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REVIEW ARTICLE

Proteases: The Industrial Biocatalyst

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Abstract

Enzymes are the biocatalysts that have become obligatory for industrial processes now-a-days. Proteases are one such class of enzyme that accounts for the largest sale (65%) of the total worldwide enzyme sale. These occur ubiquitously in all life forms viz. plants, animals and microbes but microbes are better sources from industrial point of view. Proteases are exploited in a wide range of industries like food processing, leather, brewing, silk manufacturing, photography, laundry, personal care and pharmaceutical industry. The current review describes about the various applications of proteases in these industries.

Keywords

Brewing, Enzymes, Fishery, Food Processing, Laundry, Leather, Pharmaceutical Industry, Silver Recovery

Introduction

Enzymes are biocatalysts, and by their mere presence, and without being used in the reaction, can speed up chemical processes that would otherwise run very slowly. Enzymes have become indispensable biocatalysts these days due to need for eco- friendly approach to industrial processes. Because of their high specificity in bringing about chemical changes and the minute amounts in which they are effective, emphasis is being laid down on searching newer sources of novel enzymes. Proteases represent one such class of enzymes which occupy a key position taking into account their physiological and commercial applications (Gupta *et al*, 2002).

Proteases are physiologically obligatory for living organisms and occur universally in a wide variety of plants, animals and microbes (Yandri *et al*, 2008). Although such wide variety of living systems produces proteases, generally microbial sources are preferred because of the limited space required for their cultivation, broad biochemical diversity, rapid growth and ease of genetic manipulation to generate enzymes for various applications (Kumar *et al*, 2008).

Since the advent of the study of enzymes, microbial proteases have been studied the most amongst other hydrolytic enzymes. Scientists are researching extensively on proteolytic enzymes because they hold a key position in metabolic processes of a cell and find a large array of applications in various industries (Gupta *et al*, 2002).

Among the three largest groups of industrial enzymes viz., lipases, amylases, and proteases, proteases alone accounts for 65% (Aqel, 2012) of the total worldwide sale of enzymes that has increased from 59% reported by Rao *et al* in 1998 (Fig.1). Proteases are exploited in detergent industry as an ingredient to remove protein stains (Adinarayana *et al*, 2003; Balakrishnan *et al*, 2012); leather industry for dehairing of skins and hides (Kumar and Takagi, 1999; Sharmin and Rahman, 2007; Zambare *et al*, 2007; Abidi *et al*, 2008); silk industry for degumming; food industry for baking, remove bitterness of protein hydrolysates, and manufacture of soy products; removal of protein from shrimp and crab-shell waste (Yanga *et al*, 2000) pharmaceutical industry and bioremediation process (Dube *et al*, 2001; Pastor *et al*, 2001; Gupta *et al*, 2002; Gerze *et al*, 2005; Kasana and Yadav, 2007). Among all types of proteases, alkaline proteases find maximum use in industries (Fig.2). Many researchers have reported the applications of alkaline proteases in hydrolysis of fibrous proteins of horn, feather and hair (Anwar and Saleemuddin, 1998; Giongo *et al*, 2007). Alkaline protease can also be used for synthesis of peptides, resolution of the racemic mixture of amino acids and recovery of silver from X-ray films by hydrolysis of gelatin (George *et al*, 1995; Singh *et al*, 1999; Pastor *et al*, 2001; Gupta *et al*, 2002; Pathak and Deshmukh, 2012). In pharmaceutical industry, proteases are used as ingredients of ointments for debridement of wards and in medicine preparation (Sjodahl *et al*, 2002; Najafi *et al*, 2005).

Though mesophilic proteases have large applications, thermostable proteases are advantageous in some applications where higher processing temperatures are employed. Moreover, use of thermostable proteases results

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in faster reaction rates, increase in the solubility of non-gaseous reactant and products, and reduced incidence of microbial contamination by mesophilic organisms (Nascimento and Martins, 2004; Gomes and Steiner, 2004)

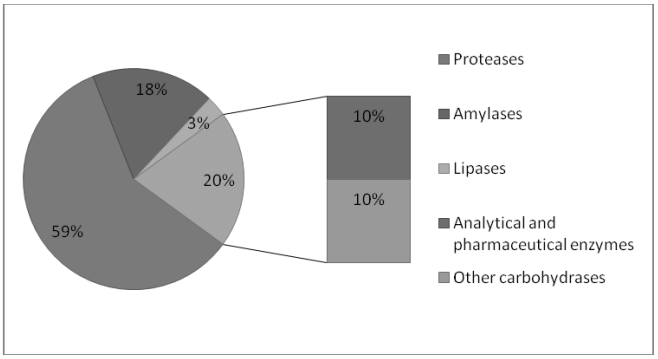


Fig.1. Exploitation of various enzymes in industrial processes (in percentage) (Rao *et al*, 1998)

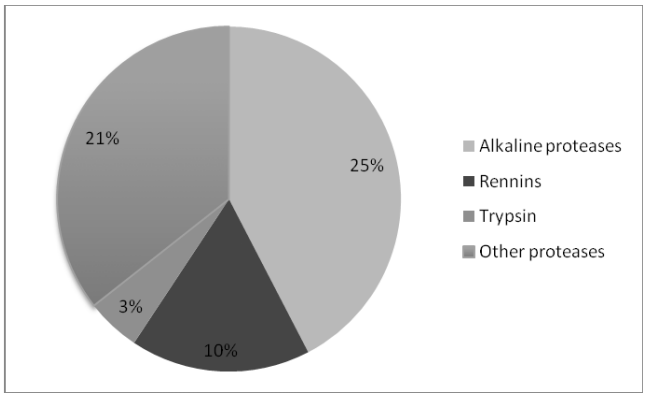


Fig.2. Use of various proteases in industrial processes (in percentage) (Rao *et al*, 1998)

Proteases are complex multi-enzyme system which catalyses the hydrolysis of amide bonds in proteins hence it has been used in the textile industry for de-gumming of silk and processing of wool (Ravel and Banerjee, 2003; Adinarayana *et al*, 2005). With the advent of new areas in Biotechnology, applications of proteases have stretched to new directions that include analytical and medical chemistry. To meet the current largely increased demand, studies on the cost-effective production of industrially important enzymes have become the need of today (Kalaiarasi and Sunitha, 2009).

The present review aims to focus on the potential industrial application of such huge variety of proteases.

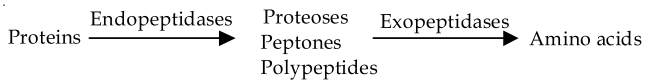
Applications of Proteases

Biotechnology is believed to be an effective substitution of traditional methods in research and manufacturing industries. Exploitation of a biological organization is beneficial over a chemical one as they are highly specific and efficient in completing a difficult chemical reaction. These undergo efficient processing with lesser number of unwanted products, easy to scale-up and hence give increased product yields. The ease and array of chemical conversions catalyzed by these biological enzymes has revolutionized the processing and product development in biotechnology industries (Sema and Veysi, 2007).

The global market for industrial enzymes was valued at $3.1 billion in 2009 and reached about $3.6 billion in 2010. The estimated market for 2011 is about $3.9 billion. BCC (Business Communications Company Research) projects this market to grow at a compounded annual growth rate (CAGR) of 9.1% and to reach $6 billion by 2016 (BCC Research, 2011).

