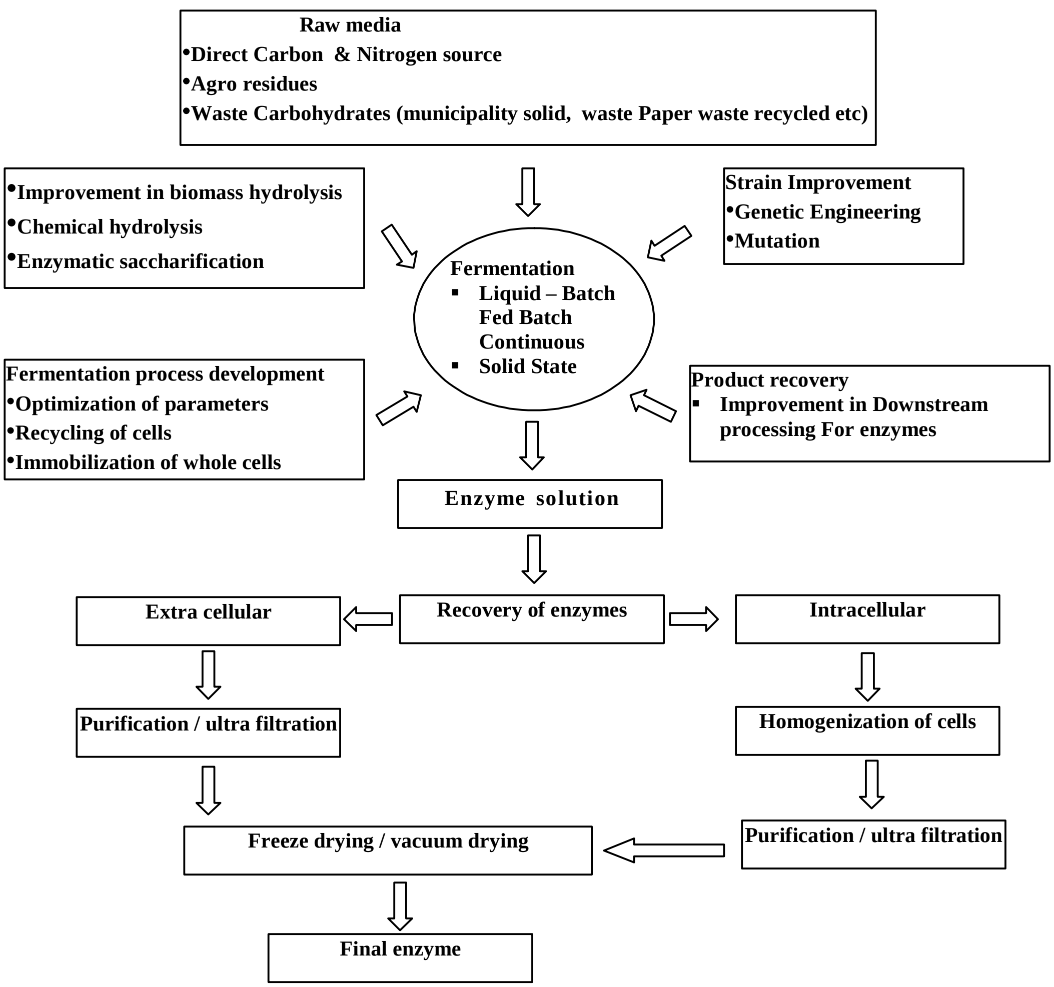

Figure 1: Schematic flow diagram for enzymes production with the future implications for the improvement in production methodologies

cheap raw materials with faster rates, requiring minimal amount of nitrogenous substances, providing high yields of preferred metabolite with less time and complications. Many molds (*Aspergillus*, *Penicillium*, *Mucor* and *Rhizopus*), and bacteria (*Bacillus sps*, *Escherichia coli*) excrete large quantities of enzymes into the surrounding medium or within cell itself[3].

