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SIIRT UNIVERSITY
INSTITUTE OF SCIENCE

PRODUCTION AND CHARACTERIZATION OF THERMOSTABLE ALPHA-AMYLASE AND XYLANASE FROM *THERMOBACILLUS SP*. VO15 AND *BACILLUS LICHENIFORMIS*. VO14

MASTER DEGREE THESIS

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THESIS ACCEPTANCE AND APPROVAL

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Signature

Mariwan Fathalla ABDALFATAH

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ABBREVIATIONS AND SYMBOL LISTS

Abbreviation	Explanation
NB	**:** Nutrient Broth
NA	**:** Nutrient Agar
NCBI	**:** National Center for Biotechnology Information
IUB	**:** International Union of Biochemistry
IUBMB	**:** International Union of Biochemistry and Molecular Biology
GRAS	**:** Generally Recognized as Safe
EC	**:** European Commission
SmF	**:** Submerged Fermentation
SSF	**:** Solid State Fermentation
UV	: Ultraviolet
M	**:** Molar
g	**:** gram
mg	**:** milligram
hr	**:** hour
min	**:** minutes
L	**:** Liter
ml	**:** milliliter
rpm	**:** revolutions per minute
DNS	**:** dinitrosalcylic acid
mM	**:** millimole
v/v	**:** volume/volume
w/v	**:** weight/volume
w/w	**:** weight/weight
sp	**:** species
BSA	**:** Bovine serum albumin
IU	**:** International Unit

Symbol	Explanation
%	: Precentage
^{o}C	: Centigrade Celsius
α	: Alpha
β	: Beta
µl	: Microliter

ÖZET

YÜKSEK LISANS

''*THERMOBACILLUS* SP. VO15 VE *BACILLUS LICHENIFORMIS* VO14'DEN TERMOSTABIL ALFA-AMILAZ VE KSILANAZ ENZIMLERININ ÜRETIMI VE KARAKTERIZASYONU''

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Bu çalışmada, Billoris (Sağlarca, Siirt) sıcak su kaynağından izole edilen termofilik izolatlar VO15 ve VO14 kullanılmış ve derin kültür fermentasyonuyla, termofilik bakteriler tarafından ekstraselular alfa-amilaz ve ksilanaz enzimleri için optimum üretim koşullarının belirlenmesi amaçlanmıştır.

Alfa-amilaz için optimum üretim koşulları inkübasyon zamanı, sıcaklık ve pH *Thermobacillus sp*.VO15 için 48 saat (12.53 U/mg), 40°C ve pH 6.0 olarak, *B.licheniformis* VO14 için 24 saat (15.98 U/mg), 45°C ve pH 7.0 olarak elde edilmiştir.

Ksilanaz için optimum üretim koşulları inkübasyon zamanı, sıcaklık ve pH *Thermobacillus sp*.VO15 için 24 saat (28.24 U/mg), 45°C ve pH 6.0 olarak, *B.licheniformis* VO14 için 24 saat (32.52 U/mg), 40°C ve pH 5.0 olarak elde edilmiştir. Azot kaynakları arasında maksimum alfa-amilaz üretimi *Thermobacillus* sp için amonyum sülfat (117.6%) ve *B.licheniformis* için maya ekstraktından (122.44%) elde edilmiştir. Surfaktant olarak Tween-40 kullanıldığında her iki bakteride de alfa-amilaz ve ksilanaz enzimlerinin üretiminde artiş kaydedilmiştir.

Sıcaklık ve pH, sıcaklık ve pH stabiliteleri, NaCl, deterjanlar, surfaktantlar ve metal iyonlarının enzim karakterizasyonuna etkisi gibi değişik parametreler test edilmiştir. Ayrıca farklı nişastaların alfa-amilaz tarafından parçalanmaları çalışılmıştır. 50ºC - 110ºC sıcaklık değerlerinde enzimlerin sıcaklık stabiliteleri karşılaştırılmıştır. *Thermobacillus sp*.VO15 tarafından üretilen alfa-amilazın 110ºC'de 30 ve 240 dakika ön inkübasyona bırakılmasından sonra enzim aktivitesini sırasıyla %95 ve %80 oranında koruduğu gözlenmiştir. Her iki bakteriden elde edilen alfa-amilazın Cd^{+2} ve Hg^{+2} tarafından aşırı şekilde inhibe edildiği, Co^{2+} tarafından aktive edildiği tespit edilmiştir.

