BIO-PROSPECTING APPROACH FOR BIOCATALYSTS FROM EXTREMOPHILES

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ABSTRACT

In the present research scenario the bioprospecting of biocatalysts from extremophiles has increased the market potential of enzymes. These enzymes are comparatively more stable and highly efficient under commercial conditions. Therefore the researchers are actively engaged in finding the most potent source for biocatalyst to satisfy the conditions with high efficacy. Enzyme technology has been adopted by a number of industries. A significant number of industrial enzymes are being produced using genetic and protein engineering tools. Moreover enzymes may be fairly imperfect than chemical catalyst when away from their natural environment; they are used to catalyze non natural processes on synthetic substrates under non-conventional conditions. The entire production scenario is gradually shifting from conventional chemical treatment method to biological means of production. The enzymatic conversion technology will play an magnificent role in future in place of biochemical process as enzymes achieve relatively high catalytic activities and high selectivity with low impact on environment some of which are reviewed in the present article.

KEY WORDS: Industrial Enzymes, Bio-catalyst, Biological Oxygen Demand, Extremophiles, Ecologically efficient process, Bioconversion.

INTRODUCTION

Biocatalysts are biological macro-molecules able to catalyze the most complex metabolic processes under the mildest environmental conditions. The nature has provided a number of key catalysts that are very specific and unique in their properties. Recently this ability of biocatalysts is being exploited for the commercial purposes (1, 2). Thus conducting a research on enzyme for the production of commercial requirement has great hope, which can be achieved and alleviated by enzyme isolated from extremophiles (3-5).

The main problem with enzyme is its solubility. Enzymes are readily soluble in aqueous solutions, after the completion of process, it is very difficult to recover enzyme from the solution. So there is immense requirement to preserve these enzyme molecules by using certain approaches like immobilization or they should be engineered before being used for catalyzing most of their practical application. (6-8) Protein engineering via genetic or chemical modification is an excellent approach to improve activity (9-11), by applying these means stability properties of industrial enzymes can be accomplished under high temperature, pH and pressure conditions (12). This unique property has been widely exploited for development of enzyme production for many industries. Enzymatic processes are ecologically efficient which has always been a first preference for all industries (13).

ENZYMES HAVE BROAD BASE FOR BIOTECHNOLOGICAL APPLICATIONS.

Enzymes can be applied for various industrial purposes but still several problems exist which affect the activity of enzyme (14). These setbacks can be put off by applying some biochemical approaches as described in table-1.

Biotechnological applications of Enzymes

Scientists have chosen to explore enzymes from thermophilic, alkalophilic and acidophilic, anaerobic bacteria, as these microorganisms are believed to be evolved under energy limited conditions on earth (15-17).

The enzyme technology is being used for many industrial purposes (18) like waste disposal, bio fuel, fruit juice preparation, paper industries, textile industry, leather industry, detergent and animal feed, etc.

ENZYME TECHNOLOGY FOR WASTE DISPOSAL

Total waste generated is primarily composed of paper products, food and yard wastes and a wide collection of organic by-products. Anaerobic processes are complex as consortia of microorganisms are responsible for the production of specific enzyme mixture necessary for effective breakdown of the polymeric substrates. The uncontrolled nature of landfill in environment results in variable populations of microorganisms. Controlled and aerobic digestion system may be used at a small scale to develop appropriate microbial consortia and to determine optimal nutrient levels and environmental parameters necessary for effective regeneration rates in large scale processing (20). Anaerobic digestion represents one of the possible technologies for Municipal Solid Waste (MSW) disposal that produce a useful energy product in a form of methane gas (biogas). The biogas product may be converted directly to electrical power as medium BTU (British thermal unit) fuel (in internal combustion engines) or may be upgraded by reducing the CO_2 content to produce synthetic natural gas (21). Applications of enzymes in municipal and cellulosic waste treatment are described in fig.1.

Figure.1. Application of enzymes in municipal and cellulosic waste treatment

Although the exact composition of MSW varies widely with respect to location season and time of day, MSW consists primarily of cellulose in the form of newspaper, wood and cardboard, food waste, and yard waste (22). The disposal of the waste through landfill is being a common practice for centuries. However landfills receiving MSW have been implicated in the contamination of groundwater, which occurs when liquids wastes or natural precipitation percolates through the waste into deep ground water. Organic acids generally accounts for 80-90% of the contamination. Landfills are estimated to contribute as much as 30-60×10^{-6} tons of methane per year to the atmosphere which is contributing to green house effect (23). Although the methane gas produced in landfills may be considered as an environmental pollution problem it has also been viewed as an opportunity for the recovery of significant supplemental energy (24). However the anaerobic digestion process in landfill sites is extremely slow and uncontrolled due to the nature of in-situ environment. Extracellular hydrolytic enzymes such as Cellulase and lipases have been shown to be effective in the host hydrolysis of anaerobic digester effluent solids (25) or pre-treatment of complex organic polymers before the digestion processes. When the Cellulases activity found in anaerobic digester, compared to few others hydrolytic environments, evidences seem to indicate that cellulose degrading enzymes in MSW fed digesters are operating under less then optimal enzyme titres (21, 25).