Industries that use enzymes for manufacture of their products invest roughly trillions of dollars annually in their commercialization. Amongst these exploited enzymes, 75% belong to hydrolytic enzymes and out of these proteases represent 65% of total of the world-wide (Aqel, 2012). The enormous range and specificity of proteases has attracted the attention of the scientists globally and has made them investigate its diverse physiological and industrial applications. In addition, proteases are playing a leading role in almost every biotechnological process as they maintain their enzymatic activity in a broad range of pH and temperature (Dubey and Jagannadham, 2003).

Industrially available proteolytic enzymes produced by microorganisms are usually mixtures of endo-peptidases (proteinases) and exopeptidases. In overly simplified form, the action of the proteases may be formulated:



In addition to microbial proteases, the plant proteases bromelin, papain, and ficin, and the animal proteases viz. pepsin and trypsin, have extensive industrial application. Because of the complex structures and high molecular weights of proteins made up of some 20 different amino acids, enzymatic proteolysis is extremely complicated. Most proteases are quite specific with regard to which peptide linkages they can split (Smith, 1951). Hence, for specific applications, it is obligatory to select the right protease complex or combination of enzymes. Usually this can only be determined by trial and error methods. By means of such experimentation, however, many and diverse uses have been found for the various proteases. For specific proteases, with appropriate conditions of time, temperature, and pH, limited as well as complete hydrolysis of most proteins to amino acids can be done (Underkofler *et al*, 1958).

Proteases are of commercial value and find numerous applications in a variety of industrial sectors:

Food Processing Industry

Augmentation of digestive capacity by inclusion of exogenous proteases in the diet can enhance protein use and is becoming increasingly important in diet formulation (Odetallah *et al*, 2003, 2005; Lei *et al*, 2006).

The rationale behind using proteases in food processing industry is that they are known to improve the digestibility, solubility, fat and water-binding properties, flavor and palatability of food. Moreover, it improves processing by reducing viscosity and improving drying. In the food processing industry, proteases are used in the production of the following:

Functional foods

a) Production of non bitter protein hydrolysates from soya, pea, maize and wheat flours etc. (Pasupuleti and Braun, 2010). Soy protein hydrolysates are used in protein fortified soft drinks and special diabetic diets. They are nutritionally excellent in terms of amino acid content of the proteins. Soy protein hydrolysates have also been shown to have various physiological and antioxidant activities. Moreover, there are numerous reports on the immunomodulating activity of enzymatic hydrolysates prepared from casein, whey and soy proteins (Kong *et al*, 2008).

b) Soy sauce: Muangthai *et al* in 2007 studied the production of soy sauce by using *Aspergillus oryzae*. They reported the production of soy sauce from varying ratios of soya bean, wheat flour and salt in which they analyzed the amino acid content and monitored that the protease content increased with fermentation time in all the combinations studied.

c) Synthesis of aspartame: A non calorific artificial sweetener named aspartame has been approved by the Food and Drug Administration. Aspartame is a dipeptide made up of L-aspartic acid and the methyl ester of L-phenylalanine. The enzymatic synthesis of aspartame has been reported from the immobilized preparation of thermolysin from *Bacillus thermoprotyolyticus*. The major industrial producers of aspartame are TOYA SODA of Japan and DSM of Netherlands (Rao *et al*, 1998).

d) Meat tenderization: One of the significant attributes of meat texture is tenderization which affects the perception of beef meat by the consumers. Meat tenderization is done by limited proteolysis of the myofibrillar and stromal proteins of muscle (Gerelt *et al*, 2000). The treatment of proteolytic enzymes like papain, bromelin, ficin and bacterial collagenase, is one of the accepted methods for meat tenderization for improved digestibility and reduced allergenicity (Ionescu *et al*, 2008). Meat recovery from bones for use as additives to canned meats and soups has also been reported (Grzonka *et al*, 2007).

e) Baking: Dough viscosity is reduced by protease treatment that assist in gluten development. Moreover, the structural properties of bread are altered on partial hydrolysis of dough proteins during baking (Moodie, 2001).

f) Production of singe cell proteins : A wide variety of wastes are used as substrates for SCP production like agricultural wastes such as hemicellulosic and cellulosic waste from plants and fibrous proteins like horn, feather, nail and hair from animals. Some protease producing microorganisms are used to convert these wastes to biomass, protein concentrates and amino acids which are utilized for production of SCP (Adedayo *et al*, 2011).

Dairy industry

a) Production and enhancing flavors of processed cheese: Animal rennet (bovine chymosin) was traditionally used as a milk-clotting agent in the dairy industry for high-quality cheese production with enhanced flavor and texture. Looking at the worldwide increment in demand of cheese and decrement in supply of calf rennet, exploration for rennet substitutes i.e. microbial rennet became the need of the hour. Presently, microbial rennet is responsible for production of one-third of all the cheese produced worldwide. Microbial rennet from microorganisms like R*hizomucor pusillus, R. miehei, Endothia parasitica, Aspergillus oryzae,* and *Irpex lactis* are used widely for cheese manufacture (Neelkantan *et al*, 1999).

b) Formation of milk protein hydrolysates (Pasupuleti and Braun, 2010)

c) Milk coagulation: The purpose of addition of milk-clotting enzymes to milk is to cleave □-casein and begin coagulation of milk (Brown and Ernstrom, 1988).

Laundry and Dishwashing Detergents

Alkaline protease added to laundry detergents plays a specific catalytic role in the hydrolysis of protein stains such as blood, milk, human sweat etc. The increased usage of the protease as a detergent additive is mainly due to its environmentally acceptable cleaning capabilities (Venil and Lakshmanaperumalsamy, 2009; Rai, 2010).

Nearly 25% of the total global sale of enzymes is because of use of protease in laundry detergents. The first utilization of enzymes in detergent named, "Burmin," dates back to 1913 and was made up of sodium carbonate and a crude pancreatic extract. In

1956, the first detergent containing the bacterial enzyme was introduced under the trade name BIO-40. Novo Industry A/S introduced alcalase, produced by *Bacillus licheniformis*; its commercial name was BIOTEX in 1960, followed by Maxatase, a detergent made by Gist-Brocades (Rao *et al*, 1998).

An enzyme can be utilized as an additive to detergent only if they are stable and active in the presence of harsh conditions generated by other detergent ingredients, such as surfactants, builders, bleaching agents, bleach activators, fillers, fabric softeners and various other formulation aids (Najafi *et al*, 2005). Balakrishnan *et al* (2012) isolated protease producing *Bacillus sp.* from green mussels (*Perna viridis*) and have reported stability of the enzyme in the presence of various detergents like Tween 20, Tween 80, Triton X100, SDS and BRIJ 35.

In a study conducted by Pathak and Deshmukh (2012), alkaline protease obtained from *Bacillus licheniformis* KBDL4 obtained from Lonar Soda Lake, India, retained 30-80% of its original activity in the presence of different detergents.

