Eco. Env. & Cons. 30 (August Suppl. Issue) : 2024; pp. (S120-S123)
Copyright@ EM International
ISSN 0971–765X

DOI No.: http://doi.org/10.53550/EEC.2024.v30i05s.020

Screening, isolation and production of alkaline protease from various agro-industrial wastes by using taguchi experimental design

Pukhraj Meena[1*] and Vikash Singh[1]

[1]Department of Food Technology, Faculty of Agriculture Science & Technology, AKS University, Satna 485 001, M.P., India

(Received 24 January, 2024; Accepted 28 March, 2024)

ABSTRACT

Proteases are the most important industrial enzymes that execute a wide variety of functions and have various important biotechnological applications. Commercial application of alkaline proteases is attractive due to the relative ease of large-scale production as compared to proteases from plant and animals. Reduction in the production cost of enzymes could greatly reduce the cost of the enzyme. Taguchi DOE methodology revealed 51% enhanced alkaline protease production compared to average performance of the fermentation and for improvement of biological processes. The use of a good reliable statistical model is essential to develop better strategies for the optimization of the fermentation process

Key words: *Alkaline protease, Fermentation, Biological processes, Taguchi DOE methodology.*

Introduction

Proteases are produced wide spread in nature such as by plants, animals and microorganisms (Rao *et al.,* 1998). More than 3000 different enzymes described to date the majority have been isolated from mesophilic organisms. These enzymes mainly function in a narrow range of pH, temperature and ionic strength. The microorganisms are an important source of enzymes, whose specific properties are expected to result in novel process applications (Kumar *et al.,* 1999).

Proteases are industrially important enzymes and constitute a quarter of the total global enzyme production. The role of enzymes in many processes has been known for a long time. Their existence was associated with the history of ancient Greece where they were using enzymes from microorganisms in baking, brewing, alcohol production, cheese making etc. With better knowledge and purification of enzymes the number of applications has increased manifold, and with the availability of engineered enzymes a number of new possibilities for industrial processes have emerged (Beg *et al.,* 2003).

Application of Alkaline Proteases

Almost all proteases are heat resistant (Mahler *et al.,* 1957) vary widely in their Specific activities. Protease catalyzes the hydrolysis of peptide bonds that link Amino acids together in the polypeptide chain forming a protein molecule. Alkaline Protease is defined as that protease that is active in a neutral to alkaline pH Range. They are either a serine protease or metalloprotease. Alkaline protease occupy a pivotal position due to their wide application in food processing (Pastor *et al.,* 2001), pharmaceutical industries (Anwar *et al.,* 1998), meat tenderization process (Wilson *et al.,* 1992), peptide synthesis (Kumar

and Hiroshi, 1999), instant formula preparation (American academy of pediatrics committee on nutrition, 1989), leather processing (George *et al.,* 1995) and in weaving processing (Helmann *et al.,* 1995).

Molecular weight of the enzyme was 30,000 Da, which is slightly higher than those of other alkaline proteases. The addition of a 5mM solution of calcium ions increased the activity by 70% at the optimum temperature of 60 ºC. Moreover, various types of alkaline protease have been characterized and their potential industrial applications have been explored. The major applications of these enzymes are in detergent formulation, the food industry, leather processing, chemical synthesis and waste management (Gupta *et al.,* 2002a).

Factor Affecting Enzyme Activity

The cost of Enzyme is also the most critical factor limiting wide use of alkaline proteases for different applications. A large part of this cost is accounted for the production cost of the enzyme. Therefore, reduction in the production cost of enzymes could greatly reduce the cost of the enzyme. In submerged fermentation up to 40% of the total production cost of enzymes is due to the production the growth substrate. In this regard, SSF which uses cheap agricultural residues have enormous potential in reducing enzyme production cost. So, studies on alkaline protease that are produced in SSF by alkaliphilic microorganisms are scarce in literature. As a result, it is of great importance to pursue such studies.

Source of Protease Production

Microorganisms are the most important sources of enzyme production. Bacillus species possess remarkable biotechnological value due to their nonpathogenicity, ability to produce extracellular protease in large amounts, easily to handle & downstream processing for enzyme purification and great commercial value.

Agro-industrial wastes: Generation and composition

Agro-industrial wastes, generally, include the wastes generated during the industrial processing of agricultural or animal products or those obtained from agricultural activities in the form of straw, stem, stalk, leaves, husk, shell, peel, lint, seed, pulp, legumes or cereals (rice, wheat, corn, sorghum and barley), bagasse from sugarcane or sweet sorghum milling, spent coffee grounds, brewer's spent grains, and many others. These wastes are mainly composed of sugars, fibers, proteins, and minerals. The chief constituents of such agro-industrial wastes include cellulose, hemicelluloses and lignin, collectively being called "lignocellulosic materials" (Mussatto *et al.,* 2012). Individual wastes display a range of relative of proportion of these major constituents. This variability entails certain level of specificity/preference of their use.