BIOFUEL

The increase in the price of petroleum feed stock has created opportunities for the development of combined biological and chemical processes that will produce liquid fuels and chemicals from alternate feed stocks such as biomass coal and gas. Near term opportunities exist in the bioconversion of cellulosic materials contained in urban, agriculture and forestry waste. Enzymatic conversion will play an ever-increasing role in future biochemical processes because enzymes achieve relatively high catalytic activities; high selectivity and they have a low impact on environment (23). Biopolymers of a carbohydrate and polyphenolic nature represent the largest reservoir of organic carbon fixed annually by plants. This shows that many segments of the annual biomass fuel easily exceed the total annual requirement for basic petrochemicals. Biomass feedstocks are highly oxygenated whereas fossil raw materials are mainly per hydrocarbons. All process for conversion of carbohydrate to hydrocarbon type materials suffer a penalty a high weight losses with the exception if short chain organic acids where a high weight yield are possible (22- 24). Conversion of biomass to ethanol is described in following schematic diagram (fig.2).

Figure.2. Schematic Diagram for the conversion of biomass to ethanol- as Biofule

The use of plant polymers biopolymers involved depolymerisation to oligomers and monomers followed by microbial and chemical conversion to liquid fuels and chemicals. The gaseous fuel which is the mixture of methane and CO_2 can be produced by fermentation of various biodegradable feed stock using consortia of anaerobic bacteria (27). The microbial conversions of sugars to lower alcohols, ketones, volatile fatty acids and acids of tricarboxylic acid cycle are very efficient because fermentative microorganisms can perform multiple enzymatic steps in very high yield and secretes the final product in the surrounding medium (23).

FRUIT JUICE INDUSTRY

Today the main objective of the fruit juice industry is to process fruits at lower cost while maintaining or improving the organoleptic qualities and stabilities to finished product (26). This ambitious objective has been made possible through the use of enzymes at different stages of the process as it is not possible to produce clear juice without enzymes the cell wall is the most important pert fruit is be broken down to make clear juice recovery easy(2, 29). So, the pectinases hemicelluloses celluloses, amylases are the important enzymes application of exogenous enzymes lead to degradation of the fruits or selective extraction of some of their components allowing creation of new types of finished products and fruit derivates. Exogenous enzymes are also tool of choice because it preserves colour and vitamins for the same time. The pectinases an and hemicelluloses are used, these are added to the mesh at 40-200 gm ton^{-1} of fruit for 30-60 minutes at 15^{0}C these enzymes decrease viscosity by pectin hydrolysis and improved press ability as the pectin gel collapses. Enzymes are also added to the juice after pressing as a depectization step pectinases hydrolyze the residual pectin and hemicelluloses leading to the clarification of the fruit by neutralization of the electrostatic charges between uronic acid proteins and tannins (32-34). The addition of fining agents and then filtration or ultra filtration, allow clarification of juice, which can be concentrated up to 70-72 Brix. The concentration of the juice increases the storage capacity and avoids microbial contamination. Pectinases, hemicelluloses and Cellulase are used o achieve almost complete liquefaction, yield release 95% as a result of solubilisation of cell wall polysaccharides (32, 33).

ANIMAL FEED

The animal feed industry is an extremely important part of the worlds agro industrial activities it is an industry that has gone through many changes in the past few years as consumers and industry itself have looked more closely than ever before into

1. How compound animal feed are produced?
2. What is the end result for the environment of the system of animal husbandry today?

In animal feed industry the microbial enzymes are used to improve the utilization of raw materials and to reduce the excretion of nitrogen, phosphorus and other nutrients into the environment. Thus the main application is that of providing enzymes capable of degrading N, S, and P found in cereals and vegetable proteins but amylases and proteases are used in diet for young animals. The various enzymes like alpha amylase protease papain, cellulase, and xylanase and beta glucanase are added to supplement diet. Enzymes are added to animal feeds either to degrade cellular fibre which has an ant nutritional effect or to supplement then animals own digestive enzymes. In the latter case supplementation with amylase and protease normally is recommended in the early stages of growth when pancreatic secretion of this enzymes due not fully match the digestive requirement thus the main enzyme application is that of providing enzymes capable of degrading N, P, S found in cereal and vegetable proteins but amylase and proteases are used in diet from young animals (35).