Over the past five decades of research, continuous improvement in industrial biotechnology has been instrumental for the incumbent of new value-added diversified products. However, this time span has been a mix of success and failures. At industrial scale, both liquid and solid state fermentation methods have been explored and successfully implemented for many bioproducts[8]. In both the fermentations, maintenance of suitable physical parameters (temperature, pH, aeration, agitation etc) and other important factors viz. inoculum age, inoculum concentration, and maintenance of sterility are crucial to get the utmost yields and productivity of enzymes[1]. In general, liquid or submerged fermentation (batch, fed–batch and continuous culture) has been more pronouncedly used for enzyme production at industrial scale. Among all, fed-batch fermentation has been found most suitable and feasible for enzymes production at industrial level. The later is commonly employed for high biomass production within specific time interval and conditions. In fed-batch fermentation, the microorganism works at low substrate concentrations with an increasing biomass production which eventually will give high enzyme yield. In batch fermentation, the microorganism works at high substrate concentration initially and a high product concentration finally. Due to low productivities and high labor intensive, batch fermentation is generally not recommended at industrial operations. Continuous fermentations have not been explored much for the enzymes production due to more chances of contamination, and are used to analyze the critical production parameters. However, it is less labor intensive and offers ease of operation than batch operation[13].

Solid state fermentation is a state of art fermentation technology which has several advantages over to liquid fermentation in many ways - higher productivity, low cost media utilization, simplicity, and easy down stream processing. However, it has some disadvantages like-difficulties in scale up, and controlling of process parameters (pH, heat, moisture and nutrient concentration). Recent advancements in reactor designing and analysis have showed the way for successful implementation of SSF for industrial enzyme production[7]. Immobilized cell systems are now used industrially for production of different metabolites. There are several advantages of using immobilized cell system over to free cell system. First, a higher cell mass per unit fermentation volume can be achieved than with batch, continuous or cell recycle system, resulting in a corresponding increase in enzyme production. Second, reduction in down stream processing costs as there is no need for cell removal making the product extraction more economical. Third, maintaining a specific growth rate and dilution rate in continuous free cell systems is not a crucial factor in immobilized systems, thus flow rates can be optimized for best system kinetics. Finally, the risk of contamination is reduced due to fast dilution rates and high cell densities[14].

After fermentation, downstream processing is essential to obtain a final product from the fermentation broth. Down stream processing is the most important part and decides the economic viability of the product[8]. In general, a biological product is either extracellular (secreted into the environment), or intracellular (retain in the cell itself). The harvesting of intracellular product is quite complicated and cost effective in comparison to extracellular products. Some steps are additional in recovery of intracellular products like cell disruption, partial purification, and addition of more centrifugation steps[15]. Till now maximum numbers of enzymes are being produced by harnessing bacteria like *Bacillus* or fungi like *Aspergillus*. However, some enzymes are still extracted from animal or plant tissues. Plant derived commercial enzymes include proteolytic enzymes like papain, bromelain and ficin and some other specialty enzymes like lipoxygenase from legumes and tubers. Animal derived enzymes include proteases like pepsin and rennin[3]. TABLE 2 shows the plant and animal tissue derived enzymes with their respective applications. Most of the enzymes are, however, produced by microorganisms in submerged cultures in large reactors or solid state fermentation conditions. The enzyme production process can be divided into following phases: selection of an enzyme, selection of a production strain, construction of an overproducing strain by genetic en-

