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REVIEW ARTICLE

Fish Waste-Potential Low Cost Substrate for Bacterial Protease Production: A Brief Review

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Abstract: Industrial biotechnology processes have recently been exploited for an economic utilization of wastes to produce value added products. Of which, fish waste is one of the rich sources of proteins that can be utilized as low cost substrates for microbial enzyme production. Fish heads, tails, fins, viscera and the chitinous materials make up the wastes from fish industries. Processing these wastes for the production of commercial value added products could result in a decrease in the cost of production. In addition, we can eliminate the pollution of the environment and health issues due to the improper disposal of these fish wastes. This review highlights the potential use of fish waste as a cheaper substrate for the production of economically important protease enzyme.

Keywords: Defatting, Fish waste, Pollution, Protease, Substrate, Value added products, Waste processing.

1. INTRODUCTION

Enzymes from microorganism are of high biotechnological interest due to their less complexity, rapid growth, ease of cultivation and genetic manipulation [1]. Proteases (EC 3.4.21-24) are enzymes capable of hydrolyzing the peptide bonds between amino acids of proteins. Proteases are one of the most important industrial enzymes, representing more than 65% of the world industrial enzyme sales [2]. Many microorganisms *Rhizopus oryzae*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Vibrio splendidus, Bacillus cereus, Bacillus licheniformis*, *Aspergillus* spp., *Penicillium* spp., *Trichoderma* spp. *Bacillus megaterium* and *Thermoactinomyces* spp. have been used to produce protease enzymes [1, 3 - 9]. Proteases with biotechnological potential have been produced by different microorganisms. Biosynthesis of enzymes by microorganisms is influenced by various factors including carbon, nitrogen sources, pH, temperature, *etc*. The economy of protease production is mainly dependent on the type of substrate, which plays a major role in decreasing the cost of production. Hence, the searching of easily obtainable, regularly available, low cost substrates is necessary to cut down the production cost. In this review, the applications of protease and the utilization of fish waste as a substrate for protease production will be discussed.

2. MICROBIAL PROTEASES

Microbial proteases are classified into different groups, namely, metallo-proteases (EC.3.4.24), aspartic-proteases (EC.3.4.23), cysteine-proteases or sulphydryl-proteases (EC.3.4.22) and serine-proteases (EC.3.4.21). This classification is based on their activity under acidic, neutral, or alkaline conditions and also depending on the characteristics of their active sites [10]. Alkaline proteases are defined as those proteases which are active in neutral to alkaline pH range. Mostly, they are either serine type or metallo-type [11]. Most of the industrially important thermostable bacterial enzymes are produced from members of the genus *Bacillus*. Mainly the strains of *B. subtilis* and *B. licheniformis* are of predominant interest as they are known to be good producers of thermostable proteases [12]. *Bacillus* species are gram-positive bacteria commonly found in soil [13] and produce proteases and other hydrolases

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either during the exponential growth phase or when the culture enters the stationary phase [14]. Microbes degrade the proteins and they utilize the degraded products as nutrients for their survival. Degradation is initiated by proteinases (endopeptidases) secreted by microorganisms followed by further hydrolysis by peptidases (exopeptidases) at the extra- or intracellular locations [15]. Each organism or strain has its own specific conditions for maximum enzyme production. Enzyme production has a characteristic relationship with the growth phase of that organism. The synthesis of protease in *Bacillus* species is controlled by numerous complex mechanisms operative during the transition state between exponential growth and the stationary phases [16]. A variety of proteases are produced by microorganisms depending on the species of the producer or the strains, even belonging to the same species.

3. APPLICATIONS OF PROTEASE ENZYME

Proteases are playing an important role in industries due to their wide application in leather and detergent industry, food and pharmaceutical industries and also in bioremediation processes [17]. Bacterial proteases are being produced in large scale due to their high stability, specificity and activity in a wide range of physical parameters [18]. More than 60% of the worldwide production of industrial enzymes are proteolytic enzymes. Among these 35% is comprised of alkaline proteases [19], which are extensively used in a wide range of industries such as food, pharmaceutical, detergent, cheese making, brewing, photography, baking, meat tenderization, cosmetics and leather [20, 21].