Enzyme must be capable of being resistant to the heat processing given to the feed s and also of transiting the digestive system intact to the point of action. Each enzyme has its intrinsic thermal stability (36). In feed the temperature routinely reaches 85^{0}C and where expanders are introduced the peak can be up to 130^{0}C. In practical feed mill conditions it is the addition of steam in pelleting which are responsible the loss of enzyme activity. if dry heat is used then all feed enzyme will be much more stable and can tolerate the temperature of 90^{0} C for up to 30 minutes without significant loss of activity when steam is applied and condenses on feed partials there is an inactivation of enzymes unless they are protected by being absorbed onto a carrier of more preferably coated with steam resistant material that hinders the excess of steam to the enzyme product is thus a combination of the intrinsic thermal ability to the enzyme and the system of

protection used. Thermal resistance is therefore one of the most important factor of governing the application of enzymes and animal feed. The realization that enzyme without protection are extremely unstable in fed processing is one of the major reason why feed enzyme today are given serious through the use of extremozyme from extremophiles microorganisms (37).
In diets producing without heat treatment the intrinsic phytase can improve phosphorous digestibility but as the level of phytase vary and enzyme is thermo labile so the phytase from thermophilic microbe can be added to feed to supplement then endogenous phytase activity then this enzyme application could become more widespread in the future(36, 38).

PAPER PULP

Growing consumer awareness and preferences toward the environmentally friendly and safe products are safe processes forged many industries to adopt environmentally friendly techniques in spite of high cost. One among such industries is pulp and paper industries (38). This growth has been fuelled by several factors, including

1. An improved understanding of the interactions between enzymes and the constituents of the pulp and paper processing.
2. An increased need for the industry to adapt environmentally benign technology
3. Development of cost effective production technology or the relevant enzymes.

The pulp and paper industry processes wood and other cellulose containing fibre. Typically a pulp is made by processing of raw fee stocks, while paper is made by combining one or more types of pulp with various non pulp additives designed to achieve the desires properties such as smoothness, opacity and colour (40-42).
Enzymes are used to aids as pulp and pare manufacturing by modifying the following types of substrates

1. The cellulosic fibre.
2. Non cellulosic constituents of the fibre, such as hemicelluloses and lipid.
3. Paper additives and contaminates such as starch, pitch and slime.

Generalized flow sheet for three major pulp production processes are shown in table 2. The enzymatic processes in the pulp and pare industry include enzymatic darkening, modification of fibre properties and bipbleaching and the deinking of the recycled pulp. The diversity of lignocellulolytic microorganism serves as a rich source of different enzymes for applications in the pulp and paper industries are summarized in Table.2
Table 2
Three major pulp production processes
One process parameter that is if primary interest in mechanical pulps and secondary fibre processing is the rate of drainage of water from the pulp. This is important because the subsequent rate of water removal on the paper machine is often rate limiting in mill production (43). The presence of fine particles in the pulp markedly decreases the rate of draining. Enzymes are used to increase the drainage of mechanical pulp and secondary fibre.

Mechanical pulps and secondary fibre processing is described in fig 3.
Figure.3. Flow diagram for mechanical pulps and secondary fibre processing
Enzymes can be use to eliminate some of the chlorine based compounds with little capital investments and are thus attractive to the industry. The research over 3 decades focussed on the ligninase enzyme

to remove lignin from the pulp and xylanase enzyme which act the xylan on the pulp the use of lignase has not been suitable for mill application because it requires several days reaction time xylanase enzyme technology has been more accepted by the industry (41- 45).Application of enzyme treatment in pulp bleaching in paper industry is described in fig.4.

Figure.4. Enzyme treatment in pulp bleaching in paper industry

Fig.4 shows that process streams at the point of enzyme treatment in a craft mill. The unbleached pulp (brown stock). Although the pulp is well washed it has sufficient residual alkali soda that its pH is 9-12 and the temperature is about 60-70^{0}C. Here we have to have enzymes, which can sustain in these harsh conditions so the extremophiles can be the best sources. The enzyme treated pulp then proceed as normal through to bleach plant, except with the decreased requirement for bleaching chemicals and this also requires changes to be made in to bleach plant control strategies (43).

TEXTILE INDUSTRY

With increasing requirement to reduce the use of chemicals in textile industry due to their high stability and very harmful nature against environment the exercise of enzymes in textile processing is rapidly mounting. By the recognition of non-toxic properties and eco friendly nature the use of enzymes is rapidly taken up by industry. Some main enzymes used in textile industry are presented in fig.5.

Figure.5. some enzymes used in textile industry

Some other advantages are more specificity and less energy requirement make the process fast and cheaper then chemicals used conventionally. Enzymes are been used in textile industry for the process such as de-sizing, scouring, bleaching, dying, and finishing in other hand the use of chemical substrates is more costly in terms of energy consumption and often harmful to environment and cause many problems.

LEATHER INDUSTRY

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. These chemicals completely dissolve the hair and open up the fiber structure.

