



Review

A brief review on production of Industrial protease through Organic waste

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Abstract: Proteases are basic in numerous natural cycles, for example, cell development, separation or sustenance, and are fundamental biocatalysts in all types of life on earth. Proteases have been broadly utilized for a huge assortment of utilizations, mostly in the cleanser and food enterprises, yet in addition in the drug business as new apparatuses for medication. In this work wild strain isolation, screening, identification, optimization of fermentation process and purification of acidic protease. The Identified microorganisms will be subjected to fermentation process optimization with the associated parameters for the acidic protease production and purification. This work will include a number of microbial strains susceptible for protease production characteristics. These agro-industrial residues are inexpensive potential substrates for protease production substrate-based media.

Keywords: protease, enzyme, solid state fermenter for enzyme, agro-industrial residues, Organic waste

Introduction:

Protease enzyme- it's also called proteinase, or peptidase, a group of enzymes that tear lengthily chain molecules of proteins in shorter fragments (peptides) and formally within their segment, amino acids. Proteolytic enzymes are found in bacteria, archaea, certain types of algae, some viruses, and plants; they are most abundant, however, in animals. Any enzyme that performs proteolysis that is begins protein catabolism by hydrolysis of the peptide bonds that link amino acid together during a polypeptide chain these enzyme passes catalytic activities in broad range of temperature 15-100 degree Celsius and pH (0-14).

Source of proteases

- Plant protease- papain, bromelain, keratinases, ficin
- Animal protease - trypsin, chymotrypsin, pepsin, rennin.
- Microbial protease-bacterial-natural and alkaline proteases,
- Fungal-acid, neutral, alkaline,
- Viral-serine, aspartic, cysteine peptidases (all are endopeptidases)

Major bacteria, fungi producing alkaline proteases Microorganisms having protease

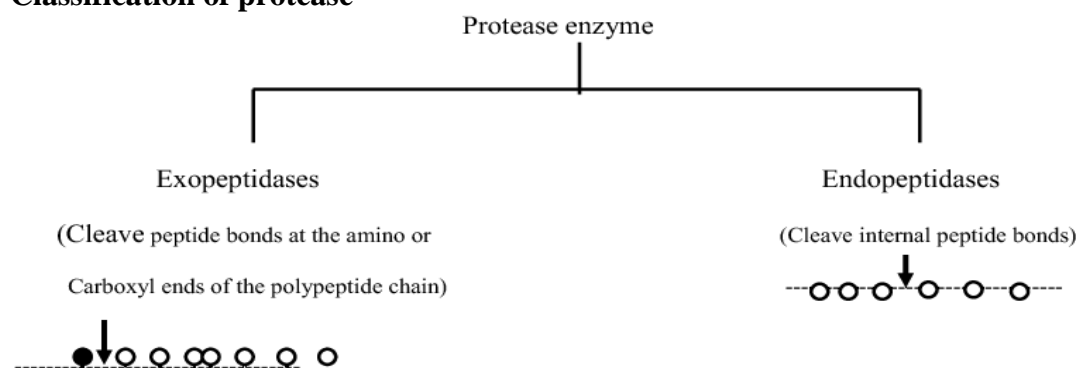
activity (Kumar CG, 1999; Aruna K et al.2014)

Bacteria- *Bacillus clausii*, *B. cereus*, *B. licheniformis*, *B. sphaericus*, *B. subtilis*, *B. sterothermophilus*, *B. mojavensis*, *B. megaterium*, *B. brevis*, *B. anthracis*, *B. thuringiensis*, *B. circulans*, *B. coagulans*, *B. marmarensis*, *B. firmus*, *B. stratosphericus*, *B. polymyxa*, *B. Lentus*, *B. alcalophilus*, *B. amyloliquifaciens*, *B. subtilis*, *B. intermedius*, *B. thermoruber*, *Bacillus pumilus*, *B. cohnii*, *B. fastidiosus*, *B. pseudofirmus*, *B. pantotheneticus*, *B. aquimaris*, *B. proteolyticus*, *B. laterosporus*, *B. coagulans*, *B. amovivorus*, *B. flexus*, *B. horikoshii*, *Pseudomonas aeruginosa*, *P. fluorescens*, *P. putida*, *Aromonashydrophila*, *Serratia*

