



Review article

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The Importance of Alkaline Protease Commercial Applications: a Short Review

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ABSTRACT

Enzymes are well known biocatalysts by which chemical reactions can be catalyzed and commercially accelerate and facilitate the processes in different industries such as detergents, food, drugs and disease detections. Among 3000 enzymes, most of them have been extracted from mesophyll microorganisms. Application of microbial enzymes belongs to Ancient Greek era, the time they used enzymes in bakery products, alcohol production, cheese production etc. one of the main group of enzymes with many applications is protease. This group of enzyme categorizes in two groups, endo and exo enzymes. With respect to this fact that a live cells take benefits from proteases, the role of them in synthesizing of live molecules in cells is a must. This article reviews alkaline proteases types as one member of endo enzymes proteases.

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1. INTRODUCTION

Microbial proteases: Proteases define as the main hydrolytic enzymes which are taking into account as vital components of all organisms like molds, plants and animals. Among them microorganisms due to fast growth, need to limit space for culturing and genetically manipulation in order to production of new enzymes with new properties and therefore microbial protease which have lower cost have contained two third of global market. While production is one of the instinct properties of all organisms but only microbes can extra cellular produce protease with commercial target. Microbes are good source of proteases since mentioned enzymes are commercially important, a bulk of researches is available in literature. Alkaline protease is produced by thermophilic basils with high resistance to pH and denaturing agents in non-aquatic media. Alkaline protease produced by alkalophylic bacillus is more resistance to High pH conditions^{1,2}. Application of Alkaline protease as an additive in washing detergents has been well reputed³. In addition, alkaline proteases are widely used in industries such as silver recovering⁴⁻⁶. However only bacillus's proteases can be used in

detergents. Bacillus varieties are also introduced successfully in decomposing of protein wastes⁷. Proteolysis defines as the process of hydrolysis of peptide bonds by protease which produces protein pieces, peptides and free amino acids. Proteases can be found in all of live organisms and are too critical and vital. This enzyme has not only important roles individually, but they have commercially and functionally increasing in different industrial branches.

Classification of Proteases: Proteases are categorizing based on their three Amin properties.

- catalyzing reaction
- the nature of catalytic site
- evolution relations

Proteases classifying into exo including carboxyl peptidase and amino peptidase and endo including Serineprotease, aspartic protease, metalo protease and cyctein thiol protease. For thousands of years, fermentative products have been consumed. Within First World War, the importance of microorganism has highlighted via introduction of some products like, enzymes, amino aacids, nucleotides, vitamins, and

organic acids. Enzymes contain the main parts of the products which are used in chemical and food industries⁸.

Proteases are the main constituents of removing contaminant of environment⁹. In addition the turnover of enzyme industry has been 220 million USD¹⁰. Alkaline protease is the main group of proteases and contain %35 percent of microbial enzymes. Affecting parameters on enzymes are microorganism, the combination of surrounded media and physicochemical parameters such as pH, temperature and oxygen transferring.

Those microorganisms can be selected with ability of high protein production and no toxin. Bacillus varieties can produce protease extra cellular in high amounts¹¹. The production of an enzyme shows relationship of a characteristic and logarithmic phase of an organism. Protease production is controlled by complicated reactions and mainly can be said that its synthesis depends on high protein rate and storing¹².

A wide group of bacteria are known as protease producer but a low group of them are commercial producer¹³. Bacillus is positive gram spore rod shape bacteria with a more heat resistance endospore compared to vegetative forms. Positive varieties are moving via flagella. They produce amylase and protease¹⁴. Some superior properties of Bacillus Subtilis in production of extra cellular protein are as below;

- being non toxic
- manipulating via genetic engineering methods
- easy growing comparing to other Eukaryote systems

The culture component of microbial media is of importance¹⁵. Mostly, microorganisms need to have amino acids, trace elements and vitamins. The culture conditions in which enzymes like proteases are enhanced their growing¹⁴.

Bacterial cells are composed of heavy polymers like polysaccharides, proteins and lipids. In order to form macromolecules, it is requested to be polymerized initial units¹⁶. In addition, biopolymers include non-organic salts such as nitrate, phosphate, and sulfate. A bacterial cell is composed of %50 Carbone, % 20 oxygen, % 14 nitrogen, %8 hydrogen, and % 3 phosphorous, % 1 sulfur¹⁷. Mainly, these enzymes are produced as a response to environmental components such as growth hormones, ions. Quality and quantity needs related to cells are of importance to create optimal growth conditions. Alkaline protease is made of % 53.8 Carbone and % 15.6 nitrogen. Production of protease is highly dependent to Carbone and nitrogen sources¹⁴. Different bacteria types can be cultured for each biophysics and biochemical targets. Regarding the bacteria requirements, different culture media have been developed¹⁸.

In case of commercial targets, combination of culture media is a way to achieve. Optimizing can help to non-utilized compounds in the final stages of fermentation. More researches have been focused on the followings;

1. Evaluation of Carbone and nitrogen nutrients on functionality and efficiency of enzymes.
2. Need to ions in fermentative medium.
3. Optimizing fermentation and media parameters such as pH, aeration temperature, agitation.

Glucose is frequently used in biological processes to produce protease. Due to catabolic loss of Glucose; protease may produce¹⁹. Mostly, microorganism can consume both organic and non-organic of nitrogen to produce amino acids, nucleic acid, proteins and other cell wall compounds²⁰. Complicated sources of nitrogen is usually used to create alkaline proteases²¹.

Ions like calcium, cobalt, copper, magnesium, are required in culture media of producing alkaline protease²². Buffering property is depends on the presence of salt. The optimized concentration of phosphate is 2g/l in case of protease production¹⁴. Regarding the buffer activity of phosphate ions leads to the pH stability of medium which is non-directly of protease synthesis. Optimization of physical parameters such as oxygen transferring, pH and temperature can effects on products from non-aerobic fermentation¹⁵.

The production of protease from waste: Waste defines as any useless material however it may that a waste can be a valuable source of other factories when we separate them from effluent²³. Solid waste is a term refers to non-domestic, industrial wastes. One of the main problems related to developed countries is human wastes. The most challenge is their availability in environment, the most concerns related to water resources. The entrance of solid and liquid domestic, agriculture and industrial wastes effect on public health. Foodstuffs also can consider as wastes in case of spoilage or non-healthiness. Proteases treat aforementioned waste sources²⁴.

Separation and purification: The time of separating enzymes is too important in calculating costs. Sedimentation is the most common methods for separating and recycling²⁵. However the sediment of aluminum sulfate has been used for a long time, but only in alkaline and neutral pH has been usable²⁶. Despite better sedimentation properties of sodium sulfate; it has been limited due to low solubility in lower temps²⁷.

Concentration: Since the amount of free cell filtrate is in low level thus removing of water is first stage. In this regards separating process are in the maximum level²⁸. Ultrafiltration is a mini layer process widely used for recycling of enzyme²⁹. This separation process is inexpensive with low level of enzymes lost and presents

both concentration and purification³⁰. However the defect of this method is sedimentation or clogging of membrane which is removable by means of detergent, treatments, and proteases of acid / base³¹. A hydrogel ultra-filter is sensitive to temperature degree of alkaline protease concentrating. The efficiency of enzyme recycling depends on temperature (the best is in 15 and 20°C).

2. CONCLUSION

Microbial metabolism is a complicated process with possibility of playing role in production of biomass. Hence evaluation of complete metabolic of process is often difficult or impossible, if the result of common fermentation is available. Achieving to produce more alkaline protease along with reduction of processing cost is too important.

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