TABLE 1: Major enzymes manufacturers in India

Company name	Products	Revenue generated (Rs. millions)
Novozyme India, Bangalore	Designer cellulases, hemicellulases, detergent, food and feed enzymes, specialty enzymes.	1000
Biocon India, Bangalore	Brewing enzymes, detergent and textile enzymes, food and feed enzymes, pharmaceutical grade enzymes	910
Advanced Biochemicals, Ahemadabad	Alpha-acetolactate decarboxylase, alpha amylase, cellulase and alkaline protease	693
Rossari Biotech Ltd., Mumbai	Food, feed and paper starch based enzymes	660
Zytex India Private Limited, Mumbai	Starch degrading enzymes, food and feed specialty enzymes	NA
Fermenta Biotech Ltd, Kullu, Himachal Pradesh	Penicillin acylases, Fermase OX 1500, Fermase PA 1500 and enantio-selective enzymes	NA
Kopran Drugs Ltd, Mumbai	Penicillin acylases and other beta lactam antibiotic associated enzymes	NA
Dalas Biotech Ltd., Bhiwadi, Rajasthan	Penicillin acylases, glutaryl-7ACA acylase and D-amino acid oxidase	NA
Osten Enzymes, Chennai	Oil degrading and trans-esterification enzymes	NA
Celestial Labs Ltd, Hyderabad.*	Alpha amylase and alkaline protease	NA
Maps India Ltd., Ahemdabad	Palkoenzyme (alpha amylase) and palkobate (alkaline protease)	NA

#NA-Not Available, *Production not yet started, all the ground work is over with the technical know- how from Institute of Microbial Technology, Chandigarh, India.

gineering, optimization of culture medium and production conditions, optimization of recovery process (and purification if needed), formulation of a stable enzyme product for application point of view[4]. Criteria used in the selection of an industrial enzyme include specific reaction rate, pH and temperature and stability in presence of inhibitors and affinity with substrates.

For the selection of most appropriate microorganism for the production of desired metabolite at plant scale, several aspects have to be considered. First, the production strain should have a GRAS-status, which is important especially if the enzyme produced by the organism is used in food/feed processes. Second, the organism should have the capability to produce high amount of the desired enzyme titers taking reasonably less time. Third, the organism should be less contamination prone with a good stability at high volume reactors and lastly, organism should be able to utilize a broad range of carbohydrate and nitrogen sources[16]. Most of the industrial enzymes are produced by a relatively few microbial hosts like *Aspergillus*, *Trichoderma, Fusarium* and *Phanerochaete* fungi, *Streptomyces* and *Bacillus* bacteria. In general, yeasts are not good producers of extracellular enzymes and are not preferentially used for the commercial production of industrially important enzymes[2].

Modern recombinant DNA technologies have led the way for developing novel traits by successfully manipulation in genetic material of hosts with desired properties. After the successful genetic manipulation in organism to overproduce the desired products, the later is optimized for fermentation process which includes-media composition, cultivation type and process conditions for maximum production of metabolites. The various parameters viz. carbon and nitrogen source and concentration optimization, and physical factors (pH, buffering agents, mass transfer rates, agitation, aeration, temperature, foam control) under different culture cultivation conditions (batch, intermittent batch, fed-batch and continuous cultivation)[4,17].

Enzyme business in India and Indian enzyme manufactures

TABLE 1 shows the main enzymes manufacturers in India. In India, the major industrial enzyme manufacturers are- Novozymes India, Bangalore, Biocon India Ltd, Bangalore, Advanced Biochemicals, Ahemdabad and Maps India, Ahemdabad. Novozymes was the market leader in this segment during 2006-07 with total sales of Rs.1000 millions. Biocon and Advanced Biochemicals were the other major players with the sales of Rs.950 and 693 millions respectively. Novozymes, Advanced Enzymes Technologies and Zytex have registered over 20% growth in 2006-07 against the previous year. Rossari registered over 60% growth while

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TABLE 2: Some important industrial enzymes and their applications[3]