Sources of Major Agro-industrial By-products

The major by-products of cereal processing are the bran and the germ obtained during both the wet and dry milling processes. During the processing of wheat, coarse and fine middling are also obtained as the by-products. The major by-products arising from the maize processing industry, besides the germ and the bran, are the gluten meal obtained during the wet milling. Apart from these, another by-product obtained during the wet milling of corn is corn steep liquor, which is used as a nutrient source in feed and fermentation processes. It is a complex broth composed of carbohydrates, proteins, organic acids, minerals, etc. (Awarenet *et al.,* 2001-2004).

Sources of Alkaline Proteases

Alkaline proteases are obtained from various microbial sources such as bacteria, fungi and certain yeasts. Of all the microbial sources, bacterial proteases are of particular interest due to their various applications in industries such as detergent, textile, leather food and feed industry. A major source of bacterial alkaline proteases is *Bacillus* species, which has been studied extensively. Some fungal species are also known to produce alkaline proteases of industrial use of which *Aspergillus* species has been extensively studied. A very few studies exist on yeast species.

Recently, alkaline proteases from mushrooms have also been studied and purified. Some halophilic sources have also been screened for the secretion of alkaline proteases. Halophilic enzymes find rapidly increasing use in biotechnological applications owing to their halotolerance, thermostability for long incubation periods and capability to retain activity in presence of high levels of organic solvents (Madern *et al.,* 2000 and Jerry *et al.,* 2001). Extracellular alkaline proteases from halophilic bacteria with high pH and thermostability, organic solvent stability and compatibility with detergents have

been reported.

Production of Alkaline Proteases

The Taguchi approach of orthogonal array experimental design for bioprocess optimization is a good reliable statistical model. Taguchi experimental design for alkaline protease production by *Bacillus clausii* and reported that this experiment design provided basic information to improve the efficiency of protease production and also supported the analysis of the main effect of each constituent of the medium and concluded that his study would serve as another example for the application of the Taguchi methodology for improvement of biological processes (Seyedeh *et al.*, 2007).

Pseudomonas aeruginosa was used to produce alkaline protease by utilizing available agricultural residues (wheat bran) via solid state fermentation (SSF). Taguchi (DOE—Design of Experiment) was successfully applied to test the relative importance of medium components and environmental factors on alkaline protease production. The optimized conditions showed an enhancement in alkaline protease activity by 28.8% (from 450.0 to 582.2579.2 U/ml). The experimental data showed 97.24% resemblance with the expected data (Meena *et al.*, 2013).

Inability of Plant and Animal Proteases

Current world demands have led to an increased interest in microbial proteases. Proteases of bacteria, fungi and viruses are increasingly studied due to its importance and subsequent applications in industry and biotechnology. Commercial application of microbial proteases is attractive due to the relative ease of large-scale production as compared to proteases from plant and animals (Rao *et al.*, 1998).

Most of the alkaline proteases that play a role in industries are thermostable as their optimal activity lies between 500°C to 700°C. The recently used statistical methods have given way to a more rapid optimization process for alkaline protease production. Other than traditional industrial uses, alkaline proteases have promising application in feather degradation and feather meal production for animal feed.

Screening and Isolation of Alkaline Proteases

All the alkaliphilic bacteria that have been screened for use in various industrial applications, members of the genus *Bacillus*, mainly strains *B. subtilis* and *B. licheniformis* were found to be predominant and a prolific source of alkaline proteases (Kumar and Takagi *et al.*, 1999).

Production of alkaline protease employing the laboratory isolates, *Bacillus* sp. under solid state fermentation. The effect of wheat bran and lentil husk was examined. Wheat bran showed highest enzyme production. Maximum yields of 429.041 and 168.640 U g^{-1} were achieved by employing wheat bran and lentil husk as substrates in 0.1 M carbonate/bicarbonate buffer at pH 10 with 30 and 40% initial moisture level at 24 h. Inoculum size and buffer solution level were found to be 20 and 25% and 0.5:1 for wheat bran and lentil husk, respectively (Uyar *et al.*, 2004).

Alkaline protease production using isolated *Bacillus circulans* under solid-state fermentation environment was optimized by using Taguchi DOE methodology. The software-designed experiments with an OA worksheet of L-27 was selected to optimize fermentation (temperature, particle size, moisture content and pH), nutrition (yeast extract and maltose) and biomaterial-related (inoculum size and incubation time) factors for the best production yields. DOE methodology revealed 51% enhanced protease production compared to average performance of the fermentation (Prakasham *et al.*, 2005).

The use of a good reliable statistical model is essential to develop better strategies for the optimization of the fermentation process (Ghaley *et al.*, 2005).

Taguchi experimental design for alkaline protease production by *Bacillus clausii* and reported that this experiment design provided basic information to improve the efficiency of protease production and also supported the analysis of the main effect of each constituent of the medium and concluded that his study would serve as another example for the application of the Taguchi methodology for improvement of biological processes (Seyedeh *et al.*, 2007).

