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Optimization of Alkaline Protease Enzyme Using Statistical Methods

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***Abstract*— the extensive usage of proteases in industries such as pharmacy, nutritious, weaving, paper production, curriery, detergent and silver recycling in crystallography (X-ray) has caused a serious attention to the production of protease enzymes. Bacillus species are been used as suitable microscopic organisms for the production of enzymes [1-2]. The design of experiments is a strong tool for improving the quality of products and eliminating the causes of quality losses, especially in the pre-production stages or in the provision of services. Today, there are new methods for performing the design of experiments. These methods have been enhanced by foresighting suitable models in comparing data collection, known as modern test design techniques [3]. The purpose of this review essay is to optimize the production protease enzyme by using the Taguchi statistical method and the effects of different sources of Carbon, Nitrogen, mineral elements, and creating different conditions such a pH, temperature, the duration of incubation and aeration in the production of protease [4].**

Keywords: Protease, Different techniques of experiment designing, Taguchi method, Optimization of Alkaline Protease Production Factors.

Introduction

Proteases are proteolytic or peptidases enzymes that have proteolytic activity. These enzymes perform protein catabolism by peptide banding hydrolysis; secretion of proteases by the bacterium may be as exotoxin which is the cause of the disease and the destruction of the cell's external coverage [1-2].

The division of proteases is based on the existing amino acid in the active station and is divided into six groups including serine protease, threonine protease, cysteine protease, aspartate protease and metallo proteases. Serine proteases have a wide range of substrates and a remarkable stereotypic activity for the steric substrate. Cysteine proteases were also identified for the first time in a tobacco plant [1].

The usage of proteases

Proteases have an extensive usage in the industry; they are included 60% of commercial enzymes and have 1/3 of the world market commercial detergents. These enzymes generate about $1.6 billion annually in the United States which it's 29% is for human's food, 15% for livestock feed , 56% of it has usage for other industries like pharmacy ,nutritious, weaving, paper production, curriery, detergent and silver recycling in crystallography(X-ray). The optimized pH of protease is between 9 and 12.The tolerance of various pH in protease depends on the available serine in active station. Proteases are produced in plants, animals and microorganisms in high amount. In protease plants such as papain from papaya tree, keratiniases are produced in some vegetal plants and bromelain from pineapple tree, and in animals proteases such as trypsin (boar pancreas), chymotrypsin (cow pancreas) and pepsin (calf stomach) are produced [1-2].

The ATCC (American Type Culture Collection) presents a catalog of bacteria and protease producing phages. Proteases are produced by Bacillus, such as : Bacillus licheniformis , Bacillus horikoshi, Bacillus sphaericus, Bacillus formis, Bacillus alkalifilus and Bacillus subtilis ,other bacteria such as Alcaligenes faecalis, pseudomonas fluorescens, Aeromonas hyphilia, and molds such as Aspergillus, Penicillium, Rhizopus and Fusarium oxysporum.[1-2-3-4].

Designing medium for protease enhancement

Designing experiments in a traditional way is a statistical approach. This method was broached by Fisher in England in 1920.For years in a row; he was responsible for analyzing the statistical data of an agricultural station in London. Fisher developed an analysis of variance (ANOVA) in an effort to improve agricultural products. The method of designing the experiments is a powerful tool for improving the quality of products and eliminating the causes of quality losses, especially in the pre-production stages or the provision of services. Nowadays, new methods have been developed to carry out the design of experiments. These methods have been upgraded by generating appropriate forecast models in comparison with data collection and are known as modern experiment designing techniques [5].

Different techniques of experiment designing

In general, experiment designing can be done in three ways: One-factor-at- a- time, Full factorial and fractional factorial designs.

One-factor-at-a-time (OFAT) method

The simplest test mode is based on one factor on the product. This method is also called one-factor method. In this way other factors are kept constant, a factor changes and at the end the best amount with an optimal response is selected. In this experiment every single factor is repeated. Before OFTA designing, there is a need for a general diagnosis to be analyzed. The limitations of this method can be noted as: if there is an interaction between the variables, the probability of reaching optimal conditions or even near them is very low. On the other hand the total time required is long, because the experiments are carried out consecutively [6].

Full factorial method

In the Full factorial method, the effects of the variables are simultaneously checked. In this method, all possible states between the variables and their levels are tested. In this way, the exact answer is obtained but because of expenditure lots of time, energy and cost, using of this method in experiment design is not recommended. The number of experiments is obtained from: N=Lm .For example, to examine the effect of four factors on two levels, the number of required test is 24 equals with 16 tests. This method is suitable for cases with the low number of experiments [6].