Enzyme derived	Enzymes	Source and origin	Industrial applications
Plant originated	Actindin	Kiwi fruit (E)*	Food and feed improvement
	Amylases	Malted barley and other frains (E)	Brewing, food and bakeries
	Bromelain	Pine apple latex (E)	Brewing and fruit juice processing
	Glucanase	Malted barley and other food grains(E)	Food, brewing and medicines
	Ficin	Fig latex (E)	Food and fruit pulp clarification
	Lipoxygenase	Soyabeans, green legumes and starchy tubers (I)**	Food, bread making and aroma generation
	Papain	Pawpaw latex and other latex yielding fruits (E)	Meat and food preparations
Animal originated	Catalase	Liver (I)	Food and medicines
	Chymotrypsin	Pancreas (E)	Leather and medicines
	Lipase	Pancreas (E)	Food, tanneries and medicines
	Rennet	Abomasum (E)	Cheese and medicines
	Trypsin	Abomasum (E)	Leather and pharmaceuticals
Fungal originated	Amylase	*Aspergillus, Trichoderma* (E)	Food, detergents and baking
	Penicillin amidase	*Aspergillus, Penicillium* (I)	Pharmaceutical and chiral compound synthesis
	Glucoamylase	*Aspergillus* (E)	Food, feed and baking
	Catalase	*Aspergillus* (I)	Food and biosensor development
	Cellulase	*Aspergillus, Trichoderma, Fusarium, Phaenerochaete* (E)	Food, detergents, and bioethanol production
	Dextranase	*Penicillium, Trichoderma* (E)	Food and confectionaries
	Glucose oxidase	*Aspergillus, Fusarium* (E)	Food and gluconic acid production
	Lipase	*Aspergillus* (E)	Food, detergent and biodiesel production
	Rennet	*Rhizopus* (E)	Drink and dairy industries
	Pectinase	*Aspergillus, Trichoderma* (E)	Drink and fruit juice clarification
	Alkaline protease	*Aspergillus, Trichoderma* (E)	Detergents and glass lens cleaning
	Raffinase	*Mortierella* (I)	Food and confectionaries
	Laccase	*Phenerochete, Cyathus, Pynoporous* (E)	Bioremediation, detoxification, pulp and paper and biosensor development
Yeasts originated	Invertase	*Saccharomyces* (E)/(I)	Sugar syrups and confectionaries
	Lactase	*Kluyveromyces* (E)/(I)	Dairy and food industries
	Lipase	*Candida* (E)	Food, textiles and medicines
	Raffinase	*Saccharomyces* (I)	Food and confectionaries
	D-amino acid oxidase	*Candida* (I)	Pharmaceuticals
Bacterial originated	Amylase	*Bacillus* (E)	Starch, detergent and baking
	Asparagine	*Bacillus* (E)	Health
	Glucose isomerase	*Bacillus* (E)	Sugar syrup and food industries
	Penicillin amidases	*Bacillus/E.coli* (E)	Pharmaceuticals (Semi synthetic antibiotic intermediates and chiral compound synthesis)
	Protease	*Bacillus* (E)	Detergents, food and in textiles
	Pullulanase	*Bacillus* (E)	Starch and food/feed synthesis

*Extracellular, ** Intracellular

Biocon registered 12% growth over the previous year's sales revenue[1].

Major enzymes and their industrial importance

TABLE 2 summarizes major large-scale enzyme applications[3,18]. Application of microbial enzymes in detergents was the first major break trough in application of enzymes. Bacterial proteases are still the most important detergent enzymes and predominantly cover the major bio-industrial business. In reality, a cocktail of enzymes comprising proteases, amylases, cellulases, lipases etc are mixed in a certain ration to formulate the final enzyme cocktail is added in detergents at a definite concentration. Other industrially important enzymes are alpha-amylases, lipases, tannases, penicillin acylases etc.

are also now being used in detergents. However, these enzymes have their own characteristics and are used for a specific application according to their native properties[3,6].