liquefaciens, *Flavobacterium balustinum*, *Exiguobacterium* sp

Fungi- *Aspergillus awamori*, *A. clavatus*, *A. flavus*, *A. fumigates*, *A. niger*, *A. oryzae*, *A. parasiticus*, *A. ustus*, *Beauveria bassiana*, *B. feline*, *Botrytis cinerea*, *Clonostachys rosea*, *Conidiobolus coronatus*, *Cordyceps militaris*, *C. sinensis*, *Fusarium oxysporum*, *Graphium putredinis*, *Mucor circinelloides*, *Penicillium camemberti*, *P. citrinum*, *P. restrictum*, *P. roqueforti*, *Phanerochaete chrysosporium*, *Rhizomucor sp.*, *Rhizopus SMC*, *R. oryzae*, *Thermoascus aurantiacus*, *T. aurantiacus*, *Thermomyces lanuginosus*, *T. lanuginosus*, *T. lanuginosus*, *Trichoderma harzianum*, *T. reeseibroad* range of temperature 15-100 degree Celsius and pH (0-14).

Classification of protease-



Exopeptidases-

Enzyme that catalyzes the removal of an amino acid from the end of a polypeptide chain, which is sub divided by aminopeptidases, carboxypeptidases, Aminopeptidases 1 from *Escherichia coli* is large protease (400000 Da). It has a broad ph optimum of seven .5 to 10.5 and requires Mg^{2+}

The *Bacillus licheniformis* amino peptidase has a molecular weight of 34000.it contain 1 gram atom of Zn^{2+} per mole and its activity is enhance by Co^{2+} ions.

Endopeptidases-

An enzyme that catalyzes the cleavage of a polypeptide or protein at interior positions

of the amino alkanolic acid chain, which is subdividing into serine proteases, Cystein proteases, Aspartic proteases, Metalloproteases serine proteases.

Application of protease enzyme-

Proteases play vital role in food biotechnology, food processing & detergent industry, leather industry, pharmaceutical industry.

Food industry

Alkaline proteases have been used in the preparation of protein hydrolysate, which has high nutritional value. There are various kinds of protein hydrolysates, which can be prepared from food proteins. The protein hydrolysate plays an important role in blood pressure regulation and is

used in infant food formulations, specific therapeutic dietary products, and the fortification of fruit juices and soft drinks.

Leather Industry

Leather processing implicated several steps such as soaking, dehairing, bating, and tanning. The major ingredient of skin and hair are proteinaceous. The use of proteases as possible choice to dangerous chemicals such as Na_2S has proved prosperous in improving leather quality & in reducing environmental pollution. Proteases are used for selective hydrolysis of non-collagenous constituents of the skin & to remove non-febrile proteins such as albumins & globulins. Currently, microbial alkaline proteases are used to ensure quick absorption of water & to decrease time required for soaking

Detergents

Proteases are one of the watched ingredients of all kinds of detergents ranging from domestic laundering to reagents used for cleaning contact lenses or artificial teeth. The use of proteases in the washing detergents accounts for almost 25% (around 13 billion tons per year) of the total international sales of enzymes. The perfect detergent protease should obtain broad substrate particularly to facilitate removal of a large diversity of stains due to blood, food & other body discharge.

Pharmaceutical Industry -

The wide variety & specificity of proteases is profitably utilized in developing effective therapeutic agents. Oral authoritative of proteases from *Aspergillus oryzae* (Luizym, Nortase) has been used as the digestive aids to accurate certain lytic enzyme Microbes are good sources of protease. Since proteases are enzymes of metabolic also as commercial importance, there is a huge literature on their biochemical & biotechnological aspects (Ward 1983; Kalisz 1988). Alkaline proteases created by thermophilic & alkaliphilic bacilli can with set & high

temperature, pH chemical denaturing agents in non-liquid environments. Serine alkaline proteases as their activity are powerfully inhibited by Di isopropyl methyl fluorophosphate & PMSF, created by alkalophilic bacillus strains are far more active & stable at considerably higher pH than the subtilisin Carlberg & BPN(EC 3,4,21,14) (Takii et al. 1990 & Joo et al.2003).