Fractional of full factorial method

Experts have developed the statistics of performance plans that are known as fractional business plans. In this method, a number of possible combinations of variables are selected and the best possible conditions are obtained after the tests are performed by evaluating the obtained answers. There may be no optimal conditions between the tested modes. If the initial study to determine the variables and their interrelations between them is done carefully, the obtained answer is optimal. Of course in this method, some interactions are eliminated in order to simplify, so the best answer may not be obtained but if the assumptions applied are close to the truth, the answer is close to the optimal answer. In this method, in addition to the lower number of experiments, due to the possibility of performing parallel experiments, the overall time also greatly decreases [6].

Taguchi method

The basis of this method was presented by Dr. Genechi Taguchi which was later used as Taguchi method to optimize researches in engineering processes. Recently, this method has been used in other areas of biotechnology, including some genetic engineering techniques. In this method a collection of tables is called orthogonal arrays for designing experiments.

The characteristics of these orthogonal arrays are that each test series has a different composition than others, and there is a balance between levels or in the other words, the variables are distributed equally in one level. The experiments are based on these arrays and are performed randomly and repeatedly to reduce errors caused by uncontrollable factors. Of course, if these factors are well known, they can be examined with a separate orthogonal array (external array). Analyzing the results in this method, evaluating the main effects and interaction can evaluate the contribution of each factor in the response. Analysis of variance is statistical [5].

In order to determine the level of each factor, degree of freedom, sum of squares, variance or mean square, the ratio of variance and total squared net are used. When the number of experiments is low, analysis of variance of manual methods is possible using existing formulas. The Qualitek-4 statistical software designed specifically for statistical analysis of the responses by the Taguchi method is proposed for a large number of experiments [5].

Advantages and limitations in the Taguchi method

To highlight the advantages and limitations of the Taguchi method, it is better to compare this method, which is a fraction of the full factorial method, with other methods, namely, the full factorial method and the one-factor method at one time. The main advantage of statistical methods in comparison to the one-factor method at one time is that in the statistical methods, the overall design of the experiments is carried

out in one step and at the beginning, and then the tests are carried out simultaneously. If, in one factor at a time method, at each stage, at first thinking about what should be tested must be done, then several tests are carried out and this process continues into several stages. It may be possible to make statistical analysis, also briefly, in the calculation of error and variance in experiments of one factor at a time, but accurate analysis of the results is not feasible.

As it is known, in the Taguchi method, the number of experiments required to study the main effects of factors independently is much less than the full factorial method and is almost lesser than the one-Factor at a time method. Of course, as mentioned, if there is a need of reviewing interaction effects between factors in the Taguchi method, the number of required experiments will be increased. It should be noted that despite the fact that all the mutual effects in the full factorial method can be investigated, since the degree of influence of the factors and interactions are not equal to each other and only some of them have a significant effect and should be investigated, performing experiments in abundant number, is only a waste of time and money. Sometimes, large number of tests also reduces their accuracy. The thing that distinguishes Taguchi method from the others, even other fractional methods of factorial, is reducing the change in quality due to the presence of intrusive (uncontrollable) factors by taking them into experiments without deleting them. Usually in industrial processes, eliminating these factors involves a large amount of money. Using the Taguchi method, it is not necessary to spend on eliminating these factors, and their effects are made by adjusting the levels and controlling the change of other factors. For this purpose, the optimal point for the factor is usually determined in such a way that, due to its minor changes, there will be no significant changes in the final quality. To eliminate the effects of disturbing factors, it is possible to design experiments for effective factors in different conditions for disturbing and repetitive factors. Another advantage of the Taguchi method, as already mentioned, is the possibility of examining the effects of disturbing agents simultaneously and in foreign array. In this way, the optimal conditions are determined with minimum variability against the change of controllable and uncontrollable factors (disturbance). Subsequently, the cost of guaranteeing the quality of goods decreases in industrial production processes. In the Taguchi method and possibly other complete fractional factor methods, given that statistical control of the process is a method for ensuring or developing quality and applied online, if the effective factors have already been identified, statistical control of the process for these factors can be done mainly. In the discussion of "experiment designing", the main advantage of the Taguchi method is its application in cases where different variables do not have the same levels, and each one is examined at different levels. The most important limitation of the Taguchi method is that it can only be used in design. In other words, once the industrial process is launched, the Taguchi method cannot be used during the work to increase product quality or to stabilize against external factors in that process, unless the development of the industrial process is contemplated. Another limitation is Taguchi's method of using it for discrete variables. In other words, this method is not suitable for optimizing continuous variables [7-8-29].