4. PROTEASE IN DETERGENT INDUSTRY

The major application of alkaline proteases is in the detergent industry due to their ability to aid in the removal of stains by hydrolyzing large protein molecules associated with tough stains. During the process of hydrolysis, the peptide bonds that hold various amino acids together to form a protein molecule are broken down, releasing smaller polypeptides and individual amino acid units. They work as scissors to cut off the stain physically piece by piece from the surface of the fabric [10]. Today, detergent proteases account for 89% of the total protease sales in the world out of which majority are alkaline proteases from *Bacillus* species [22]. The characteristic features of a good detergent protease are compatible with components such as surfactants, perfumes and bleaches in the detergent [23], good activity at washing pH and temperature [24], compatibility with the ionic strength of the detergent solution, stain degradation and removal potency, stability and shelf life [25, 26].

5. PROTEASE IN LEATHER INDUSTRY

Another important application of alkaline proteases is in the leather industry for dehairing. The alkaline nature of enzyme speeds up the swelling of hair roots, after which, the protease attacks the hair follicle protein facilitating the easy removal of hair [27]. Collagen is the main leather making protein, which exists along with other globular and fibrous proteins. The non-collagenous constituents have to be partially or completely removed in order to process leather. The extent of removal of these constituents determines the durability and softness of the leather. Earlier, various steps in leather processing such as soaking, liming, dehairing, deliming, bating, degreasing and pickling used to be carried out using toxic chemicals like lime, sodium sulphide, salt, and solvents which contributed to environmental pollution [28]. Proteases are good substitutes for these harmful chemicals because of their eco-friendly nature [29].

6. PROTEASE IN OTHER INDUSTRIES

Alkaline proteases are also used for the preparation of protein hydrolysates of high nutritional value which is widely used as feed additives [30]. The commercial protein hydrolysates are derived from casein, whey and soy protein. Alkaline proteases are also used in meat processing. SEB Tender 70, a commercially available protease is extensively used in meat tenderization to break down collagens in meat to make it more palatable for consumption [31].

Proteolytic enzymes are also used in the management of industrial and household wastes [9] and to remove pollutants [32] by solubilizing protein wastes and contaminants. X-ray films contain 1.5-2% silver by weight and proteolytic enzymes are used for recovering silver bound to gelatin in X-ray and used photographic films [33, 34]. Alkaline proteases are also used for contact lens cleaning [35], for the isolation of nucleic acid in molecular biology [36], pest control [37], degumming of silk [38, 39] and selective delignification of hemp [40]. In medicine, protein hydrolysates are administered to patients with digestive disorders and food allergies [41].

7. WASTE MATERIALS AS A SUBSTRATE FOR PROTEASE PRODUCTION

Industrial procedures are harsh and are often carried out in extreme conditions for which the enzymes have to be

more stable. The production of these commercial enzymes is carried out using expensive raw materials and techniques. Therefore, there is high demand in detecting inexpensive substrates for producing stable enzymes under cost effective conditions. Considerable interest has been shown in utilizing agricultural wastes as a substrate for microbial products [42]. Different waste materials have been used as substrates for protease production. Low-cost agricultural residues such as dried powder of wheat bran, rice bran and sugarcane bagasse, sugarcane molasses [43] and rice mill wastes [44] have been utilized as substrates for protease production. No defined medium has been standardized for the maximum production of proteases from different microbial sources.