Amylases are used in primarily in starch based industries, baking and detergents to remove starch based stains. Amylases hydrolyse gelatinized starch into simpler monomeric constituents, which tends to stick on textile fibres and bind other stain components. Cellulase is actually an enzyme complex capable of degrading crystalline cellulose to glucose. Now they are indispensable for detergent applications also. In textile washing cellulases first loosens the cellulose/hemicellulose microfibrils and finally break down into simpler carbohydrates, which can be dissolved and gets removed during washing from cloths. Alkaline cellulases are produced by *Bacillus* strains and neutral and acidic cellulases by *Trichoderma*, *Aspergillus*, and *Humicola* fungi[8].

Starch breaking enzymes include a variety of microbial enzymes. Pullulanase is an additional de-branching enzyme, which act on starch polymer to yield glucose and other monomeric constituents. Alpha amylase is commercially produced from *Bacillus sp*. while beta amylase is commercially produced from barley grains and used for the production of the disaccharide maltose.

Enzymes have wide application in drinks and are used in fruit juice manufacturing. Fruit cell wall needs to be broken down to improve juice liberation. Addition of pectinase, xylanase and cellulase improve the recovery of the juice from the fruit pulp. Moreover, pectinases, amylases and laccases are now commercially applying in juice clarification and stabilization.

Brewing is an also important area for application of enzymes. Enzymes like-alpha amylases, beta glucanases, neutral proteinase, pectinases, papain etc. are integral part of brewing processes. These enzymes can be used to help in starch hydrolysis, solving out the filtration problems caused by beta-glucans and hydrolysed proteins present in malt, and eventually controls hazing during maturation and storage of alcoholic drinks. Lipase, an industrial enzyme is applied in fat processing industries, medicines and in biodiesel production. The most important lipase in the market was originally obtained from *Humicola lanuginose*. Now, it is successfully produced on large scale by cloned *Aspergillus* harboring the *Humicola* gene.

Oxidative enzymes are now directly being used to bleach textiles and paper pulp. Laccase-a polyphenol oxidase from white rot fungi has shown promising results in this field. Also they are now commercially being used in dye de-colorization, de-inking and for detoxification of sugar syrups, fruit juice stabilization etc. Xylanases along with laccases are now integral part of pulp and paper industries in making of fine quality paper.

Animal feed improvement by application of enzymes is a very potential and interesting area for application of enzymes. The first commercial success was addition of beta-glucanase and xylanase into grains based feed diets. Xylanases were found to be the most effective ones in this case particularly in cheek feed improvement. Usually a feed-enzyme preparation is a multienzyme cocktail containing glucanases, xylanases, proteases and amylases. Enzyme addition reduces viscosity, which increases absorption of nutrients, liberates nutrients either by hydrolysis of non-degradable fibres or by liberating nutrients blocked by these fibres, and reduces the amount of faeces.

Another type of important feed enzyme is phytase. Phytase is a phosphoesterase which liberates phosphate from phytic acid which is a common compound in plant based feed materials. This enzyme is a revolutionary addition in this aspect. The net effect is reduced phosphorous in faeces resulting in reduced environmental pollution.

Leather industry uses combined enzymes consisting of tannanolytic, proteolytic and lipolytic enzymes in leather processing. The use of these enzymes is primarily associated with the structure of animal skin for dehairing and de-wooling. The application of these enzymes results in a more environmentally benign process and eventually improves the quality of the leather by cleaning and softening the stronger leather surface.

Some novel enzyme applications

In addition to large volume enzyme applications, there are a large number of specialty applications for enzymes. These include use of enzymes in analytical applications, flavor production, protein modification, and personal care products, recombinant DNA-technology and in fine chemical production[6]. TABLE 3 summa-