The key parameter for the best performance of a protease in a detergent is its pI. It is known that a protease is most suitable for this application if its pI coincides with the pH of the detergent solution. Esperase and Savinase T (Novo Industry), produced by alkalophilic *Bacillus* spp., are two commercial preparations with very high isoelectric points (pI 11); hence, they can withstand higher pH ranges. Moreover, taking into account the consciousness for energy conservation, proteases active at lower temperatures are more useful. In addition to an active and stable protease, a blend of lipase, amylase, and cellulase is projected to improve the performance of laundry detergents (Rao *et al*, 1998).

Personal Care

Application of proteases for oral care has been reported for removal of plaque & odour causing deposits from teeth & gum tissue, helps in teeth whitening (Berg *et al*, 2001). Moreover, proteases are also employed for skin exfoliation and enhancement of natural hair colour & texture. Gelatin hydrolysates are prepared utilizing alkaline proteases which are in turn used in the cosmetic industry as additives to shampoos and ointments (Gupta and Ramnani, 2006).

Leather Industry

Proteases are used in leather industry for processing hides & skins, bating & de-hairing processes (Najafi *et al*, 2005). Alkaline proteases are envisaged to have extensive applications in leather industry. In a tannery, hide is subjected to a series of chemical treatments prior to tanning and finally converted to finished leather. Proteases may play a vital role in these treatments by replacing these hazardous chemicals especially involved in soaking, dehairing and bating (Puvankrishnan and Dhar, 1986). Increased usage of enzymes for dehairing and bating not only prevents pollution problems, but is also effective in saving energy. In general, proteases play a vital role in leather processing starting from soaking of hides to finished products (Sachidanandham *et al*, 1999; Mukhtar and Haq, 2007). In advanced tanneries, soaking is usually performed with combination of proteolytic enzymes that are optimally active in the alkaline or neutral pH (Ward, 1985; Christner, 1995; Godfrey and West, 1996). The conventional method for depilation has now been clearly recognized to be environmentally objectionable accounting for the discharge of 100 % of sulfide and 80 % of the suspended solids in the tannery effluent (Peper and Wyatt, 1989).The enzymes catalyze the breakdown of the protein keratin in the hair and allow the hair to be easily removed. The advantages of using proteases for dehairing of skins are several like reduction of the sulfide contents in the effluent, recovery of the hair/ wool which is of good quality, an increased yield of leather area, easy handling of the pelts by workmen, simplification of the pretreatment, the elimination of the bate in the deliming stage and finally the production of a good quality pelts/leather (Dhar, 1974; Mitra and Chakraverty, 1998; Kamini *et al*, 1999; Mukhtar and Haq, 2008).

Increased usage of enzymes for dehairing and bating not only prevents pollution problems but also is effective in saving energy (Gupta *et al*, 2002). Novo Nordisk manufactures three different proteases, Aquaderm, NUE, and Pyrase, for use in soaking, dehairing and bathing, respectively.

Medicines

The broad range and specificity of proteases provides a great advantage in developing effective therapeutic agents.

a) Proteases from *Aspergillus oryzae* are used as digestive aid to cure several lytic enzyme deficiency syndromes by oral administration (Raimi *et al*, 2010).
b) Alkaline and neutral protease formulations are used to produce stable suspensions of tryptic and peptic casein hydrolysates using soy proteins and whey powder, that are used as a diabetic product (Hermansen *et al*, 2001) and in treatment of cystic fibrosis (Nissen, 1976).
c) Healing of burn wounds: Clostridial collagenase or subtilisin is used in amalgamation with broad-

spectrum antibiotics for treatment of burns and wounds. *Bacillus subtilis* 316M has been reported to synthesize elastoterase, a serine protease which has the property of hydrolyzing a wide variety of animal proteins such as collagen and elastin. An elastoterase immobilized on a bandage is suggested for the treatment of burns a purulent wounds, deep abscesses etc. (Kudrya and Simoenko, 1994). According to Kim *et al* (1996), alkaline protease obtained from *Bacillus* sp. CK-114 showed fibrinolytic activity and hence can act as a thrombolytic agent.

d) Treatment of Acute Lymphoblastic Leukiemia: L-asparginase (EC 3.5.1.1) hydrolyzes L-asparagine to aspartic acid and ammonia. This L-asparagine is required for protein synthesis by tumor cells. With introduction of L-asparginase, the cells are deprived of this essential amino acid and hence results in cytotoxicity of tumor cells (Jain *et al*, 2012).
e) Treatment of skin ulcers: Proteolytic enzymes support the natural healing process of ulcers by efficient removal of the necrotic material (Sjodahl *et al*, 2002; Najafi *et al*, 2005).

Pharmaceutical Industry

a) Proteases find application as biopharmaceutical products. These are used in contact lens cleaning formulations (Anwar and Saleemuddin, 2000).
b) Preparation of animal feed: Partial hydrolysis of proteins with special cocktails of proteases designed to produce mixtures of small peptides has resulted in products that can improve animal productivity (Cahu *et al*, 1998, 1999; Lindemann *et al*, 2000; Zambonino *et al*, 1997; Lei *et al*, 2006).
c) Hard surface cleaning formulations: Onaizi *et al*, (2009) reported cleaning performance of surfactant and biosurfactant-based formulations with and without protease against protein stains from solid surfaces. They found that 2 ppm subtilisin A and 2 ppm sodium dodecyl benzyl sulphonate combination showed the best cleaning performance.
d) Trypsin, a serine protease plays an important role in animal cell culture in detaching adherent cells from the culture vessel. Chiplonkar *et al*, 1985 reported the application of alkaline protease from *Conidiobolus coronatus* in animal cell culture.

Fishery

Proteases are used in fish industry for modification of fish protein to recover fish protein from fish waste which is used in fish manure preparation. These enzymes are also used to produce oil, fish solids and fish solubles from non edible fishes. He *et al* in 2006 reported digestion of a shrimp *Acetes chinensis* by a crude protease from *Bacillus* sp. SM98011 and the antioxidant activity of the hydrolysate so produced were found to be high.

Silk Degumming

Utilization of protease in silk industry is least explored and only a few patents have been filed describing silk degumming by proteases (Kanehisa, 2000). Sericin contains approximately 20-30% of total raw silk and its major role is to wrap the fibroin. In presence of sericin the fibres of silk are rough and become soft and lustrous after its removal (Padamwar and Pawar, 2004). Conventionally, sericin was removed from the inner core of fibroin by shrink-proofing and twist-setting methods, using starch (Kanehisa, 2000). But the process is expensive. Alternatively, a traditional method of degumming was performed in an alkaline solution containing soap which was a harsh treatment because the fiber itself is attacked (Singhal *et al*, 2012). Therefore, use of enzyme preparations, such as protease can be an alternative method, for degumming the silk prior to dyeing.

Protease treatments can modify the surface of wool and silk fibres to provide new and unique finishes (Najafi *et al*, 2005).

Photography : Silver Recovery

In photographic films, the amount of silver varies between 1.5% and 2.0% by weight even after fixing and developing processes. It has been reported earlier by Shankar *et al* in 2010 that 25% of the world's silver needs are supplied by recycling and rest 75% of this is obtained from photographic waste. The waste films are hence a good source of silver, one of the precious noble metals. The emulsion layer on X-ray film contains silver and gelatin, it is possible to break down the gelatin layer using proteases and release the silver (Nakiboglu *et al*, 2001). Moreover, the polyester base can also be recycled.