Optimization of Alkaline Protease Production Factors

Compounds of the growth medium of a bacterium that differs from one organism to another, plays an important role in the production of extracellular alkaline protease, and therefore the compositions required for the culture medium with their concentrations should be optimally optimized. The researchers' efforts mainly focus on the effects of different nutrients of carbon and nitrogen as substrates that affect the enzyme's efficiency, the need for bivalent metallic ions in the culture medium and the optimization of fermentative and environmental parameters such as pH, temperature and aeration rate. No growth medium has been created for the best protease production from various microbial sources, and each organism has special conditions for maximum enzyme efficiency. The production of the enzyme shows a definite

relationship due to the growth stage of the organism. Protease synthesis in Bacillus species is controlled by complex mechanisms that are in the phase of growth and stagnation[9]. the secretion of proteases is highly dependent on the growth medium and the factors affecting the optimal production for other species are different. Therefore, it is important to determine the most suitable culture and environment parameters in order to maximize growth [10]. Production of extracellular protease in microorganisms is heavily influenced by the components of the culture medium, such as variation in the C / N ratio, the presence of some metabolizable sugars such as glucose and metal ions. In addition, a number of other physical factors such as aeration, temperature, PH, incubation period, and inoculum concentration also affect the amount of protease production. [9]. Temperature: Temperature is a vital parameter that varies from an organism to another for maximize cell growth and enzyme production. The temperature control mechanism for producing the enzyme is not easily understandable. Although studies by Frankena et al. showed that there is a link between enzyme synthesis and energy metabolism in Bacillus, which is controlled by temperature and oxygen uptake [11]. The temperature affects the production of the enzyme by altering the physical properties of the membrane, which has been shown by Usharani and Muthuraj on Bacillus laterosporous [12].

The optimum temperature for the alkaline protease produced by Bacillus has been reported. The optimal temperature for the production of protease by Bacillus cereus and Bacillus coagulants is 30 ° C and the lowest optimum temperature for Bacillus seroclaning and Cinerea Bacillus is 28 ° C. The temperature of 37 ° C has been reported as the optimum temperature for the production of protease by a number of Bacillus species such as Amovivorus Bacillus[11]. The data from the study on PE-11 Bacillus subtilis showed that the maximum production of protease at 47 ° C and the best temperature for producing protease was 60 ° C. The results show that the maximum protease production for PCSIR EA-3 Bacillus is at 35 ° C, and this bacillus cannot produce protease below 25 ° C. On the other hand, a significant reduction in the production of the enzyme was observed at 40 ° C, and this bacillus did not produce any proteases at 50 ° C [13]. In another study on Bacillus SP MIG showed that the optimum temperature for producing the enzyme was 30 ° C, and the increase in temperature would reduce the production of the enzyme. High temperatures at 40 and 45 degrees cause a loss of 37 to 77 percent of the enzyme[14]. In another study on Bacillus SP MIG showed that the optimum temperature for producing the enzyme was 30 ° C, and the increase in temperature would reduce the production of the enzyme. High temperatures at 40 and 45 degrees cause a loss of 37 to 77 percent of the enzyme[14]. The effect of temperature on N2 Bacillus subtilis also showed that the highest production of protease is at 40 ° C and the minimum production is at 80 ° C[15] Ray et al. (1992) reported that Temperature can regulate the synthesis and secretion of extracellular protease by microorganisms [14].

pH: pH is the most important physical factor determining the rate of biological process [15]. The production of alkaline protease by microbial strains strongly depends on the extracellular pH, since this parameter significantly affects the enzymatic processes and the transmission of various components throughout the cell membrane [16]. Bacillus subtilis Bacillus subtilis needs an alkaline pH to grow and produce enzymes and can grow Above the 12 to 5 / 6 range. Maximum enzyme production was observed at pH 10.5. Similar effects on protease production were achieved by researchers: Tari (2006), Banerjee (1999) and Shikha et al. (2007). Protease higher than pH: 12 will not be produced. The highest production of protease by brevis B., B.pantotheneticus and Bacillus spl18 was at pH 10.5, 10, 10, respectively. The maximum production of protease in Ve1 Bacillus and Bacillus subtilis species has been reported at an optimum pH of 9. Bacillus subtilis has a wide range of pH and, according to researches, it seems that the protease enzyme is stable against pH changes. Similar results have been reported by Genckal for l21 Bacillus sp [15]. Sarkar et al. reported that the maximum amount of enzyme production was obtained when the pH of the culture medium was about 6, whereas for Bacillus subtilis 38, the maximum production of protease was reported at pH 6.5. According to the results of Qadar et al. (2009), the maximum production of protease is at pH 7 and the