With enzyme-assisted de-hairing, it is possible to reduce the chemical requirements and obtain a cleaner product and a higher area yield with fewer chemicals in the wastewater. Since the enzyme does not dissolve the hair as the chemicals do, it is possible to filter out the hair, thus reducing the chemical and biological oxygen demand of the wastewater. Additionally the Skins contain proteins and fat between the collagen fibres that must be all or partially removed before the skins can be tanned. To make the leather pliable, it is necessary to subject the skin to an enzymatic treatment before tanning to selectively dissolve certain protein components. This is called bating (46).

Traditionally, the degreasing (removal of fat) 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 to the leather industry. The enzymatic degreasing process replaces the solvent-based process. Since the enzyme interferes less with the skin structure, the enzymatic process also results in a product with improved quality, for example improved tear strength and more uniform colour. Use of enzymes in leather processing shown in fig.6.

This process has many benefits like it replaces solvent-based system, lowers volatile organic chemical load. And higher quality leather – the improved tear strength should be very meaningful to anyone with leather furniture.

Figure.6. Application of enzymes in leather processing

DETERGENT INDUSTRY

Enzymes are very important components of detergent since last four decades. They are still in process of development with progress of biotechnology. Enzymes such as protease, amylase, lipase, and cellulose are frequently used as additive components of detergent to remove dirt caused due to protein, starch and lipids. Cellulose improves the quality of fabric by removing surface microfibrills, with the conservation of appearance and colour. Detergents used in textile industry must be very mild for the fabric so they require some less harsh additives for cleaning. The main enzymes been used in detergent industry are illustrated in fig.7.

Figure.7. Application of enzymes in detergent industry

Enzymes work more efficiently than any chemical catalyst even when present in very small quantity. They significantly decrease the amount of surfactants used in detergent with maintaining its efficiency.

Another advantage of using enzymes instead of chemicals is the reduced consumption of water. Often when chemical used to wash clothing require much more quantity of water because of repeated rinsing but with use of enzymes usually the single rinse is enough to clean completely (47).

CONCLUSION

These are only few examples from the number of uses of enzymes in industry they are such efficient tools provided by nature that can be applied to perform industrial process with more cleanly and eco-friendly way. Current status of the use of enzymes is still requiring paying more attention so that more safe and neat processes can be developed to maintain and enhance the quality of life and natural resources.

Over the last 20 years, many doubts with respect to bio-catalysis have been expressed, challenging that:

(i) Enzymes only feature limited substrate specificity;
(ii) There is only limited availability of enzymes;
(iii) Only a limited number of enzymes exist;
(iv) Protein catalyst stability is limited;
(v) Enzyme reactions are saddled with limited space–time yield; and
(vi) Enzymes require complicated co-substrates such as cofactors.

Biotechnology and bio-catalysis differ from conventional processes not only by featuring a different type of catalyst; they also constitute a new technology base. The *raw materials base* of a biological process is built on sugar, lignin, or animal or plant wastes; in biotechnology, unit operations such as membrane processes, chromatography, or bio-catalysis are very common, and the product range of biotechnological processes often takes in chiral molecules or biopolymers such as proteins, nucleic acids or carbohydrates.

Cost and margin pressure from less expensive competitors and operation with emphasis on a clean (or less polluted) environment are two major developments.

Fewer processing steps, with higher yields at each step, lower material and energy costs, and less waste are the goals. Biotechnology and biocatalysis often offer unique technology options and solutions, they act as *enabling technologies*; in other cases, biocatalysis has to outperform competing technologies to gain access. Extremozymes can play a great role to achieve all these goals.

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Table 1 Biotechnological applications of Enzymes

Applications	Current problem	Approaches
Starch processing, Brewing and fermentation, Backing and food processing, Forest products, Detergent, Waste treatment, Bioleaching, Drugs.	Limited uses, Unstable biocatalysts, Low efficiency and product yield, High cost and safety	Increase thermo stability and environmental capability, Increased specificity and activity, Develop thermophilic enzymes, Screen for new organisms, Clone and over express in industrial host, Site directed mutagenesis

Table 2 Three major pulp production processes

Operation	Mechanical pulp	Recovery pulp	Chemical
Feed stock	Virgin fibre	Secondary pulp	Virgin fibre
Handling	Cleaning chip sizing	Shredding	Cleaning chip sizing
Pulping	Mechanical action on fibre (Which improves strength of pulp)	Repulp	alkali craft or acid sulphate
Refining & dewatering	Integrated closely with pulping (Enzyme are used for dewatering)	Often required	usually after bleaching
Bleaching	Mild with hydrogen peroxides	Mild	severe with strong Oxidants and
Paper making	News paper product At lower cost	Blend with mechanical pulp	fine performance