Alkaline proteases from many *Bacillus* strains have been reported be effective in decomposing the gelatin layer present on X-ray films resulting in silver extraction like *Bacillus* sp. B18 (Fujiwara *et al*, 1991; Najafi *et al*, 2005), *B. coagulans* PB-77 (Gajju *et al*, 1996) and *Bacillus licheniformis* KBDL4 (Pathak and Deshmukh, 2012).

Brewing Industry

Proteases allow removal of protein remains of yeasts, making the beer look clearer and hence easily filterable (Paranthaman *et al*, 2009). Beer appears cloudy at nearly freezing temperatures because of the presence of proteins. This condition is known as chill haze. It

is not indicative of a bad beer but does not appeal the costumers. Moreover, presence of excessive protein adversely affects the shelf life of the beer. In order to avoid this, proteases are added during the fermentation processes. SEBclear manufactured by Specialty Enzymes and Technologies® is a liquid enzyme preparation produced by *Trichoderma* and supplemented with protease produced from the fruit of *Carica papaya* that inhibits post-chilling haze formation.

Management of Household and Industrial Waste

In recent times, proteases are finding application in waste management. In aquatic bodies, they degrade waste that are proteinaceous in nature and lowers the BOD. Use of protease for degrading the waste generated by food processing industries and household activities can help solve the problem of waste management. An example of this is the management of waste feathers generated from poultry slaughterhouses (Deivasigamani and Alagappan, 2008).

Traditionally used chemical and mechanical hydrolysis of keratin wastes such as hair is successful but they have many drawbacks of as they demand a lot of energy input and also pollute the environment. Hence, enzymatic degradation using keratinases, a kind of protease, is an attractive method for the same (Ramnani *et al*, 2005).

Proteases are physiologically necessary for living organisms and occur ubiquitously in a broad diversity of organisms such as plants, animals and micro-organisms (Rao *et al*, 1998; Gupta *et al*, 2002). Commercial proteases are derived from all these sources viz., animal tissues, plant cells and microbial cells via fermentation. The well-known proteases of plant origin are papain, bromelain, keratinases, and ficin while the most common proteases of animal origin are pancreatic trypsin, chymotrypsin, pepsin and rennin. The use of plants as a source of proteases is governed by several factors such as the availability of land for cultivation, the time-consuming processes and the suitability of climatic conditions for growth whereas the production of proteases in animals depends on the availability of livestock, which in turn is governed by political and agricultural policies. Due to these factors, plant and animal proteases are unable to meet current world demands and the attention is shifting towards microbial proteases (Rao *et al*, 1998).

Microbial enzymes are more beneficial over the enzymes derived from plants or animals because of their great variety of catalytic activities, stability, ease of genetic manipulation, continuous supply due to absence of seasonal fluctuations, possible high yields, rapid growth of microorganisms in inexpensive media, more convenient and safer protection methods (Hasan *et al*, 2006).

Proteases of microbial origin are among the main hydrolytic enzymes and have been studied extensively since the inception of enzymology. There is an increased interest in the study of proteolytic enzymes as they not only play a central role in the cellular and metabolic processes but have also gained a significant attention in the industrial community due to their wide variety of applications (Gupta *et al*, 2002; Barindra *et al*, 2006).

More than 3000 different enzymes have been described to date and majority of them have been isolated from mesophilic organisms. These enzymes mainly function in a narrow range of pH, temperature and ionic strength. Moreover, the technological application of enzymes under demanding industrial conditions makes the currently known enzymes non-recommendable. For example the proteases used in a detergent formulation should ideally have a high level of activity over a broad range of pH and temperatures. For the same purpose alkaline proteases from high yielding strains have been studied extensively. One of the major drawbacks of enzymes recovered from thermophiles is the instability at alkaline pH and thermo liability of enzymes recovered from alkalophiles (Griffin *et al*, 1992). Thus, the search for new microbial sources is a continual exercise, where one must respect biodiversity. The microorganisms from diverse and exotic environments called as extremophiles, are an important source of enzymes, whose specific properties are expected to result in novel process applications (Kumar and Takagi, 1999; Adinarayana *et al*, 2003).

Only about 2% of the world's microbes have been reported as enzyme sources. Bacterial strains are generally more exploited for enzyme production as they offer higher activities compared to yeasts (Frost and Moss, 1987) and tend to have neutral or alkaline pH optima (Table 1) and are often thermostable (Table 2) (Venil and Lakshmanaperumalsamy, 2009).

There are number of microbes associated with protease production but very few are recognised as commercial producers. *Bacillus* species are specific producers of extra cellular proteases and is by far the most popular source of commercial alkaline proteases (Ward, 1985; Kalisz, 1988; Outtrup and Boyce, 1990; Kumar and Takagi, 1999).

Samples from different places and sources have been isolated and screened. Screening and isolation of proteolytic bacteria have been carried out from soil samples of Ikogosi warm spring (SW, Nigeria) (Olajuyigbe and Ajele, 2005), Nadiad industrial area, Gujarat (Chudasama *et al*, 2010) from different regions of Bangalore (Siddalingeshwara *et al*, 2010), from dog dung (Sharmin and Rahman, 2007), from sub- Antarctic marine sediments of Isla de Los Estados (Argentina) (Olivera *et al*, 2007), from Egyptian Soda Lake (Ibrahim *et al*, 2007) and so on.

Table 1. pH optimum of various proteases produced from *Bacillus sp*.

Source	Substrate	Optimum pH	Reference
Bacillus sp. B21-2	Casein	11.5	Fujiwara and Yamamoto, 1987
Bacillus sp. No. AH-101	Casein	12-13	Takami *et al*, 1989
Bacillus sp. B18'	Casein	13.0	Fujiwara *et al*, 1991
Bacillus licheniformis MIR29	Casein	12.0	Ferrero *et al*, 1996
Bacillus sp. AR-009	Casein	10	Gessesse, 1997
Bacillus licheniformis	Casein	9.0	Manachini and Fortina, 1998
B. brevis	Azocasein	10.5	Banerjee *et al*, 1999
Bacillus sp. SB5	Casein	10.0	Gupta *et al*, 1999
Bacillus sp. NCDC 180	Casein	11-12	Kumar *et al*, 1999
Bacillus sphaericus	Azocasein	10.5	Singh *et al*, 1999
B. pumilus UN-31-C-42	Casein	10.0	Huang *et al*, 2003
Bacillus sp. JB-99	Casein	11.0	Johnvesly and Naik, 2001
Bacillus sp. RGR-14	Casein	11.0	Oberoi *et al*, 2001
Bacillus sp. P-2	Casein	9.6	Kaur *et al*, 2001
Bacillus sp. SSR1	Azocasein	10.0	Singh *et al*, 2001
Bacillus horikoshii	Casein	9.0	Joo *et al*, 2002
Bacillus mojavensis	Casein	10.5	Beg and Gupta, 2003
Bacillus clausii I-52	Casein	11.0	Joo *et al*, 2003
Bacillus subtilis megatherium	Casein	10	Gerze *et al*, 2005
Bacillus sp. GUS1	Casein	8-10	Seifzadeh *et al*, 2008
Bacillus thuringiensis cc7	Casein	8.5	Chudasama *et al*, 2010
Bacillus subtilis	Casein	8.0	Das and Prasad, 2010
Bacillus sp. B001	Azocasein	10.0	Deng *et al*, 2010
Bacillus subtilis	Casein, Gelatin	8.0	Naidu, 2011
Bacillus licheniformis KBDL4	Casein, Gelatin	8.0-12.0	Pathak and Deshmukh, 2012

Conclusion

Industrial and household processes are fetching more and more reliance on enzymes because of their ability to catalyze a wide array of chemical processes. The major benefits of enzyme usage are generation of superior quality product, cheaper manufacturing cost, reduced amount of waste and lesser energy consumption. The major dependence among all is on proteases that account 65% of the total enzyme sales in the world. It is now clear from the above mentioned description of different applications of protease that it has the potential to be exploited further in the future. Furthermore, being isolated from biological sources these are safer compared to the harsh chemical compositions.