Anahtar Kelimeler: Alfa-amilaz; *B.licheniformis;* derin kültür fermentasyonu; katı faz fermentasyonu;.ksilanaz; *Thermobacillus* sp.

ABSTRACT

MSc THESIS

PRODUCTION AND CHARACTERIZATION OF THERMOSTABLE ALPHA-AMYLASE AND XYLANASE FROM THERMOBACILLUS SP. VO15 AND BACILLUS LICHENIFORMIS. VO14

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Thermophilic strains VO15 and VO14 were isolated from the hot-spring water of Billoris (Sağlarca), Siirt in Turkey, were used in this work. The study was aimed to produce and determine the optimum condition for extracellular alpha-amylase and xylanase by thermophilic bacteria under submerged fermentation (SmF).

The optimum condition for production alpha-amylase by SmF, for *Thermobacillus sp*.VO15 and *B.licheniformis* VO14, time of incubation recorded at 48 and 24 hours (12.53 and 15.98 U/mg), temperature 40°C and 45°C and pH 6.0 and 7.0, respectively. The optimum condition for production xylanase, for *Thermobacillus sp*.VO15 and *B.licheniformis* VO14, time of incubation recorded at 24 hours for both (28.24 and 32.52 U/mg), temperature 45°C and 40°C and pH 6.0 and 5.0, respectively. The maximum alpha-amylase production by *Thermobacillus* sp and *B.licheniformis* were found with ammonium sulfate (117.6%) and yeast extract (122.44%), respectively. The maximum alpha-amylase and xylanase production were obtained with Tween-40 for both bacteria.

Various parameters such as temperature and pH, temperature and pH stability, NaCl, detergents, surfactants and metal ions on influence of enzyme characterization were tested. In addition degradation of different starches by alpha-amylase was assayed. Temperature values (50ºC - 110ºC) were examined to compare the differences of the stabilities. The alpha-amylase produced by *Thermobacillus sp*.VO15, at 110ºC keep more than 95% and 80% of its activity after 30 and 240 min, respectively. alpha-amylases were strongly inhibited by Cd^{+2} and Hg^{+2}. Both enzymes activated by Co^{2+}.

Keywords: Alpha-amylase; *B.licheniformis*; submerged fermentation; solid state fermentation**;** *Thermobacillus sp*; Xylanases.

1. INTRODUCTION

Enzymes were determined in the second half of the 19th century and have considering broadly used in an amount of industrial developments. Enzymes are known for their specificity, high efficiency and ability to work in environmental conditions and provide a solution to these challenges. It is clear that enzyme technology can be used to progress a usable, further environmental friendly, economical competitive process (Panneerselvam and Elavarasi, 2015; Abdel-Naby, 1993). Enzyme are biocatalysts that play an important role in metabolic and biochemical reactions (Anbu et al, 2017). Microorganisms are the primary source of enzyme production because they are cultured in large quantities in short span of time and genetic manipulation can be done on bacterial cells to enhance the enzyme production (Gopinath et al, 2017). In addition, the microbial enzymes have been paid more attention due to their active and stable nature than enzymes from plant and animal (Anbu et al, 2017).

There are two types of fermentation processes recently used for the manufacture of enzyme solid substrate fermentation (SSF) and/or submerged fermentation (SmF). Through this fermentation, they can produce different enzymes depending on the microorganism used. Bacteria and fungi are the most studied organisms that are capable of producing the enzyme (Ratna and Mustafa, 2011).

The principal objective of this thesis is to study the optimal condition for the alpha-amylase and xylanase production and characterization by submerged fermentation (SmF). The fermentation are carried out by using *B.licheniformis* VO14 and *Thermobacillus sp* VO15. The parameters have been studied; time of incubation, temperature, pH, a size of inoculums, which are the most important factors that affect alpha-amylase and xylanase production through fermentation. In order to achieve this aim, the specific objectives are:

1) Discover the effectiveness of carbon and nitrogen groups as a substrate for *B.licheniformis* VO14 and *Thermobacillus sp* VO15 alpha-amylase and xylanase production.

2) Identify the optimum condition of fermentation for submerged fermentation (SmF).

3) Determine enzymatic activity and stability of alpha-amylase and xylanase production from submerged fermentation (SmF) by *B.licheniformis* VO14 and *Thermobacillus sp* VO15.