8. FISH WASTES AND ITS SIGNIFICANCE

Fisheries generate a large amount of solid waste such as whole fish waste, fish head, viscera, tails, skin, bones, blood, liver, gonads, guts and some muscle tissues and the liquid waste consists of wastewater used during fish processing [17]. The average composition of fish waste consists of head (21%), gut (7%), liver (5%), roe (4%), backbone (14%), fins and lungs (10%) [45]. These wastes are rich in organic contents such as protein, bioactive peptides, collagen, calcium, gelatin, oil and enzymes which make this disposal complicated and more expensive [46]. Improper discarding by incineration and sea dumping can lead to pollution and other environmental issues [47].

Almost 75% of the worldwide fish production is utilized for human consumption and the rest 25% is considered as fish waste [17]. Fish wastes have been used conventionally to produce high protein rich animal feed by fermentation [48] and also for composting purposes [49]. However, recent advances in industrial biotechnological processes have paved way for economical and highly beneficial utilization of these wastes for mankind. Fish oil, which is rich in polyunsaturated fatty acids, is considered to be a healthy food product that has been produced from fish waste [50]. Fish skin or, cartilage provides excellent raw materials for the production of gelatin, which is used in food and pharmaceutical industries [25]. Fish hydrolyzates with high biological properties can be used in several fields ranging from medicine to aquaculture [51]. Fish wastes are available throughout the year and are rich sources of carbon and nitrogen, which can be effectively utilized for the synthesis of value added products through fermentation using microorganisms. Fish waste consists of 58% protein, 19% fat and trace amounts of minerals, mainly copper, phosphorus, magnesium, sodium, potassium, calcium, iron, zinc and manganese [52]. These elements are very useful for the growth of microbes as they act as cofactors for various metabolic activities. Commercially used substrates for protease production are casein, meat, gelatin and soy [4]. These expensive substrates are the reason for the high production cost of the enzyme and therefore, finding a suitable low cost medium and optimization strategies would economically benefit the production process on a large scale.

9. FISH WASTES AS SUBSTRATE FOR PROTEASE PRODUCTION

So far, fish wastes such as whole body, heads, viscera, chitinous materials from fish with shells, and also fish wastewater which are rich in specific growth factors and amino acids were used as substrates for enzyme production. However, each of the above said substrates was processed by different ways into a suitable form that could make up for the medium [17]. Most of the time, the fish wastes including head and viscera were first cooked, pressed to remove excess water, minced thoroughly and dried at different conditions and finally made into a powder, in case it is needed in solid form [5]. In several studies, fish wastes were boiled with water and the supernatants were used as substrate [53]. In addition, it was also reported that fish wastes subjected to defatting can enhance protease production due to the lipid free nature of the substrate [54]. Raw fish wastes have also been subjected to chemical treatments using acids, alkali and enzymes in order to obtain protein hydrolysates. After treatment, the processed wastes either in powder form or as supernatant were added to the basal medium for the production of enzyme. Some studies using fish waste as a substrate for protease production have been listed in Table **1**.

B. subtilis and *P. aeruginosa* were reported to produce significant amount of proteases when cultured in a medium containing powder from heads and viscera of sardines [4, 5]. Different processing methods were used on the viscera of rainbow trout, swordfish, squid and yellow fin tuna to produce peptones [56]. When this peptone is added in the medium to cultivate *Vibrio* species, it yielded high quantities of protease than media containing commercial peptones. *B. subtilis*, *P. aerigunosa*, *B. cereus*, *B. licheniformis* and *V. parahaemolyticus* showed enhanced protease activity in the medium supplemented with cuttle fish powder dissolved in fish wastewater [57]. Similarly, an increased level of protease activity by *B. cereus* was observed when cultured in media containing defatted tuna waste over raw tuna waste [54]. Evaluation of protease activity by *B. cereus* was also investigated on substrates including acid and alkali hydrolysates of tuna [53]. Acid hydrolysates were prepared by extraction using water, followed by hydrolysis in a dilute

acid, while alkali hydrolysates were prepared by recovering proteins through chemical extraction and isoelectric precipitation using HCl and sodium hexametaphosphate [58].