The use of alkaline protease as an active element in washing detergent is well known (Sinha & Satyanarayan, 1991). Alkaline protease is also generally used in leather processing medical disorder, improvement of silver from X-ray films (Fujiwara et al, 1991), meat, photographic & dairy industries (Layman 1986 & Kalisz 1988), still only protease from bacillus have been found satisfactory in detergents & which is the single greatest application of these enzymes, a pharmaceutical product such as contact lenses enzyme disinfectants & enzyme debris, now in the application the food & drug organization has authorized a number of enzyme disinfectants of use with soft contact lenses. These disinfectants contain the active enzymes papain, pancreatin, subtilisins.

The photolytic enzymes also propose a gentle & selective debridement, encouraging the natural restorative technique in the successful local management of skin ulcerations by the effective removal of the necrotic material. Bacilli species have been successfully used in cleavage of proteinaceous wastes into useful biomass by proteases have also been displaying by many investigators. Most of the proteases used in waste bioconversion are alkaline proteases, a protease producing microorganism bacillus subtilis Y-108 used for deproteinisation of crustacean wastes in the preparation of chitin (Germano et al.2003).

Genetic Engineering of Alkaline Proteases:

Alkaline proteases from alkaliphilic bacilli sp 221 have been reported by (Harikoshi 1971) because of the first enzyme with alkaline pH optimum produced by an alkaliphilic microorganism. This strain was identified as *Bacillus alcalophilusvedder* (ATCC 24522). The bacillus strain 221 produces an alkaline protease that features a high optimum pH (11.5-12) Thermostability at highly alkaline pH and stability to detergents. Since the primary report on alkaline protease from alkaliphilic bacillus sp 221 was published there are extensive studies of the properties of alkaline protease from other stains (Horikoshi 1971). As the first enzyme with alkaline pH optimum produced by an alkaliphilic microorganism. This strain was identified as *Bacillus alcalophilusvedder* (ATCC 24522).the bacillus strain 221 produces an alkaline protease from alkaliphilic Bacillus sp 221 was published there are extensive studies of the properties.

Gene for alkaline serine protease from neutrophilic bacilli like *Bacillus amyloliquifaciens*, *Basillus licheniformis* (*subtilisin Carlsberg*), *Bacillus subtilis* and *Basillus subtilis* var. *amylosacchariticus* (Yoshimoto et al.1988) are cloned and sequenced. Nucleotide and amino acid sequence of those subtilisin type enzyme share significant homology aminoalkanoic acid sequence of those subtilin type enzymes share significant homology although these enzymes are distinct from one another in their enzymatic and physicochemical properties. The gene encoding 221 alkaline protease to also structural and functional comparisons with the genetic approach. The cloning sequencing and expression of the gene for the 221 alkaline protease have been done.

Solid State Fermentation of Microbes-

Solid-state fermentation of microbes-SSF is an old technique of cultivating the microorganism on a solid substrate without

free flowing of water. the research about SSF has been neglected not only due to the recognition of the submerged cultures process but also for the difficulties related to the measurement of parameters in SSF, like microbial biomass substrate Consumption concentration of the products, formed also because the measurement of the physical properties of the system for example instance measurement of growth of the organism in solid culture is far harder than in liquid culture. In past 15 to twenty years there has been a resurgence in research in batch solid-state fermented like the assembly of protein-enriched animal feed from starchy material single cell protein from agriculture and forestry waste ethanol from cassava roots or from sugar beets.

SSF is generally simpler process and requires less pre processing energy than submerged fermentation .further the initial capital costs are less for SSF. Other advantages are superior productivity, low wastewater output and improved product recovery. Unfortunately, SSF is usually slower because of the diffusion barriers imposed by the solid nature of the fermented mass. However, the metabolic processes of microorganisms are influenced to a great extent by the change of temperature, pH substrate, moisture, the supply of air, inoculums concentration etc. these conditions vary widely from species to species for each of the organism .so it becomes very important to know the environmental conditions of the microorganism for maximum production.