1.1. Submerged Fermentation (SmF)

Submerged fermentation (SmF) the substrate using a free flow of liquid as a medium. The bioactive compounds are secreted in the medium of fermentation. The substrate is used positively soon. So they need to be either replaced or integrated constantly using nutrients. This technique of fermentation is quite suited for microorganisms that need high humidity like bacteria (Subramaniyam and Vimala, 2012).

1.2. Solid State Fermentation (SSF)

SSF involves the culture microorganisms on a solid substrate, such as apple shell, wheat bran, rice husk, banana peel, orange peel and barley husk. The substrate is selected in base availability, particle size, and moisture content. Smaller substrate particles have a larger area for a propagation of microorganisms, however, if it is too small, respiration efficiency is compromised and poor growth and, therefore, poor production of enzymes. Enzymes that can be produced by SSF include alpha-amylase, cellulase, xylanase, protease, pectinase, etc (Onosakponome, 2017).

1.3. Enzymes

Enzymes are a purified forms protein that is produced as intracellular and extracellular compound and may or may not possesses a protein, not a group prosthetic (Vallery and Devonshire, 2003). The rate of enzyme reaction increases among high specificity and catalyzes the biochemical reaction (Keith and John, 2000). All enzymes are known proteins (except ribozymes) that are composed of high molecular weight produced by a chain of amino acids bound by peptide bonds. The enzymes are divided into the types of catalyst and reaction substance (called substrate) that play. It’s generally used to add 'ase' to the end of the name of the main substrate on the enzyme actions (Bennett and Frieden, 1969) like xylanase is acted on xylan, lipases on lipids.

1.4. Microbial Enzymes

Same all plant and animal cells, the microbe’s cells produce the enzyme that is oxidizing, hydrolysing, or reducing metabolic, but the production volume of enzymes in different species and strain different.

Thus, for specific enzymes production commercially, its necessary select a particular strain with high enzymatic production activity. Microbial sources of the enzymes are more beneficial than their plant and animal sources due to lower costs production than others, large-range production, for manipulation in the genetic better field, accelerated growth of crops, using less material, respect for the environment and cause a wide physical and chemical characteristics area, so they are in various industrial applications preferred (Hasan et al, 2006; Kumari et al, 2012).

1.5. Innovation and the Development of Enzyme

Determining the exact discovery of enzymes is difficult in the days of development enzymes, previously enzymes used for the basic necessities of the life by human (Salis et al, 2007). Spallanzani in 1783, said hawks gastric juice may dissolve the flesh (Sumner and Somers, 2014). After years later, observations several similar were made. In 1814, Russian chemist Kirchhoff noted a component of "glutinous" (i.e.Proteinaceous) wheat was obtained by altering starch into sugar (Segel, 1975). Subsequently, Kirchhoff established the frameworks for the revelation of amylase. Some early investigations were directed in 1830 Rabiquet, Boutron and Chaland found the hydrolysis of amygdalin by better almonds (Ehrlich and Newman, 2008). Jons Jakob the Barzelius Swedish chemist, who made "catalysis" word when he noticed that a few chemicals add to speeding the reaction response frequency (Singh, 2007). In 1831, the diastatic act on ptyalin salivary by Leuchs (Segel, 1975). The "enzyme" term in the principle used by Kuehne derives from "yeast" the Greek word. General, the primary revelation of an enzyme is credited by Jean François Persoz and Anselme Payen scientists, in 1833, they tried and the malt extracted aqueous ethanol and precipitated labile hydrolysed heat-stimulated starch. They called their portion 'diastasis' (Whitehurst and Van Oort, 2010). Amylase is the primary enzyme fungal source that it produces industrially, which has been used for the digestive disorders treatment as a pharmaceutical in 1894 (Pandey et al, 2010).

1.6. Alpha-amylase

Alpha-amylase (EC 3.2.1.1) is catalyze enzymes that hydrolysis of starch internal alpha-1,4-glycosidic bonds in products starch such as low units of molecular weight of maltose, maltotriose, glucose (Kandra, 2003; Rajagopalan and Krishnan, 2008). Amylase enzymes are more significant and in biotechnology great importance. Framing a kind of modern enzyme that has around 25% of the catalyst enzyme world's advertise (Reddy et al, 2003; Burhan, 2003; Rao et al, 1998). A substantial number of microbial amylases are industrially accessible today, and the starch hydrolysis microbial amylases approximately followed chemical hydrolysis in the starch processing manufacturing. Amylase microorganism has a broad spectrum of industrial applications and are more stable than when prepared from plants and animals (Tanyildizi et al, 2005; Veille and Zeikus, 2001).