enzyme activity gradually drops in pH above 5.7 and below 5.6. It is likely that pH above 8-7/5 leads to accumulation of metabolites, which, as a result, deactivates the enzyme [12]. According to the findings of the Sepahi and colleagues, protease activity is different with the initial pH of the culture medium. The highest level of protease activity was detected in cultures that grow at pH 8 [17]. In the same study, Das and Prasad stateded the best pH for production of protease [16]. The effect of pH on Bacillus subtilis N2 showed that favorable pH for the production of protease is 9 and production reduces at higher pH. According to researches by mukesh et al. In 2012, Tsujibo et al. (1999) at higher pHs, the growth rate decreases and the enzyme will be deactivated [14]. Also, according to Rao et al., Cell growth of microorganisms and protease production depend on the initial pH of the culture medium [18]. Maximum enzyme production is reported by Bacillus sp CAMA4 at optimized pH 8. Similar results were obtained by Padmapriya et al. (2012) in the production and purification of protease from marine bacillus species, Geethanjah and Subash in optimizing the production of Bacillus subtilis protease.In agreement with this result they have a same report that pH8 is optimal for producing the maximum enzyme [19].

Carbon source: A carbon source is needed for all biosynthetic activities that results in production, such as formation of the product and cell survival. Carbohydrates are the oldest sources of carbon and energy for microbial fermentation. Between carbohydrates, the most common are sugars, which are used. Most microorganisms are able to take glucose. Polysaccharides such as starch and cellulose are used by a limited number of microorganisms. Hydrolysis of pectin is done by some bacterias and molds. A limited number of microprocessors use fats to supply their energy sources if they do not have the proper carbon source, such as sugar. In general, aerobic microorganisms hydrolyze fats more than anaerobic. In the absence of more suitable energy source, microorganisms use proteins hydrolysis, peptides, and amine acids to supply their energy sources [20]. The demand and the need for different sources of carbon vary from organism to the other organism and Even within the same species isolated from different sources is different. Glucose is the best carbon source for the production of protease from the Bacillus CAMA14 species.These findings are matching with reports that are offered for the production of proteas [19]. Carbon sources significantly affect the production of enzymes, and the most commonly used substrate has been reported as casein [8]. According to the studies conducted on Bacillus Sp MIG, it has been found that adding wheat bran, starch molasses, or maltose increases enzyme production, since wheat bran is one of the inexpensive and available resources and has a great importance in the medium. It is estimated that the wheat bran price for a liter of culture medium is $ 0.002. Studies done by Chauhan and Gupta (2004) and Fang et al. In 2001 on alkaline protease showed that adding starch to the medium induces enzyme synthesis [13]. In a study by Ciahi and Jebel Ali, it was shown that maltose and galactose use has a better growth outcome than starch consumption; although starch was the best substrate for the production of enzymes, it was suggested that favorable conditions for the production of protease are not necessarily the optimal conditions for growth. These observations are in contradiction with previous studies by Camlia Rocha et al. which showed that starch is the best source of carbon for the growth and production of protease [12]. A study on carbon sources such as maltose, glucose, lactose, starch and fructose on Ns Bacillus subtilis was investigated to produce alkaline protease and revealed that the maximum production of enzymes is due to glucose consumption. Samarntarn et al. Reported in 1999 that the production of Protease is high by microbes in the presence of carbohydrate sources, especially lactose [14].