Figure.1. Application of enzymes in municipal and cellulosic waste treatment

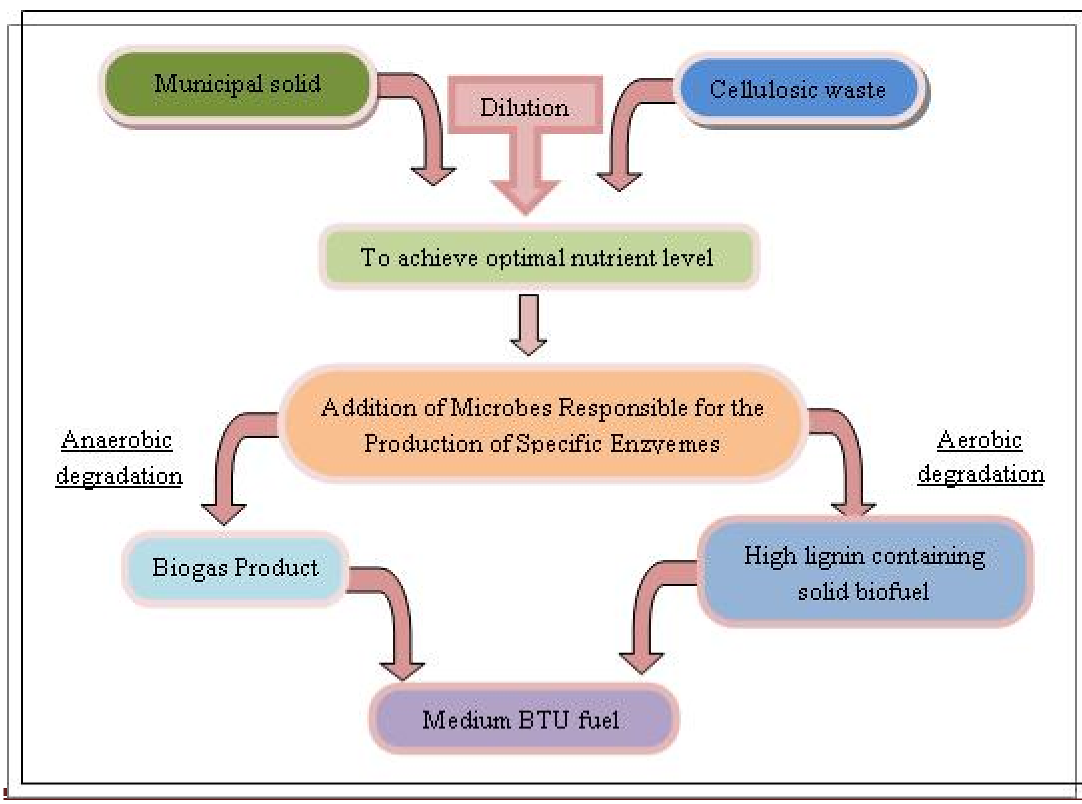

Figure.2. Schematic Diagram for the conversion of biomass to ethanol- as Biofulel