TABLE 3: Some novel applications of enzymes

Application area	Enzyme	Mechanism of Action	Catalysed action
Analytical methodology	Peroxidases	Changing in NAD (P)/NAD(P)H proportions	Detection of released H_2O_2 after peroxidase catalysed reaction spectrophotometrically
	Alkaline phosphatases	Antigen-antibody complex	Detection of color forming reaction p-nitrophenyl phosphate or peroxidase
	H_2O_2 producing oxidative enzymes	Biosensors based mechanism, glucose oxidation catalysis	Detection of peroxide and oxygen consumption
Personal care products	Proteases and Lipases	H_2O_2 disinfectants of contact lens, Catalase degrades H_2O_2	Contact lens cleaning
	Glucose amylase (GA) and Glucose oxidase (GO)	GA liberates glucose from starch oligomers; GO converts glucose to gluconic acid	Toothpaste disinfection
Recombinant DNA technology	Restriction endo, exo nucleases, ligases, topoisomerases, gyrases etc.	Nucleic acid synthesis, degradation of nucleic acids	Gene cloning for making genetically manipulated microorganisms for enhanced enzyme titers
Fine Chemicals synthesis	Cellulases, xylanases, lipases, leucine dehydrogenases	Coordinated action of multienzymes and co-factor regeneration	Bioethanol, biodiesel, xylitol butanediol, and organic acids production
Chiral, pure amino acids and aspartame synthesis	Glucose isomerases, Penicillin acylases, Thermolysin proteolytic enzymes	Amide bond acylation, Racemization of N-protected aspartic acid and phenyl alanine methyl esters formation	Pharmaceuticals, agrochemicals, sweetner chiral compounds and D-phenylglycine production
Rare sugars	Glucose isomerases, cellulases, xylanases and hemicellulases	Isomerisation, cellulolytic, and xylanolytic reactions	Synthesis of low calorific sweetners, L-ribulose, L-ribose, D-xylulose, D-lyxose and 4-carbon sugars synthesis
Biodiesel, bioethanol production, alcohol separation and fatty acid production	Lipases, cellulases and hemicellulases	Transesterification, beta1-4, alpha 1-4 likage cleavage of holocellulose polymer from plants, enantiomeric nucleophile based catalytic reactions	Transesterification of fatty acids, biodiesel, fuel ethanol production, and separation of fatty acids
Asymmetric synthesis	Proteases, lipases, lyases, catalases, hydroxynitrilases	Enantioselective addition reactions, additions of double bond to some reactive groups and chiral based specific reactions	Formation of chiral compounds, alpha keto acid precursors and L-aspartic acid production
Oligosaccharide synthesis	Glucosyl transferases, glycosidases, sucrases	Coupling and deprotection of building blocks, polymerization and hydrolytic reaction	Oligosaccharide, dextran production, thermolysin synthesis for cosmetics, medicines and functional foods

rizes some novel applications of specialty enzymes.

Long term benefits from industrial enzymes production

Development of any indigenous product using cheap sources and with existing technologies and skilled professional is always beneficial in many aspects. Here we briefly summarize some important benefits achieved by developing industrial enzymes production and in turn fulfilling the current market demand.

- Safe and clean environment option over to chemical mediated conversion processes
- Cost effective and time savings with faster conversion rates and high reproducibility
- Increased employment opportunities
- Less dependency on foreign imported enzymes by increasing more indigenous production
- Saving of foreign exchange reserves
- Key role in future alternative fuels- bioethanol and biodiesel production
- Increased research opportunities and teaming up of biotechnologists, chemists, chemical engineers, computational professionals etc.

CONCLUSION

The application of modern techniques of genetics, proteomics and metabolic engineering is crucial for the production of novel and specific enzymes after exploiting the 'booster' microorganisms. The use of enzymes in place of routine chemical mediated processes is a green and sustainable alternative to convert them environmentally benign processes[5]. Extensive search for new enzyme variants in organisms that grow in extreme conditions (high temperature, high alkaline or acidic and salt concentrations) are of more interest due to their increased applications under harsh conditions. These features of enzymes will make more industrially friendly with broad applications. Other options like protein reengineering, construction of recombinant microorganism libraries may be helpful in insightful spatiality designing feasible for broad applications. Also, the use of genetic engineering in construction of novel and high enzyme yielding hosts withstanding against all odds are certainly the cutting edge advantages and will determine the future of enzyme research.

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