Table 2. Optimum temperature and thermostabilities of various proteases produced from *Bacillus sp*.

Source	Optimum temperature	Reference
Bacillus sp. No. AH-101	80ºC	Takami *et al*, 1989
Bacillus sp. B18′	85ºC	Fujiwara *et al*, 1991
B. stearothermophilus F1	85ºC	Rahman *et al*, 1994
B. subtilis IIQDB32	55ºC	Varela *et al*, 1997
Bacillus licheniformis	70ºC	Manachini and Fortina, 1998
B. brevis	60ºC	Banerjee *et al*, 1999
Bacillus sp. SB5	70ºC	Gupta *et al*, 1999
Bacillus sp. NCDC 180	55ºC	Kumar *et al*, 1999
Bacillus sphaericus	55ºC	Singh *et al*, 1999
Bacillus JB-99	70ºC	Johnvesly and Naik, 2001
Bacillus sp. P-2	90ºC	Kaur *et al*, 2001
Bacillus sp. RGR-14	60ºC	Oberoi *et al*, 2001
Bacillus sp. SSR1	40ºC	Singh *et al*, 2001
Bacillus horikoshii	45ºC	Joo *et al*, 2002
Bacillus mojavensis	60ºC	Beg and Gupta, 2003
B. pumilus UN-31-C-42	55ºC	Huang *et al*, 2003
Bacillus clausii I-52	60ºC	Joo *et al*, 2003
Bacillus sp. GUS1	70ºC	Seifzadeh *et al*, 2008
Bacillus sp. B001	60ºC-70ºC	Deng *et al*, 2010
Bacillus subtilis	40ºC -50 ºC	Naidu, 2011
Bacillus licheniformis KBDL4	60ºC	Pathak and Deshmukh, 2012

References

Abidi, F., Limam, F., Nejib, M.M. (2008) Production of alkaline proteases by *Botrytis cinerea* using economic raw materials: Assay as biodetergent. *Process Biochem* **43**:1202-1208.

Adedayo, M.R., Ajiboye, E.A., Akintunde, J.K., Odaibo A. (2011) Single Cell Proteins: As Nutritional Enhancer. *Adv Appl Sci Res* **2(5)**:396-409.

Adinarayana, K., Ellaiah, P., Prasad, D.S. (2003) Purification and partial characterization of thermostable serine alkaline protease from a newly isolated *Bacillus subtilis* PE-11. *AAPS PharmSciTech* **4**:E56.

Adinarayana, K., Jyothi, B., Ellaiah, P. (2005) Production of alkaline protease with Immobilized cells of *Bacillus subtilis* PE-11 in various matrices by entrapment technique. *AAPS PharmSciTech* **6(3)**: E391-E397.

Anwar, A., Saleemuddin, M. (1998) Alkaline Protease: A review. *Bioresour Technol* **6(3)**: 175-183.

Anwar, A., Saleemuddin, M. (2000) Alkaline protease from *Spilosoma obliqua*: potential applications in bioformulation. *Biotechnol Appl Biochem* **31(2)**:85-89.

Aqel, H. (2012) Phenotypic and protease purification of two different thermophilic *Bacillus* strains HUTBS71 and HUTBS62. *Ann Biol Res* **3(4)**:1747-1756.

BCC-Business Communications Company Research, Inc., (2011). In: Report Global Markets for Enzymes in Industrial Applications, BIO030G.

Balakrishnan, P., Thamaraichelvan, R., Rajendran, N., Flanet, R. (2012) Production and purification of alkaline serine protease from marine *Bacillus* species and its application in detergent industry. *Eur J Appl Sci* **4(1)**: 21-26.

Banerjee, U.C., Sani, R.K., Azmi, W., Soni, R. (1999) Thermostable alkaline protease from *Bacillus brevis* and its characterization as a laundry detergent additive. *Process Biochem* **35**: 213-219.

Barindra, S., Debashish, G., Malay, S., Joydeep, M. (2006) Purification and characterization of a salt, solvent,

detergent and bleach tolerant protease from a new gamma Proteobacterium isolated from the marine environment of the Sundarbans. *Process Biochem* **41(1)**: 208-215.

Beg, K.B., Gupta, R. (2003) Purification and characterization of an oxidation- stable, thiol-dependent serine alkaline protease from *Bacillus mojavensis. Enzyme Microb Tech* **32:** 294-304.

Berg, C.H., Kalfas, S., Malmsten, M., Arnebrant, T. (2001) Proteolytic degradation of oral biofilms in vitro and in vivo: potential of proteases originating from *Euphausia superba* for plaque control. *Eur J Oral Sci* **109(5)**:316-324.

Brown, R.J., Ernstorm, C.A. (1988) Milk Clotting Enzymes. In: Jenners R, Marth EH, Wong NP, Keeney M (ed.). *Fundamentals of Dairy Chemistry* **3**:609-633.

Cahu, C.L., Zambonino Infante, J.L., Escaffre, A.M., Bergot, P., Kaushik, S. (1998) Preliminary results on sea bass (*Diecentrarchus labrax*) larvae rearing with compound diet from first feeding. Comparison with carp (*Cyprinus carpio*) larvae. *Aquaculture* **169:**1–7.

Cahu, C.L., Zambonino Infante, J.L., Quazuguel, P., Gall, M.M.L. (1999) Protein hydrolysate vs. fish meal in compound diets for 10-day-old sea bass *Dicentrarchus labrax* larvae. *Aquaculture* **171**:109–111.

Chiplonkar, J.M., Gangodkar, S.V., Wagh, U.V., Ghadge, G.D., Rele, M.V., Srinivasan, M.C. (1985) Applications of alkaline protease from *Conidiobolus* in animal cell culture. *Biotechnol Lett* **7**:665–668.

Christner, J. (1995) Modern enzyme applications in the beamhouse. *World Leather* **10**: 60-64.

Chudasama, C.J., Jani, S.A., Jajda, H.M., Patel, H.N. (2010) Optimization and production of alkaline protease from *Bacillus thuringiensis cc7. J Cell Tissue Res* **10(2)**:2257-2262.

Das, G., Prasad, M.P. (2010) Isolation, purification & mass production of protease enzyme from *Bacillus subtilis. Int Res J Microbiol* **1(2)**:26-31.