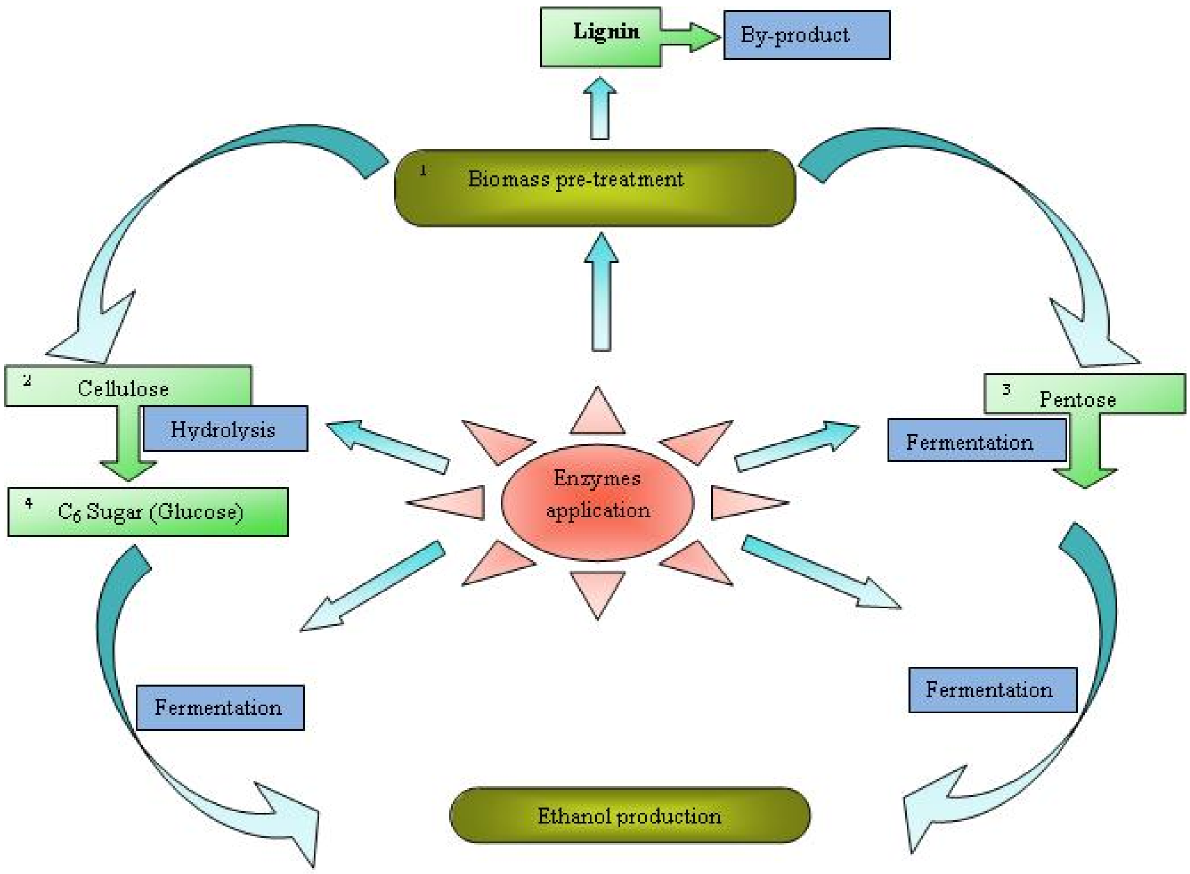

Figure.3. Flow diagram for mechanical pulps and secondary fibre processing

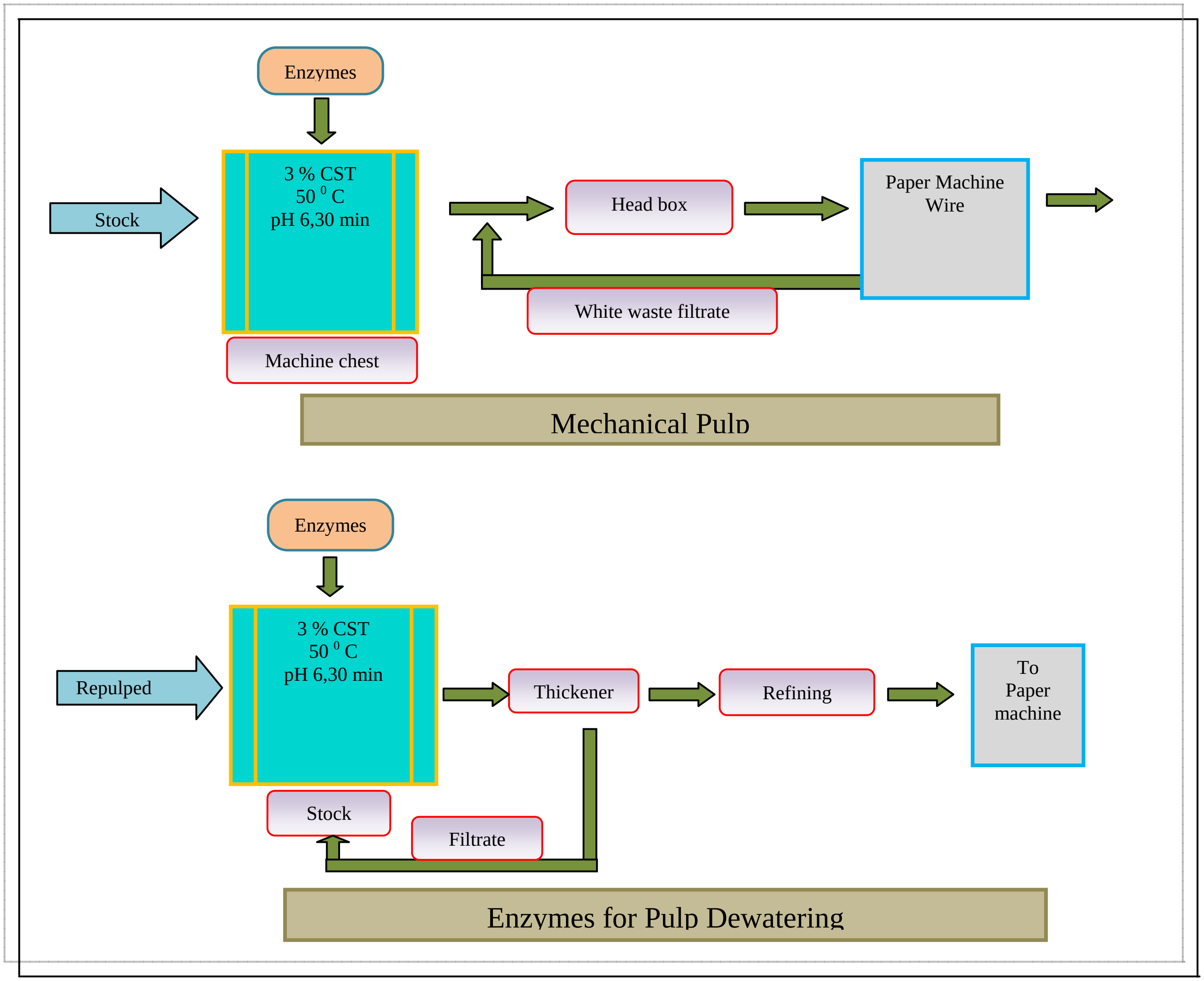