Microorganisms are the most important for the production of enzymes. Choosing the right microorganism plays a key role in the highly desirable product (Oboh, 2005). The production of an enzyme for manufacturing utilize, the separation, and characterization of new assuring strains utilizing the economical source of carbon and nitrogen in the continuous process (Panneerselvam and Elavarasi, 2015). Microorganisms have become increasingly critical as industrial enzymes producers (Prakash and Jaiswal, 2010). Due to its biochemical diversity and the simplicity with which the enzyme concentration can be enhanced by genetic and environmental manipulation, they immediately begin to replace the enzyme, which was occasionally isolated from eukaryotic complexes (Panneerselvam and Elavarasi, 2015; Reda, 2010).

In the recent past, there has been broad research into production the microbial of alpha-amylase. There are two main purposes for growing concern in microbial sources.

1) Microorganism growth is fast and this, in turn, accelerates of the enzyme production. Microorganisms are easy to control with respect to plants and animals. They want less space and serve as the most profitable sources (Gupta et al, 2003).

2) Microorganisms can be used through genetic industrial or different means. They may undergo improved deformation, mutation, and other such changes that can optimize the alpha-amylase production.

In addition, the microorganisms can be adapted to meet the needs of growing industries and to achieve enzymes with desired properties such as thermostability, these characteristics, thermostable alpha-amylase are desired as reducing the risk of contamination and reducing the reaction at higher temperatures (Konsoula and Liakopoulou, 2007).

1.7. Xylanase

Xylanase (endo.1,4-β-D-xylanohydrolase, E.C 3.2.1.8) is an hydrolytic enzyme involved in the depolymerization of xylan, major hemicellulose cellular renewable polysaccharide wall of the plant. It is produced by bacteria (Kiddinamoorthy et al, 2008), fungi (Nari et al, 2008), actinomycetes and yeast (Ninawe et al, 2007). Due to the growing biotechnological significance of thermostable xylanases, several thermophilic fungi and bacteria for xylanase production have been studied (Singh et al, 2003; Maheshwari et al, 2000). It is applied to a variety of industries, including food, feed, textiles, fuel, detergents, paper and waste treatment.

1.8. Structural and Functional Characteristic of Alpha-amylase

Alpha-amylase alpha-1, 4-glucan-4-glucoidrolase can be seen in a plant, microorganisms and higher organisms (Kandra, 2003). Alpha-amylase belongs to a family of amylases that catalyzes endo-hydrolysis of the shorter starch oligosaccharides, with alpha-D-(1-4) glycoside bonds (Brayer et al, 1995; Iulek et al, 2000). Neither terminal glucose remains nor alpha-1, 6-bonds can be cleaved by alpha-amylase (Whitcomb and Lowe, 2007). The final products of the alpha-amylase action are oligosaccharides of variable length in alpha-configuration and alpha-limit dextrin (Van der Maarel et al, 2002). These are a combination of maltose, maltotriose oligosaccharides and branched 6-8 units of glucose containing alpha-1, 4 and alpha-1, 6 linkages (Whitcomb and Lowe, 2007). Other amylolytic enzymes involved in the starch decomposition process but the contribution of alpha-amylase is the most important for the beginning of the process (Tangphatsornruang et al, 2005).

Alpha-amylase has a three-dimensional structure accomplished of binding to a substrate and the action of particular catalytic groups beyond, raise the glucoside bonds breakdown (Iulek et al, 2000).

1.9. Alpha-Amylase Production

Although the parts of particular fermentation processes selected by different manufacturers vary, there are two fundamental procedures for the alpha-amylase production (Busch et al, 1997; Haseltine et al1996). Submerged fermentation (SmF) and solid state fermentation (SSF). On an industrial scale, most enzymes are produced by the submerged fermentation method (SmF). However, in recent decades, there has been a growing tendency towards the use of the solid state fermentation method (SSF) to produce various enzymes derived from thermophilic microorganisms (Fadel, 2000; Robinson et al, 2001).

Alpha-amylase preparation by (SmF) has been studied and depended on a variety of physical and chemical factors. The submerged fermentation (SmF) approach has traditionally been utilized for the production of industrial enzymes, because to the ease of control of different factors, for example, pH, temperature, aeration and oxygen transfer and humidity (Gangadharan et al, 2008; Singhania et al, 2009).