Enzymes Produced By SSF

Type of strain, culture conditions, nature of the substrate and availability of the nutrient are a critical factor affecting the yield and will be taken into consideration for choosing a specific production technique. Ideally, most know microbial enzymes are often produced under SSF systems. Literature survey reveals that much work has been administered on the

assembly of an enzyme or industrial importance, like proteases, celluloses, ligninases, xylanases, pectinases, amylases, glucoamylases etc and attempts re phenolic acid esterases, microbial rennets, acyl hydrolase, alpha -L-arabinofuranosidase etc using SSF system. Ssf has numerous advantages. It can produce concentrated enzyme solutions (Muniswaran et al 1994), it is an alternate cultivation system for the assembly of high-cost microbial products and fungi are widely utilized in SSF within the practical production of enzyme or chemicals.

In recent years, there are increasing attempts to supply different sort of proteases like acid neutral, alkaline pro through SSF route, using agro-industrial residues as shown in the table, which also shows the spectrum of microbial cultures employed for the production of proteases in solid-state fermentation system (Chakraborty and Srinivasan.,1993; Mitra et al ,1995; Ikasari and Mitchell,1994 and Germane et al.,1998)

Solid State Fermentation of Proteases

A lot of data is out there with fungal proteases. However little information is out there on the bacterial solid-state fermentation. There are reports available on bacterial proteases obtained with SSF protease account for nearly 60% of the economic market within the world. They find application during a number of biotechnological techniques, viz in food processing and pharmaceuticals, leather industry, detergent industry, etc Mitra et al (1994) reviewed production of proteolytic enzymes in SSF systems. From their Scale-up studies for the production of alkaline protease-various bioreactor types are utilized in SSF processes for over a decade including patched beds, rotating drums, gas-solid fluidized beds and various stirred bioreactors (Pandey,1990). However in 1990 no successful large scale bioreactor had been developed other than the 1 lit capacity stirred bioreactor.

A situation caused by the quantities strategies for the planning and operation of huge scale SSF bioreactors (David et al. 2000). This lack is evidenced by the qualitative nature of a review addressing the proportion of SSF processes published in 1991 (Lonsane et al., 1992). Although proportion methods for submerged liquid fermentation (SLF) were well developed, ranging from successful rules of thumb to semi fundamental methods. These methods couldn't be applied on to SSF bioreactors thanks to the differences within the physical structures of the systems. Heat removal may be a major consideration within the design of SSF bioreactors, whereas in aerobic SLF processes the availability of O₂ is typically the key problem to be overcome (David et al., 2000). Today significant advances are made towards the developments of quantitative proportion strategies for the ssf bioreactors, through mathematical modeling of the biological phenomena and mass transfer and warmth transfer phenomena, which occur (David et al. 2000).

Solid-state fermentation has gained importance recently thanks to several advantages over submerged fermentation. Bioreactor design aspect, which is important criteria, however, hasn't been given enough attention by the researcher of solid-state fermentation and therefore the present state of data doesn't indicate a perfect sort of bioreactor for solid-state fermentation. During a fermentation process, the bioreactor provides the environment for the expansion and activity of the microorganism which performs the biological reactions. During the number of fermentation, it should be capable of preventing the discharge of internal biomass/media into the environment also as preventing entry of foreign substances into the reaction media.

An ideal fermenter should have several characteristics, in particular, the fabric of

construction should be non-toxic and ready to withstand pressure (generally pressurized steam for sterilization). It shouldn't be suffering from chemical corrosion. There should be proper arrangements for aeration /agitation, with sampling, charging and discharging ports. a cooling mechanism could also be required to regulate the generated metabolic energy. Further, bioreactor systems should be capable of operating under aseptic conditions.

In many cases, although composting is treated as a non –sterile (natural inoculum) ssf (solid-state fermentation in the absence or near absence of free water), development in composting technology is in some ways well beforehand of SSF with pure cultures.