Nitrogen source: Nitrogen forms 4 to 14 percent of the dry weight of bacteria and fungi. A wide range of organic and inorganic compounds can be used to estimate the need for microorganisms to nitrogen. The revived nitrogen element is in an amino group. Most prokaryotes can absorb and regenerate the oxidized forms of these elements, named nitrate. The most suitable source of nitrogen for microorganisms is ammonium minerals. Some microprocessors also have the ability to use N2. Most industrial microorganisms can consume organic and non-organic sources. Non-organic nitrogen is used in the form

of ammonium salts, which are often ammonium sulfate and phosphate hydrogen, diammonium or ammonia. Ammonia is also used to regulate fermentation pH. Nitrogen sources include amine acids, proteins and urea. Nitrogen is often used in crude forms that are commonly used by other factories such as corn grinding, extracts, yeasts, peptons and soybeans [20]. Pure amine acids are used only in certain conditions, which are commonly used as precursors for specific products. Bacillus lichen formis can use amino and immunogenic nitrogen, arginine, asparagine and glutamine forms by the enzymes of arginine deaminase, arginase, asperginaz and glutaminase. The use of yeast extract as a source of nitrogen has a beneficial effect on the production of protease [20]. The use of other organic compounds as a source of nitrogen, such as polypheton, also increases the production of protease.In Bacillus lichen formis and Bacillus coagulans, 57 production decreases during sporulation after 24 hours. Production starts in liquid medium and reaches its maximum after 44 hours of production [21]. Meat extracts are also used in Bacillus species as sources of nitrogen. Including inorganic nitrogen sources wich are used to produce protease enzyme , ammonium carbonate can also be mentioned [21]. In microorganisms, organic and inorganic nitrogen metabolizes the primary production of amino acids, nucleic acids, proteins, and cell wall components. The production of alkaline protease strongly depends on the availability of carbon and nitrogen sources in the culture medium. Both sources regularly affect the synthesis of the enzyme, in the study of protease production by Bacillus subtilis, various sources of nitrogen including yeast extract, meat extract, soybean peptone, ammonium sulfate and casein were used. Among the various sources of nitrogen, casein was the best source for inducing alkaline protease production. Between sources of mineral nitrogen, nitrate sodium also is the best source of nitrogen for the production of alkaline proteases,While other ammonium compounds inhibit the production of enzymes. These results are corresponded with recent findings on Bacillus species for casein, ammonium compounds and sodium nitrate [15]. Patel et al. and Prakasham reported that production of protease in Bacillus sp and Bacillus subtilis increases with organic nitrogen sources such as peptone. While the study on Bacillus subtilis revealed that peptone surprisingly inhibited the production of alkaline protease [15]. In research by patel et al. (2005) and Krishnaveri et al. (2013), it was found that yeast extract inhibited the production of alkaline protease by Bacillus subtilis.While yeast extract showed the maximum effect on increasing the production of enzymes in Bacillus sp and Bacillus licheniformis [2-22]. In the research done about bacillus sp MIG, it was found that yeast extract is the best source of organic nitrogen and sodium nitrate and potassium as the best sources of inorganic nitrogen [13]. Soybeans, casein hydrolyzate, and casein support the enzyme's high activity. Peptone and urea decrease the activity of the enzyme by 20 to 47 percent. Using 1% fill in the culture medium with 1% wheat shell protects the enzyme production, but the incubation period lasts 4 days [13]. Naile and johnvesly (2010) reported that protease production by Bacillus sp.JB-99 was supported by nitrogen nitrogen, sodium nitrate and potassium, while ammonium nitrogen completely inhibits the production of the enzyme.

The effect of the source of peptone nitrogen on the produced protease by Bacillus SP.PCSIR EA-3 was interesting, and it was observed that the best source of nitrogen is peptone between other sources, followed by it there are: the extract of meat and yeast extract [12]. The results showed that corn steep liquer causes maximum production of protease and growth in Bacillus sp.CR-179. The use of corn in the culture medium decreases the cost of enzyme production [17].

In a research conducted on Ns Bacillus subtilis, it was found that organic nitrogen sources increase protease production relative to mineral nitrogen sources. From the sources of organic nitrogen, the extract of meat had the highest production [14].

Incubation time: The production of protease increases at the end of the logarithmic phase and the early phases of stagnation, but it's highest amount is in the early phase of stagnation, and production decreases with the death of the cells. Synchronization of sporulation and production of protease has been observed in