Table 1. Protease activity of microbial strains grown in media containing various fish waste substrates prepared by different processes.

Fish raw materials	Substrate preparation	Microbial strains	Protease activity (U/ml)	References
Heads and viscera of sardines	Cooked, pressed, minced and dried (80 °C, 24–48 h) and powdered	*B. subtilis* *P. aeruginosa MN7*	720 7,800	Ellouz *et al*. [4] Triki-Ellouz *et al*. [5]
	Ground with water and centrifuged to recover supernatant and used to prepare the peptone	*V. anguillarum* *V. splendidus*	35-68 9-30	Vázquez *et al*. [6]
Cuttlefish by-products diluted in fish waste water	Washed, boiled for 10-15 min, pressed, minced, dried (80 °C, 24–48 h) and powdered.	*B. subtilis,* *P. aerigunosa,* *B. cereus,* *B. licheniformis,* *V. parahaemolyticus*	178 1680 487 407 1607	Souissi *et al*. [7]
Raw tuna waste	Cooked, bones removed, pressed to remove water and fat, minced, dried (80 °C, 24–48 h) and powdered	*B. cereus*	74.77	Esakkiraj *et al*. [54]
Defatted tuna waste	Extracted using chloroform/methanol	*B. cereus*	134.57	Esakkiraj *et al*. [54]
Acid-hydrolyzed tuna waste	Extracted using water and hydrolysed with dilute acid.	*B. cereus*	60.37	Esakkiraj *et al*. [54]
Alkali-hydrolyzed tuna waste	Chemical extraction and iso-electric precipitation.	*B. cereus*	65.96	Esakkiraj *et al*. [54]
Whole sardinella powder (WSP), meat sardinella powder (MSP) and combined heads and viscera sardinella powder (CHVSP) from *Sardinella aurita.*	Cooked, pressed, minced, dried and powdered for WSP and CHVSP. Head and viscera removed, boiled, bones removed, cooked, pressed, minced, dried (80 °C, 24–48 h) and powdered for MSP.	*B. cereus BG1*	3000 for WSP 5273 for CHVSP 800 for MSP	Sellami-Kamoun *et al*. [55]

Shells rich in chitinous materials are also considered as waste when coming to fish processing and these are dried, ground and sieved to form a fine powder with very small diameter, to be used as substrate in the basal medium for microbial growth and enzyme production. Some of them include shrimp and crab shell powder, shrimp shell powder, chitin flakes of shrimp and crab shell and squid pen powder [6]. Several studies have been conducted on the use of these chitinous materials in protease production and the choice of the type of chitinous substrate to be added depended on the strain of the bacteria and the nutrient sources already available. One study reported that chitinous materials could be modified to enhance protease activity by treating with chemicals like acids or alkali [7].

One of the major limitations is to maintain the consistency of the composition of fish waste. In the case of same species, it can vary widely with respect to the region and the season of the catch. If different species are involved, much larger variations could be expected. Segregation of wastes from different species in large quantities is a tedious undertaking. Despite the known benefits of using fish waste as potential protease producing substrate, there are some constraints, especially with regard to the total cost efficiency of a scaled up process. It is true that cheap and simple substrates can reduce the cost of the material, energy consumption and labour, but at the same time sustaining high yield is an important factor that needs consideration.

CONCLUSION

Industrial procedures are harsh and are often carried out in extreme conditions for which the enzymes have to be more stable. The production of these commercial enzymes is carried out using expensive raw materials and techniques. Therefore, there is a high demand for finding inexpensive substrates to produce stable enzymes under cost effective conditions. However, it would be more economical, if these low cost substrates needed very few treatments and also if they could be used as a complete growth medium without any supplements. One such cheap substrate could be fish wastes that are generated in large quantities all throughout the year and all over the world. This brief review based on published literature summarises the importance of using fish waste as a potential medium for protease production.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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