Deivasigamani, B., Alagappan, K.M. (2008) Industrial application of keratinase and *soluble* proteins from feather keratins. *J Environ Biol* **29(6)**:933-936.

Deng, A., Wu, J., Zhang, Y., Zhang, G., Wen, T. (2010) Purification and characterization of a surfactant-stable high-alkaline protease from *Bacillus* sp. B001. *Bioresour Technol* **101**:7100–7106.

Dhar, S.C. (1974) Production and application of enzymes in the pretanning process of leather manufacture. *Leather Sci* **21**: 39-47.

Dube, S., Singh, L., Alam, S.T. (2001) Proteolytic anaerobic bacteria from lake sediments of Antarctica. *Enzyme Microb Technol* **28**:114–121.

Dubey, V.K., Jagannadham, M.V. (2003) Procerain, a stable cysteine protease from the latex of *Calotropis procera. Phytochemistry* **62**:1057–1071.

Ferrero, M.A., Castro, G.R., Abate, C.M., Biagori, M.D., Siòeriz, F. (1996) Thermostable alkaline proteases of *Bacillus licheniformis* MIR 29: isolation, production and characterization. *Appl Microbiol Biotechnol* **45**:327-332.

Frost, G.M., Moss, D.A. (1987) Production of enzymes by fermentation. In: Rehm, HJ, Reed G, Kennedy JF *Biotechnology* (ed.) VCH, Weinheim **7a:**65 – 211.

Fujiwara, N., Yamamoto, K. (1987) Production of alkaline protease in a low-cost medium by alkalophilic *Bacillus* sp. and properties of the enzyme. *J Ferment Technol* **65(3):** 345-348.

Fujiwara, N., Yamamoto, K., Masui, A. (1991) Utilization of a thermostable alkaline protease from an alkalophilic thermophile for the recovery of silver from used X-ray film. *J Ferment Bioeng* **72 (4)**:306-308.

Gajju, H., Bhalla, T.C., Agarwal, H.O. (1996) Thermostable alkaline protease from thermophilic *Bacillus coagulans* PB-77. *Indian J Microbiol* **36**:153–155.

George, S., Raju, V., Krishnan, M.V.R., Subramanian, T.V., Jayaraman, K. (1995) Production of protease by *Bacillus amyloliquefacian* in solid-state fermentation and its application in unhairing of hides and skins. *Process Biochem* **30**: 457-462.

Gerelt, B., Ikeuchi,http://www.sciencedirect.com/science/article/pii/S0309174000000607 - AFFB Y., Suzuki,http://www.sciencedirect.com/science/article/pii/S0309174000000607 - AFFB A. (2000) Meat tenderization by proteolytic enzymes after osmotic dehydration. *Meat Sci* **56(3)**:311–318.

Gerze, A., Omay, D., Guvenilir, Y. (2005) Partial Purification and Characterization of Protease Enzyme from *Bacillus subtilis megatherium. Appl Biochem Biotechnol* **121–124**:335–346.

Gessesse, A. (1997) The use of nug meal as a low-cost substrate for the production of alkaline protease by the alkaliphilic *Bacillus* sp. AR-009 and some properties of the enzyme. *Bioresour Technol* **62(1-2)**:59-61.

Giongo, J.L., Lucas, F.S., Casarin, F., Heeb, P., Brandelli, A. (2007) Keratinolytic protease of *Bacillus* species isolated from the Amazon basin showing remarkable dehairing activity. *World J Microb Biot* **23**: 375-382.

Godfrey, T., West, S. (1996) *Industrial Enzymology,* Macmillan Publishers Inc., New York **2**.

Gomes, J., Steiner, W. (2004) The biocatalytic potential of extremophiles and extremozymes. *Food Technol Biotech* **42**: 223-235.

Griffin, H.L., Greene, R.V., Cotta, M.A. (1992) Isolation and characterization of an alkaline protease from the marine shipworm bacterium. *Curr Microbiol* **24**:111-117.

Grzonka, Z., Kasprzykowski, F., Wiczk, W. (2007) Cysteine Proteases. *Industrial enzymes: Structure, function and applications*. Poliana J, MacCabe AP (Eds), Springer; **1**:180-197.

Gupta, R., Gupta, K., Saxena, R.K., Khan, S. (1999) Bleach-stable, alkaline protease from *Bacillus* sp. *Biotechnol Lett* **21**:135-138.

Gupta, R., Beg, Q.K., Lorenz, P. (2002) Bacterial alkaline proteases: molecular approaches and industrial applications. *Appl Microbiol Biotechnol* **59(1)**: 15-32.

Gupta, R., Ramnani, P. (2006) Microbial keratinases and their prospective applications: an overview. *Appl Microbiol Biotechnol* **70(1)**:21-33.

Hasan, F., Shah, A.A., Hameed, A. (2006) Industrial applications of microbial lipases. *Enzyme Microb Tech* **39(2)**:235 – 251.

He, H., Chen, X., Sun, C., Zhang, Y., Gao, P. (2006) Preparation and functional evaluation of oligopeptide-enriched hydrolysate from shrimp (*Acetes chinensis*) treated with crude protease from *Bacillus* sp. SM98011. *Bioresour Technol* **97(3)**:385-390.

Hermansen, K., Sondergaard, M., Hoie, L., Carstensen, M., Brock, B. (2001) Beneficial effects of a soy-based dietary supplement on lipid levels and cardiovascular risk markers in type 2 diabetic subjects. *Diabetes Care*; **24**, 228–233.

Huang, Q., Peng, Y., Li, X., Wang, H., Zhang, Y. (2003) Purification and characterization of an extracellular alkaline serine protease with dehairing function from *Bacillus pumilus*. *Curr Microbiol* **46**:169-173.

Ibrahim, A.S.S., EI-Shayeb, N.M.A., Mabrouk, S.S. (2007) Isolation and identification of alkaline protease producing alkaliphilic bacteria from an Egyptian Soda Lake. *J Appl Sci Res* **3**:1363-1368.

Ionescu, A., Aprodu, I., Pascaru, G. (2008) Effect of papain and bromelin on muscle and collagen proteins in beef meat. *The Annals of the University Dunarea de Jos of Galati Fascicle VI – Food Technology* **II(XXXI):** 9-16.

Jain, R., Zaidi, K.U., Verma, Y., Saxena, P. (2012) L-Asparaginase: A Promising Enzyme for Treatment of Acute Lymphoblastic Leukiemia. *People's Journal of Scientific Research* **5(1)**: 29-35.

Johnvesly, B., Naik, G.R. (2001) Studies on production of thermostable alkaline protease from thermophilic and alkaliphilic *Bacillus* sp. JB-99 in a chemically defined medium. *Process Biochem* **37**:139-144.

Joo, H., Kumar, G., Park, G., Kim, K.T., Paik, S.R., Chang, C. (2002) Optimization of the production of an extracellular alkaline protease from *Bacillus horikoshii. Process Biochem* **38**:155-159.

Joo, H.S., Kumar, C.G., Park, G.C., Paik, S.R., Chang, C.S. (2003) Oxidant and SDS-stable alkaline protease from *Bacillus clausii* I-52: production and some properties. *J Appl Microbiol* **95**:267-272.