other studies [23]. Understanding cell growth period in relation to the production of crude enzymes may be an important factor for identifying the suitable incubation period for maximum productivity of enzymes. In a study on Bacillus sp CAMA 14, the highest enzyme production was obtained in 48 hours of cell growth. And after this time, due to the decrease in microbial growth that is available due to reduced resources, and the increase in the cell death, the enzyme production decreases [19]. The incubation period plays a significant role in the maximum production of enzymes. According to the reports, Bacillus subtilis, PE-11 showed maximum production of protease in 48 hours after inoculation, while 3411 Bacillus subtilis showed the maximum production in 72 hours and Bacillus sp. K-30 showed up to 96 hours. Studies on Bacillus sp PCSIR EA-3 showed that the production of protease depends on cell growth. Cells began to multiply within 6 hours of incubation and peaked at 48 hours. The cells then entered the inertia phase and then decreased. Maximum enzyme production was obtained at the end of the logarithmic phase and the beginning of the resting phase, and then the number of available organisms decreased [5]. The role of incubation time with different species of Bacillus has been studied. According to Krishnaveni et al. (2012), the highest protease production for firmus TAP5 Bacillus was observed between 60-64 hours. The researcher also found 48 hours to produce the maximum protease in RMK Bacillus subtilis [16]. Tari and Genckal reported a maximum 96-hour production of protease in Bacillus species 21 and 18 [24].

Metal ions: Microorganisms require specific mineral elements for growth and metabolism. Usually enough amounts of cobalt, copper, iron, manganese, molybdenum, and zinc in water are present as impurities in other components of the medium. For example, corn kernels contain large amounts of mineral elements that usually eliminate the need for low-energy elements. In some materials, the amount of calcium, magnesium, phosphorus, potassium, sulfur and chlorine is low for the needs of bacteria. So these materials are added to the environment in the form of pure salts. Metal ions, which bind to the active site of the enzyme, cause protein stability. For example, in neutral proteases like thermolysis, which binds to the active site by bacterial heat broth bacillusthemoplatonic 58, activates the protease enzyme stability. The presence of copper and zinc ions in this bacterium also leads to the persistence of alkaline phosphatase. In subtilisin BPN, the presence of calcium ions in the N terminal section results in the stability of protease enzyme. Other ions, such as Ba2 +, Co2 +, Hg2 + and Mn2 +, also contribute to the protease enzyme stability. Excessive ion uptake reduces the activity of alkaline proteases. The use of Mn ion in the culture medium of Bacillus stearotermophilus 59 F1 increases the activity of this bacterium. Increasing the concentration of calcium in the cytoplasmic membrane to more than 2 μmol also results in the production of spores, given that detergents use triphosphate as a softening factor. The use of proteases that require calcium is not appropriate [23] in a study on Bacillus sp MIG, the effect of various metal ions on enzyme activity was investigated. Mercury and silver ions inhibit enzyme activity by about 70-50%. Metal ions such as Mn2 +, Mg2 +, CO2 +, Ca2 + increase or sustain the activity of the enzyme, and these cations play an important role in the stability of the protease structure and enzyme protection against thermal degradation [13]. Between metal ions, Magnesium chloride increases the production of protease in NS Bacillus subtilis [14].

Inoculum amount: The amount of inoculum has a critical role in the production of protease enzyme. In the study on Bacillus subtilis, the amount of inoculum was reported as 5%. In parallel to these results, the inoculation rate for Bacillus subtilis L18 was reported as 5%. The amount of this inoculation was reported 2% in Bacillus species, according to Chauhan and Gupta (2004) reports [15]. The results on Bacillus sp.CAMA14 showed that the best inoculation rate was 2.5%. Further increasement in inoculation rate for microorganisms is not desirable due to changes in the availability of oxygen and competition for access to limited resources that ultimately reduce protease production. These findings are similar to those of Elibol et al. (2005) and Kalaiarasi and Sunitha (2009) who reported that 2.5% of the inoculum for the production of protease is desirable [14].

Aeration and stirring: The change in stirring rate affects the amount of mixing in the flask or bioreactor, as well as the availability of nutrients. The desired yield of alkaline protease for B.subtilis ATCC 14416 and B. licheniformis was reported to be 200 rpm [25-26].

The rate of aeration indirectly affects the level of insoluble oxygen in the medium. The maximum aeration rate in Bacillus tequilensis and B. proteolyticus CFR3001 and Bacillus and Bacillus circulans MTCC 7942 was reported as 100 rpm. These results indicate that oxygen supply is an important limiting factor for the growth and synthesis of protease [27].

Conclusion

Taguchi statistical method is a successful statistical method for determining the important link between the culture medium and culture factors for the production of protease enzyme. Selection of suitable sources for increasing the production of protease enzyme is one of the reasons for increasing the production of this enzyme. Due to the widespread application of proteases in the industry, such as the food industry, the industries include pharmacy, knitting, paper production, curriery (used in soaking, disinfecting, fat removing and tanning) , detergent and silver recycling in crystallography (X-ray) The need for this enzyme is rising in the world daily.

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