Optimization of fermentation conditions, especially chemical and physical factors, are essential for the development of fermentation procedures due to its influence on the economy and process feasibility (Francis et al, 2003). We have studied the function of different factors including pH, temperature, carbon and nitrogen source, surface acting agents, surfactants, metal ions and detergents for alpha-amylase production. The properties of each alpha-amylase, such as thermostability, pH stability, and pH profile should be adapted to an application (Gupta et al, 2003).

1.10. Xylanase Production

The xylanase enzyme is produced by a wide range of microbes including bacteria and fungi (Ahmed et al, 2009). The microorganisms which will be used for xylanases production should be adequate yields and should be not produced toxins or any other undesired products (Uday et al, 2016). In the production of the xylanase by submerged fermentation (SmF) substrates are dissolved or suspended in an aqueous medium, then mixed by air flow or agitator for fermentation to occur in a homogeneous environment that can be controlled strongly. For a production of high levels of an enzyme xylanase, optimizing the growth parameters is a major importance in industrial enzymology (Guha et al, 2013).

Several studies are focusing on attaining enzyme stability under various fermentation condition which is usually temperature, pH, time and actions in order to meet the basic of the industries (Nkohla et al, 2017).

1.11. Biotechnology and Industrial Applications of Alpha-amylase

Amylase is at the center of the most important hydrolysis enzyme for all starch-based industries (Prakashand Jaiswal, 2010). Alpha-amylase advertising is the oldest with the primary use in 1984 as a pharmaceutical aid for the treatment of digestive disorders (Aehle and Misset, 1999; Drauz et al, 2012). A large number of alpha-amylase microbial enzymes begin to be used in various sectors of the industries.

1.11.1. Industries processing of starch

The mainstream alpha-amylase market in the starch application, utilized for starch hydrolysis in the starch-liquefaction process that changes starch into glucose and fructose sugars (Nielsen and Borchert, 2000). Enzymatic conversion of starch contains all; Gelatinization, including the dissolution of starch granules, thus forming a suspension; Liquefaction includes the production of glucose and maltose from promote hydrolysis (Ogasahara et al, 1970). The method needs the use of a greatly thermostable alpha-amylase for starch liquefaction, depending on the temperature which can act at temperatures about 70-100°C (Regulapati et al, 2007).

1.11.2. Food industries

Through the advance of present day biotechnology, the food industry has experienced significant changes. There are many reports on genetically modified enzymes that have been safely used in the food business. Amylase is widely used in the food industry as processed starch syrup (Zeman and McCrea, 1985). This enzyme can be added to the bread to degrade the starch in smaller dextrins, adding alpha-amylase to the dough results to increase fermentation speed and reduce the viscosity of the dough, volume, and consistency of the product (Anthea et al, 1993). It also generates extra sugar in the dough, which improves flavor, crust color, and toast quality.

In addition to produce fermentable compounds, alpha-amylase also has an anti-staling effect in bread and improves soft retention of bakery products by increasing the shelf life of these products (Ogasahara et al, 1970).

1.11.3. Biofuel industries

Ethanol is the most widely used liquid biofuel for the production of bioethanol. Starch is the most widely used substrate due to its low price and the raw material readily available in most regions of the world (Sanchez and Cardona, 2008). In this production, the starch must be solubilized and then subjected to two enzymatic steps to obtain fermentable sugars (Moraes et al, 1999). The conventional process for bioconversion of starch in ethanol saccharification, where starch is converted into sugar using a microorganism or amylolytic enzymes such as glucoamylase and/or alpha-amylase, followed by fermentation, in which the sugar becomes (Chi et al, 2009; Oner, 2006). Between alpha-amylase bacteria obtained from *Bacillus licheniformis* thermotolerant bacteria or manipulated *Bacillus subtilis* or *Escherichia coli* strains used in the first phase of suspending starch hydrolysis (Saxena et al, 2007).