Figure.4. Enzyme treatment in pulp bleaching in paper industry

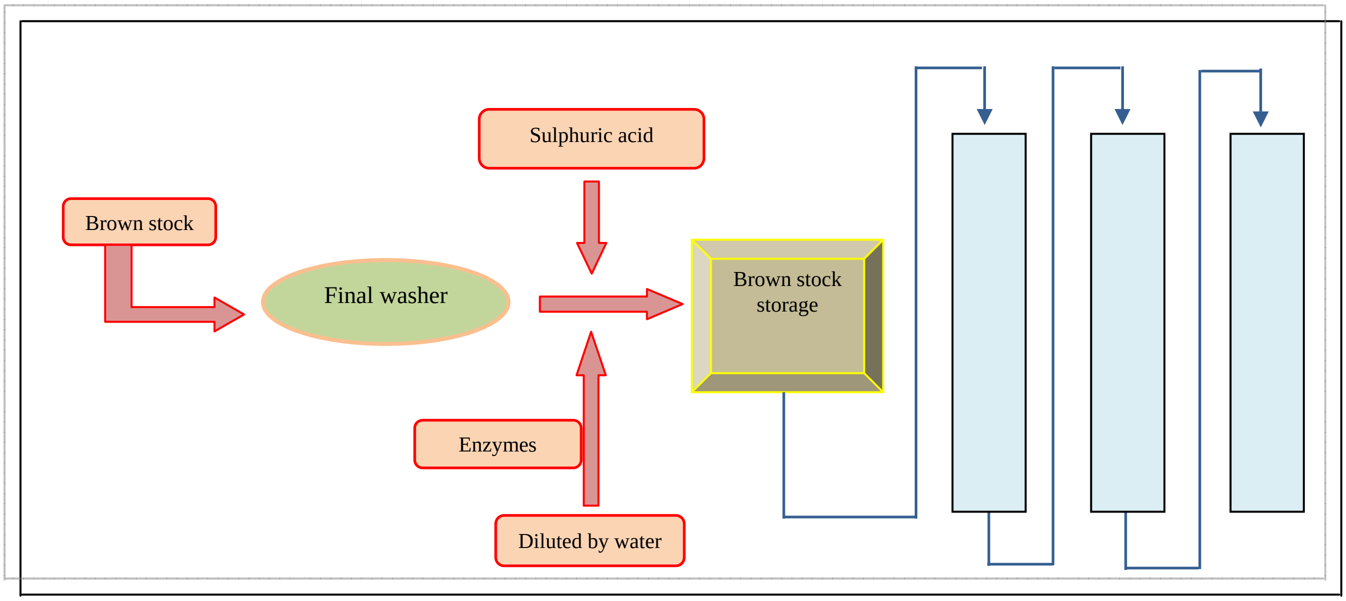

Figure.5. some enzymes used in textile industry

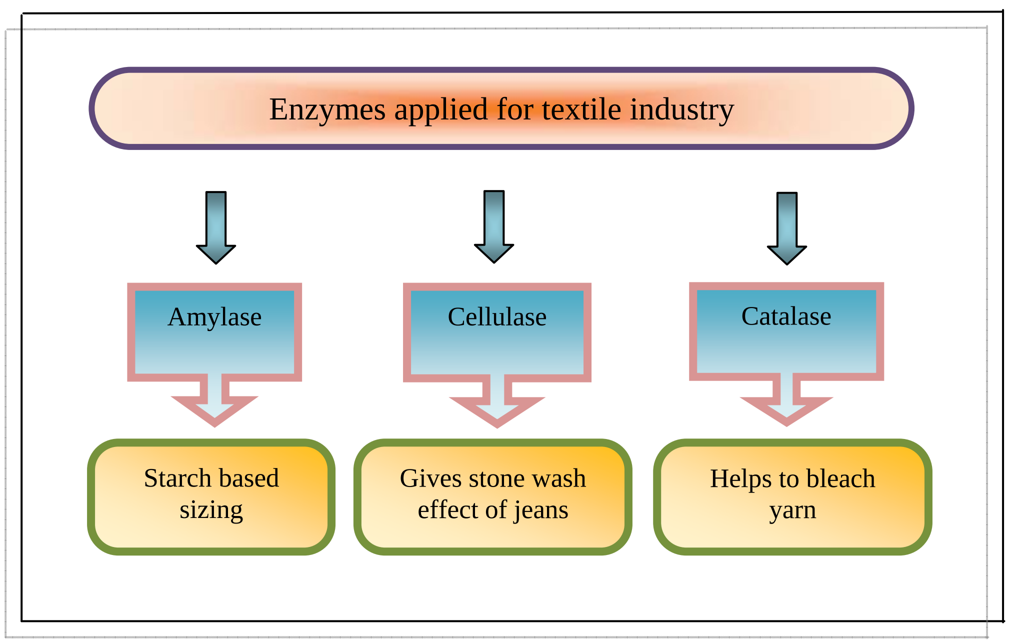