Kalaiarasi, K., Sunitha, P.U. (2009) Optimization of alkaline protease production from *Pseudomonas fluorescens* isolated from meat waste contaminated soil. *Afr J Biotechnol* **8(24)**:7035-7041.

Kalisz, H.M. (1988) Microbial proteinases. *Adv Biochem Eng Biotechnol* **36**: 1-65.

Kamini, N.R., Hemachander, C., Geraldine, S.M.J., Puvanakrishnan, R. (1999) Microbial enzyme technology as an alternative to conventional chemicals in leather industry. *Curr Sci* **77**:80-86.

Kanehisa, K. (2000) Woven or knit fabrics manufactured using yarn dyed raw silk. US Patent 6,080,689.

Kasana, R.C., Yadav, S.K. (2007) Isolation of a psychrotrophic *Exiguobacterium* sp. SKPB5 (MTCC 7803) and characterization of its alkaline protease. *Curr Microbiol* **54:**224–229

Kaur, S., Vohra, R.M., Kapoor, M., Beg, Q.K., Hoondal, G.S. (2001) Enhanced production and characterization of a highly thermostable alkaline protease from *Bacillus* sp. P-2. *World J Microb Biot* **17**:125-129.

Kim, W., Choi, K., Kim, Y., Park, H., Chol, J., Lee, Y., Oh, H., Kwon, I., Lee, S. (1996) Purification and characterization of a fibrinolytic enzyme produced from *Bacillus* sp. strain CK 11-4 screened from chungkook-jangs. *Appl Environ Microbiol* **62**:2482–2488.

Kong, X.Z., Guo, M.M., Hua, Y.F., Cao, D., Zhang, C.M. (2008) Enzymatic preparation of immunomodulating hydrolysates from soy proteins. *Bioresour Technol* **99**:8873–8879.

Kumar, A.G., Nagesh, N., Prabhakar, T.G., Sekaran, G. (2008) Purication of extracellular acid protease and analysis of fermentation metabolites by *Synergistes* sp. utilizing proteinaceous solid waste from tanneries. *Bioresour Technol* **99**:2364–2372.

Kumar, C.G., Takagi, H. (1999) Microbial alkaline proteases from a bioindustrial viewpoint. *Biotechnol Adv* **17:** 561-594.

Kumar, C.G., Tiwari, M.P., Jany, K.D. (1999) Novel alkaline serine proteases from alkalophilic *Bacillus* spp.: purification and some properties. *Process Biochem* **34(5)**:441-449

Kudrya, V.A., Simoenko, I.A. (1994) Alkaline serine proteinase and lectin isolation from the culture fluid of *Bacillus subtilis*. *Appl Microbiol Biotechnol* **41(5)**:505-509.

Lei, X., Blake, J.P., Forsberg, C.W., Fox, D.G., Grabau, E., Mroz, Z., Sutton, A.L., Walker, W.R., Webb, K. (2006) Reviewers: Matthews JC, Shears SB, Veum T, CAST Board Liaison: Bell AW Biotechnological Approaches to Manure Nutrient Management, *CAST* **33**.

Lindemann, M.D., Cromwell, G.L., Monegue, H.J., Cook, H., Soltwedel, K.T., Thomas, S., Easter, R.A. (2000) Feeding value of an enzymatically digested protein for early-weaned pigs. *J Anim Sci* **78**:318–327.

Manachini, P.L., Fortina, M.G. (1998) Production in sea-water of thermostable alkaline proteases by a halotolerant strain of *Bacillus licheniformis*. *Biotechnol Lett* **20**:565-568.

Mitra, P., Chakraverty, R. (1998) Proteolytic enzymes as depilatory agents in the tannery, *Proceedings of National Symposium on Frontiers in Applied and Environmental Microbiology*; 195-197.

Moodie, P. (2001) Traditional Baking Enzymes – Proteases, *Enzyme Development Corporation (EDC)*:1-10.

Muangthai, P., Upajak, P., Patumpai, W. (2007) Study of protease enzyme and amino acid contents in soy sauce production from peagion pea and soy bean. *KMITL Sci Tech J* **7(S2)**:106-112.

Mukhtar, H., Haq, I. (2007). Optimization of volume of fermentation medium for the production of alkaline protease by an EMS mutant strain of *Bacillus subtilis* IH-72. *Pak J Bot* **39(7):** 2705-2715.

Mukhtar, H., Haq I. (2008) Production of alkaline protease by *Bacillus subtilis* and its application as a depilating agent in leather processing. *Pak J Bot* **40(4)**:1673-1679.

Naidu, K.S. (2011) Characterization and Purification of Protease enzyme. *JAPS* **1(3)**: 107-112.

Najafi, M.F., Deobagkar, D., Deobagkar D. (2005) Potential application of protease isolated from *Pseudomonas aeruginosa*. *Electron J Biotechnol* **8(2)**:197-203.

Nakiboglu, N., Toscali, D., Yasa, I. (2001) Silver recovery from waste photographic films by an enzymatic method. *Turk J Chem* **25**:349-353.

Nascimento, W.C.A., Martins, M.L.L. (2004) Production and properties of an extracellular protease from thermophilic *Bacillus* sp. *Braz J Microbiol* **35**:1-2.

Neelkantan, S., Mohanty, A.K., Kaushik, J.K. (1999) Production and use of microbial enzymes for dairy processing. *Curr Sci* **77**:143-149.

Nissen, J.A. (1976) Enzymic hydrolysis of proteins for increased solubility. *J Agric Food Chem* **24(6)**:1090–1093.

Oberoi, R., Beg, Q.K., Puri, S., Saxena, R.K., Gupta, R. (2001) Characterization and wash performance analysis of an SDS-stable alkaline protease from a *Bacillus* sp. *World J Microb Biot* **17**:493-497.

Odetallah, N.H., Wang, J.J., Garlich, J.D., Shig, J.C.H. (2003) Keratinase in starter diets improves growth of broiler chicks. *Poult Sci* **82**:664–670.

Odetallah, N.H., Wang, J.J., Garlich, J.D., Shig, J.C.H. (2005) Versazyme supplementation of broiler diets improves market growth performance. *Poult Sci* **84**:858–864.

Olajuyigbe, F.M., Ajele, J.O. (2005) Production dynamics of extracellular protease from *Bacillus* species. *Afr J Biotechnol* **4(8)**: 776-779.

Olivera, N., Sequeiros, C., Nievas, M. (2007) Diversity and enzyme properties of protease producing bacteria isolated from sub-Antarctic sediments of Isla de Los Estados, Argentina. *Extremophiles* **11**:517-526.

Onaizi, S.A., He, L., Middelberg, A.P. (2009) Rapid screening of surfactant and biosurfactant surface cleaning performance. *Colloids Surfs B Biointerfaces* **72(1)**:68-74.

Outtrup, H., Boyce, C.O.L. (1990) Microbial proteinases and biotechnology. In: WM Fogarty and CT Kelly (Eds). *Microbial Enzymes and Biotechnology*. Elsevier Science Publishers **2**:227-254.

Padamwar, M., Pawar, A.P. (2004) Silk sericin and its applications: A review. *J Sci Ind Res* **63**:323-329.