Although there are numerous designs for industrial fermenters using liquid fermentation, only limited development has been made in processes using SSF. Recent developments in process control, using sophisticated computer-controlled monitoring in liquid fermentations has given dynamic control of the method, while solid-state fermentation lags far behind. Examples of successful scaling – up of the processes using defined medium include those from shake flasks TP small reactors for producing a recombinant therapeutic protein (Prakasham et al, 2006), and from shake –flask to pilot fermenters for the assembly of hepatitis B surface antigen by recombinant *Penicillium pastoris*. Employing a chemically defined medium and a computer-controlled fed-batch strategy, Knorr et al., (1991), reportedly experienced no problem in scaling up a recombinant *Escherichia coli* process from 70 l to the 300 l and therefore the 1500 l scale, achieving high cell density (more than 100 g dry cells/l). The spectrum of microbial cultures employed for production of protease in solid-state fermentation systems-

Isolation of Strain, Production And Characterization Of Alkaline Protease-

The thermoalkalophilic *Bacillus* sp. Jb 99 was isolated from sugarcane molasses and was cultured and maintained on chemically defined medium. The optimum pH and temperature are 10 and 50-degree Celsius (Johnvesly and Naik 2001). Citric acid was the major carbon source and yielded a maximum of 127n 80u/ml, were found best carbon sources, while NaNO₃ (12780 U/ml) and KNO₃ Were found best nitrogen sources.

The complete suppression or repression of synthesis of alkaline protease also noticed with 1% (w/v) glucose. The stain was also grown well in several agro-industrial substrates 1% (w/v), pigeon pea waste, pineapple waste; orange peel waste, sugarcane bagasse, rice bran and wheat bran were added in preliminary studies to work out the assembly of alkaline protease.

In the presence of 1% (w/v) pigeon pea waste, pineapple waste and orange rind waste, maximum protease activity 12,430+120 u.11450+95 u and 10560+80 u/ml was obtained, respectively. Pigeon pea waste was found to be most potential substrate for production of a thermostable alkaline protease from *Bacillus* sp. Jb -99 (Jahnvesly et al., 2002).

India may be a major pigeon pea growing country within the world having 80% of the planet production .pigeon pea grains (rich in nitrogen content and carbon source) are rich in protein (20.4%) soluble sugar (5.2%), oligosaccharides like stachyose, raffinose, verbascose and polyphenolic compound (Johnvesly et al., 2002). pigeon pea processing units (DHAL MILLS) are located in an around Gulbarga city, Karnataka, India. These agro-industrial residues are inexpensive potential substrates for protease production substrate-based media. The enzyme secretion was growth associated. It reached a maximum activity after 15h

fermentation and remained constant for 36h of incubation. These results are almost like reports on the production of alkaline and warmth stable protease from an alkalophilic *Bacillus* sp (Yeoman and Edwards 1997 and Gessesse 1997)

The optimum temperature and pH for proteases activity was 70-degree Celsius and 11.0 respectively. the addition of 10 mM Ca^{2+} enhanced the optimum temperature 80-degree Celsius and retained 78% activity even after 1 heat treatment at 80-degree Celsius. Proteolytic activity was completely inhibited by 1 mM PMSF and TPCK showed that it seems to be trypsin-like serine alkaline proteases. the enzyme activity was enhanced within the presence of 10 Mm metal ions namely Mn^{2+} , Mg^{2+} , Cu^{2+} AND Co^{2+} and activity also inhibited within the presence of 10 Mm metal ions like Fe^{3+} , Hg^{2+} and Zn^{2+} .the enzyme is stable within the presence of fifty H_2O_2 .

Conclusion:

The production of protease using organic waste will create a new path for researcher working in areas of industrial microbiology and food biotechnology. This will also includes the paths for its industrial application like tanneries for unhairing hides and pelts and as an immobilized cell for the assembly of the enzyme. This study will focus on acidic aspects of the Protease with greater stability. The studies will focus on coming innovation to this research and its applications.

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