Figure.6. Application of enzymes in leather processing

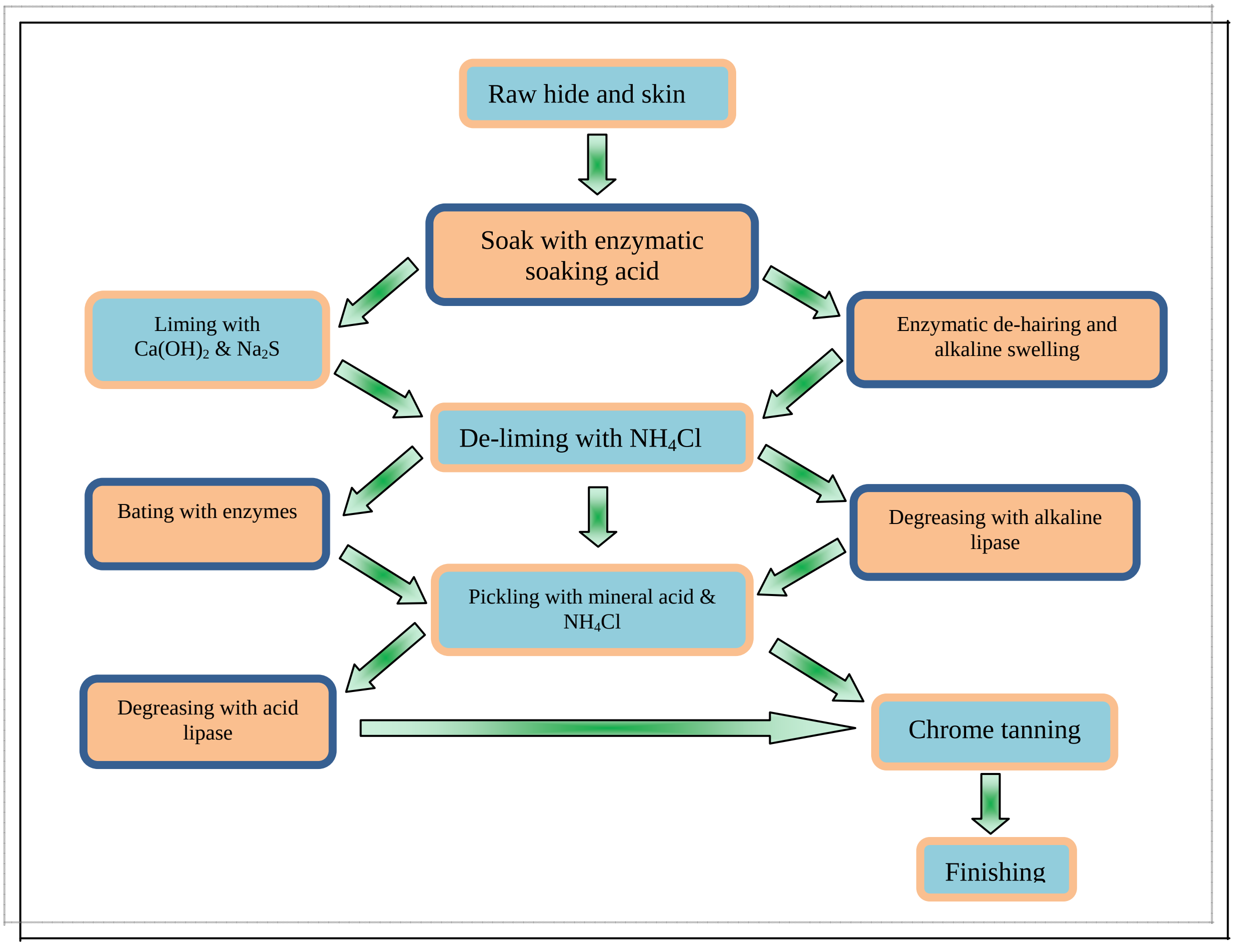

Figure.7. Application of enzymes in detergent industry