1.11.4. Detergent industries

Detergent industries are the main users of an enzyme, both in volume and in value. The use of the enzyme in detergent formulations improves detergents to remove difficult spots and make the detergent safe for the environment (Hmidet et al, 2008). Amylase is the second type of enzyme used in the enzymatic detergent formulation and 90% of all liquid detergents containing these enzymes. This enzyme is the hydrolysis of glycosidic bonds in starch polymers, commonly found in foods such as chocolate, pasta, baby food, fruit, sauce, and barbecue sauce (Mitidieri et al, 2006). As colored strains, suppression is of interest both detergent and dishes, as it is known that starch can be an attraction for many types of particular soils. The suitability of any hydrolytic enzymes for inclusion in the formulation depends on its stability and compatibility with detergent components (Kirk et al, 2002). An example of alpha-amylase used in the detergent industry derived from Aspergillus or Bacillus (Ramasesh, 1982).

1.11.5. Textile industries

In the textile industry, textile resistance is improved by rotating the starch paste fabric. It also prevents loss of rotten friction, cutting and generating static electricity on the rope, giving softness to the surface of the rope because it becomes low. After weaving the cloth, starch is removed and the cloth will be cleaned and dyed (Feitkenhauer, 2003). Starch on the cloth is generally removed by applying alpha-amylase. Alpha-amylase removes selectively the size. Amylase of Bacillus strain has been used in the textile industry for a long time (Haq et al, 2010).

1.11.6. Paper industries

Using alpha-amylase in the paper and cellulose industry is the modification of coated paper starch, that is, to produce a high starch molecular weight of low viscosity. The coating serves to make the paper surface sufficiently smooth and strong to improve the quality of the paper to be written. In this application, the viscosity of natural starch is too high for paper sizing and this can be partially modified to degrade the polymer with alpha-amylase in a batch or continuous process (Fryer and Asteriadou, 2009). Starch is a good sizing agent for paper finishing, improving the quality and erasability, besides being a good paper coating. The size improves stiffness and paper strength (Brunenberg et al, 1996). Active cold alpha-amylase is also very useful for the paper industry by reducing the viscosity of the starch in a suitable paper coating (Kuddus, 2014).

1.11.7. Clinical and medicinal applications

Alpha-amylase would potentially be useful in the pharmaceutical and adequate chemical industry if it could prepare enzymes with suitable qualities (Fogarty and Kelly, 1980). Interestingly, the first industrial enzyme production is a fungus amylase source in 1894, which was utilized as a pharmaceutical aid for the digestive disorders treatment (Pandey et al, 2000). Synthetic and natural biodegradable polymers are used to control the release of the drug or to certain drugs for which solubility can be affected by the alteration in gastrointestinal pH, a method is needed to stimulate the release of the drug. Biodegradable polysaccharide forms are of concern because the degradation of a natural product such as starch is naturally present in the body of human (Kost and Shefer, 1990).

Alpha-amylase (per-gelatinized starch) and cross-linked starch was used as a hydrogel. It has been reported that the higher stiffness of starch decreases the release rate of the drug. The frequency of release also depends on the action of alpha-amylase included in the dissolution medium. The increase of alpha-amylase to a compressed cross-linked amylase can modify the release kinetics of the drug (Domoulina et al, 1999).

1.11.8. Elimination of environmental pollutants

Starch is a polysaccharide that is broadly spread in world as a reservation of energy collected in several plants and occurs largely in the waste produced by the processing of vegetable raw materials (Barbesgard et al, 1999). Starch treatment of waste is produced in large amounts and cause contamination issues (Wu et al, 2008). To clean contaminant starch substances can microbial amylolytic enzymes or microorganisms that produce amylolytic enzyme used (Jurado et al, 2003). For this reason, alpha-amylase enzymes from yeast and bacteria can be used (Mobini-Dehkordi et al, 2011).

1.11.9. Molecular applications

It has become the test report of essential genes to the study of genetic regulatory components and gene expression (Aubel et al, 2001). In molecular biology, the presence of alpha-amylase can serve as a further system for choosing a positive reporter integration plus resistance to antibiotics. Insertion of foreign DNA into this gene produces a loose amylolytic activity in the host cell can be assayed using a simple and economical iodine staining procedure (Arican, 2008; Emmanuel et al, 2000).

1.12. Biotechnological and Industrial Applications of Xylanases

The possible application of xylanase to biotechnology involves nutrition treatment to increase digestibility, biobleaching of wood pulp, processing of food to enhance the clarification and conversion of lignocellulosic substances into raw materials and fuels (Subramaniyan and Prema, 2002). Xylanase has shown immense potential to increase the production of various beneficial products in most economic processes.