Paranthaman, R., Alagusundaram, K., Indhumathi, J. (2009) Production of Protease from Rice Mill Wastes by *Aspergillus niger* in Solid State Fermentation. *World J Agric Sci* **5(3)**:308-312.

Pastor, M.D., Lorda, G.S., Balatti, A. (2001) Protease obtention using *Bacillus subtilis* 3411 and amaranth seed meal medium at different aeration rates. *Braz J Microbiol* **32(1)**:1-8.

Pasupuleti, V.K., Braun, S. (2010) State of the Art Manufacturing of Protein Hydrolysates, Pasupuleti VK, Demain AL (eds.). *Protein Hydrolysates in Biotechnology* 11-32.

Pathak, A.P., Deshmukh, K.B. (2012) Alkaline protease production, extraction and characterization from alkaliphilic *Bacillus licheniformis* KBDL4: A Lonar Soda Lake isolate. *Indian J Exp Biol* **50**:569-576.

Peper, K.W., Wyatt, K.G.E. (1989) Enzymatic unhairing of heavy hides. *J Ind Leather Technol Assoc* **36**: 214-233.

Puvankrishnan, R., Dhar, S.C. (1986) Recent advances in the enzymatic depilation of hides and skins. *Leather Sci* **33(7)**:177-191.

Rahman, R.N.Z.A., Razak, C.N., Ampon, K., Basrim, M., Yunus, W.M.Z.W., Salleh, A.B. (1994) Purification and characterization of a heat-stable alkaline protease from *Bacillus stearothermophilus* F1. *Appl Microbiol Biotechnol* **40**:822-827.

Rai, M.P. (2010) Enhanced production of laundry detergent compatible *Bacillus* protease in aqueous two-phase system, *IJBB* **6(5):**811–817.

Raimi, O.G., Kappo, M.A., Fajana, O.O., Oku, S.N., Adeniji, M.A. (2010) Alkaline protease from maggots: a likely source of industrial enzyme, *Journal of Cell and Tissue Research* **10(3)**:2419-2422.

Ramnani, P., Singh, R., Gupta, R. (2005) Keratinolytic potential of *Bacillus licheniformis* RG1: structural and biochemical mechanism of feather degradation. *Can J Microbiol* **51(3)**:191-196.

Rao, M.B., Tanksale, A.M., Ghatge, M.S., Deshpande, V.V. (1998) Molecular and biotechnological aspects of microbial proteases. *Microbiol Mol Biol Rev* **62**:597-635.

Ravel, H., Banerjee, S.E. (2003) Effect of enzyme and chemical pretreatments on the properties of silk. *J Textile Assoc* **64**: 65-69.

Sachidanandham, R., Kumar, I.A.R., Krishnan, M.R.V., Jayaraman, K. (1999). Mathematical model based estimation of volumetric oxygen transfer coefficient(s) in the production of proteolytic enzymes in *Bacillus amyloliquefaciens*. *Bioprocess Eng* **21(4)**: 319-322.

Seifzadeh, S., Sajedi, R.H., Sariri, R. (2008) Isolation and characterization of thermophilic alkaline proteases resistant to sodium dodecyl sulfate and ethylene diamine tetraacetic acid from *Bacillus* sp. GUS1. *Iran J Biotech* **6(4)**:214-221.

Sema, A.F., Veysi, O. (2007) The obtainment of alkaline serine protease from *bacillus* sp. isolated from solid through the solid-state fermentation technique by using rind of the *Citrullus lanatus* L. (Watermelon) and *Cucumis melo* L. (Melon). *D.Ü.Ziya Gökalp Eðitim Fakültesi Dergisi* **9**:104-114

Shankar, S., More, S.V., Laxman, R.S. (2010) Recovery of silver from waste x-ray film by alkaline protease from *Conidiobolus coronatus*. *KUSET* **6(1)**:60-69.

Sharmin, F., Rahman, M. (2007) Isolation and characterization of protease producing *Bacillus* strain FS-1. *Agricultural Engineering International: the CIGR e-journal* **9**.

Siddalingeshwara, K.G., Uday, J., Huchesh, C.H., Puttaraju, H.P., Karthic, J., Sudipta, K.M. (2010) Screening and characterization of protease from *Bacillus* sp. *IJABPT* **2(1)**:575-581.

Singh, J., Vohra, R.M., Sahoo, D.K. (1999) Alkaline protease from a new obligate alkaophilic isolate of *Bacillus sphaericus*. *Biotechnol Lett* **21**: 921-924.

Singh, J., Batra, N., Sobti, R.C. (2001) Serine alkaline protease from a newly isolated *Bacillus* sp. SSR1.*Process Biochem* **36**:781-785.

Singhal, P., Nigam, V.K., Vidyarthi, A.S. (2012) Studies on production, characterization and applications of microbial alkaline proteases. *IJABR* **3(3)**:653-669**.**

Sjodahl, J., Emmer, A., Vincent, J., Roeraade, J. (2002) Characterization of proteinases from *Antarctic krill (Euphausia superba)*. *Protein Expres Purif* **26(1)**:153-161.

Smith, E.L. (1951) Proteolytic enzymes. *In The enzymes*, Sumner JB, Myrback K (eds), Academic Press, Inc., New York **1(2)**:793-872.

Takami, H., Akiba, T., Horikoshi, K. (1989) Production of extremely thermostable alkaline protease from *Bacillus* sp. No. AH-101. *Appl Microbiol Biotechnol* **30**:120-124.

Underkofler, L.A., Barton, R.R., Rennert, S.S. (1958) Production of microbial enzymes and their applications. *Microbiological Process Report* **6**:212-221.

Varela, H., Ferrari, M.D., Belobrajdic, L., Vázquez, A., Loperena, M.L. (1997) Skin unhairing proteases of *Bacillus subtilis*: production and partial characterization. *Biotechnol Lett* **19**:755-758.

Venil, C.K., Lakshmanaperumalsamy, P. (2009) Taguchi experimental design for medium optimization for enhanced protease production by *Bacillus subtilis* HB04. *e-Journal of Science & Technology*; **4**:1-10.

Ward, O.P. (1985) Proteolytic enzymes. In: M Moo-Young (Ed) *Comprehensive Biotechnology: The practice of biotechnology current commodity products*. Pergamon Press Oxford **3**: 789-818.

Yandri, Y., Suhartati, T., Herasari, D., Hadi, S. (2008) The chemical modification of protease enzyme isolated from locale bacteria isolate, *Bacillus Subtilis* ITBCCB148 with Cyanuric Chloride-Polyethylenglycol. *Eur J Sci Res* **23(1)**:177-186.

Yanga, J.K., Shihb, I.L., Tzenge, Y.M., Wanga. S.L. (2000) Production and purification of protease from a *Bacillus subtilis* that can deproteinize crustacean coasts. *Enzyme Microb Tech* **26**:406-413.

Zambare, V.P., Nilegaonkar, S.S., Kanekar, P.P. (2007) Production of an alkaline protease and its application in dehairing of buffalo hide. *World J Microb Biot* **23**: 1569-1574.

Zambonino Infante, J.L., Cahu, C.L., Peres, A. (1997) Partial substitution of di- and tripeptides for native proteins in sea bass diet improves *Dicentrarchus labral* larval development. *J Nutr* **127**:608–614.