Conclusion

Alkaline proteases with keratinolytic activity are finding an interesting application in the feather degradation for reducing poultry waste and feather meal production for fertilizers, glues, biodegradable films, and foils. Through this review, an attempt has been made to highlight the recent advances in the field of alkaline proteases and their applicability.

The utilization of agro-industrial wastes for microbial fermentation not only improves the process economics, but can also reduce the environmental

issues related with their disposal. Although various microbial sources have been used for enzyme production, yet new and efficient microbial sources are needed for the optimal utilization of agro-industrial wastes to achieve higher yields of the enzymes. Furthermore, the biotechnological techniques such as genetic engineering of the micro-organisms, enzyme engineering, and other techniques would play an important role in the near future.

Conflict of Interests

The authors declare that they have no conflict of interests.

References

Anwar, A. and Saleemuddin, M. 1998. Alkaline proteases: a review. *Bioresource Technology*. 64: 175.

Awarenet, 2001-2004. *Handbook for the Prevention and Minimization of Waste and Valorization of By-products in European Agro-Food Industries.*

Barindra, S., Debashish, G., Malay, S. and 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, Q.K., Sahai, V. and Gupta, R. 2003. Statistical media optimization and alkaline protease production from *Bacillus mojavensis* in a bioreactor. *Process Biochemistry*. 39: 203.

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

Ghaly, A.E., Kamal, M. and Correia, L.R. 2005, Kinetic modeling of continuous submerged fermentation of cheese whey for single cell protein production. *Bioresource Technol.* 96: 1143-1152.

Gupta, R., Beg, Q.K, Khan, S. and Chauhan, B. 2002a. An overview on fermentation, downstream processing and properties of microbial alkaline proteases. *Applied Microbiology and Biotechnology*. 60: 381.

Helmann, J.D. 1995. Compilation and analysis of *Bacillus subtilis* SA dependent promotes sequences: evidence for extended contact between RNA polymerase and upstream promotes DNA. *Nucl. Acids Res*. 23: 2351-2360.

Jerry, E. 2001. Biotechnological uses of archaeal extremozymes. *Biotechnol Adv*. 19(4): 261-278.

Kumar, C.G. and Hiroshi, T. 1999. Microbial alkaline protease from a bioindustrial vino point. *Biotechnol. Adv*. 17: 561-594.

Kumar, C.G. and Tiwari, M.P. 1999. Use of alkaline proteases for ultrafiltration membrane cleaning. *Biotechnology Techniques*. 13: 235.

Kumar, C.G. 2002. Purification and characterization of a thermostable alkaline protease from alkalophilic *Bacillus pumilus*. *Letters in Applied Microbiology*. 34: 13.

Madern, D., Ebel C. and Zaccai, G. 2000. Halophilic adaptation of enzymes. *Extremophiles*. 4(2): 91-98.

Meena, P., Tripathi, A.D., Srivastava, S.K. and Jha, A. 2013. Utilization of agro-industrial waste (wheat bran) for alkaline protease production by *Pseudomonas aeruginosa* in SSF using Taguchi (DOE) methodology. *Biocatalysis and Agricultural Biotechnology*. 2: 210-216.

Mehler, H.A. 1957. *Introduction to Enzymology,* Academic Press Inc. New York.

Mohsen, F.N., Dileep, D. and Deepthi, D. 2005. Potential application of protease isolated from *Pseudomonas auriginosa* PD100. *Electronic Journal of Biotechnology.* 8: 197-203.

Mussatto, S.I., Ballesteros, L.F., Martins, S.F. and Teixeira, J.A. 2012. Use of agro-industrial wastes in solid state fermentation processes. *Intech, Europe*. 121-140.

Prakasham, R.S., Rao, C.S., Rao, R.S. and Sarma, P.N. 2005. Microbial alkaline proteases: Optimization of production parameters and their properties. *Biotechnology Progress*. 21(5): 1380-1388.

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

Rao, M.B., Tanksale, A.M., Ghatge, M.S. and Deshpande, V.V. 1998. Molecular and biotechnological aspects of microbial proteases. *Microbiology and Molecular Biology Reviews.* 62: 579-635.

Seyedeh, F.G.O., Fatemeh, T., Bagher, Y. and Fereshteh, E. 2007. Enhancement of alkaline protease production by *Bacillus clausii* using Taguchi experimental design. *African J Biotechnol.* 6(22): 2559 -2564.

Uyar, F. and Baysal, Z. 2004. Production and optimization of process parameters for alkaline protease production by a newly isolated *Bacillus* sp. under solid state fermentation. *Process Biochemistry*. 39(12): 1893-1898.

Wilson, S.A, Young, O.A, Coolbear, T. and Daniel, R.M. 1992. The use of protease from extreme thermophilic for meat tenderization. *Meat Sci.* 32: 93-103.