The central possibilities are the production of enzyme, gas, solvents, and sugar syrup, which can be utilized as such or as a raw material for other microbiological

processes (Kuhad and Singh, 1993; Niehaus et al, 1999; Beg et al, 2001). Xylanases' application in the industrial.

1). Xylanase is in biobleaching of kraft pulps, the enzyme application increases fibrillation of the pulp and water retention, beating the time in reduced virgin pulps, restoring the junction, increasing in recycled fiber freeness and selective removal of xylan from pulps dissolution. Xylanases are also useful in providing the dissolution of cellulosic pulps for the production of rayon and wood pulps biobleaching (Bajpai et al, 1994; Srinivasan and Rele, 1999).

2). The application of xylanase in food production is the major agro-food sector, xylanases are utilized in animal feed with protease, amylase, galactosidases, pectinase, cellulase, and lipase. These enzymes break arabinoxylans into the feed ingredients, reducing the viscosity of the raw material (Della and Kadowaki, 2017). Arabinoxylan found in grain cell walls has an anti-nutritional impact on poultry. When the components are present in soluble form, they can increase the viscosity of ingested food, interfering with the mobility and absorption of other components (Polizeli et al, 2005).

3). Xylanases are used in the food industry. In the production of bread, enzymes are added to improve their softness and durability (Sharma and Kumar, 2013). With the use of xylanase, there has been an increase in the volume of increased bread water absorption and improved fermentation resistance (Zhan et al, 2014).

4). Present xylan in large quantities in the waste from agricultural and food industries. Therefore, xylanases are applied for the reduction of xylan to xylose into the wastewater. The development of an efficient enzyme hydrolysis process allows new perspectives for the treatment of hemicellulose waste (Rain and Nand, 1996).

5). Alkaline xylanases were used in the detergent industry. They are additives in the cleansing formulation and, therefore the removal of stains of plant origin (Kamal et al, 2004).

6). Xylanases can be applied to promote cell wall maceration for the production of plant protoplasts (Wong et al, 1988).

7). Xylanases are poorly used in the pharmaceutical industry. Some xylanases are added in combination with an enzyme complex (hemicellulases, protease, and others) as a dietary supplement or to treat indigestion (Polizeli et al, 2005).

1.13 Thesis Outline

This thesis organized into five chapters. The chapters 1 and 2, introduction and literature, respectively. Chapter 3 refers to materials and methods for the selection and identification of bacteria, focused on alpha-amylase and xylanase, finding optimized conditions such as pH, temperature, incubation time, carbon and nitrogen source for the production of amylase and xylanase. Chapter 4 presents the results of the experiment. Chapter 5 discussion and recommendation.

2. LITERATURE REVIEW

2.1. Bacillus

The genes Bacillus endospore forming aerobes and facultative anaerobes that typically move by peritrichous flagella (Alexander and Midoza, 2008). They are observed in various ecosystems like the human body, water, air and various species of Bacillus are found in soil (Vos et al, 2009). Ferdinand Cohn who was German Botanist first reported the Bacillus genus in the 1872 (Zhang, 2011). They are rod shape, Gram-positive arranged in chain or pairs (Ludwing et al, 2015). Occur in defer environmental condition such as high temperature, high pH and high salt (Joan and John, 2011).

Bacillus species are applied in many agricultural and industrial processes that use the range of physiological properties and its ability to produce enzymes, antibiotics, and other medical, pharmaceuticals metabolites. Bacitracin and polymyxin are two kinds of antibiotics derived from bacillus species. Several species of bacillus, they are spore-forming aerobic stain gram positive coloring exception of some species, the vast majority have no potential pathogen and have never been associated with diseases in humans or animals, and they have important microbiological applications (Turbull et al, 1990).

2.1.1. *Bacillus licheniformis*

Bacillus licheniformis is gram-positive bacteria, spore-forming, and saprophytic organism in an ecosystem. This species is a close relative of *Bacillus subtilis*, unlike many other bacilli, which are predominantly aerobic, *Bacillus licheniformis* is a facultative anaerobic, which also allows growing many ecological niches (Alexander, 1977; Veith et al, 2004). There are many commercial and agricultural uses for *Bacillus licheniformis* and are extracellular products. The species has been made for decades in the production of industrial enzymes including various proteases, alpha-amylase, penicillinase, pentosanase and numerous pectolytic enzymes. *Bacillus licheniformis* alpha-amylase used in the detergent industry (Rey et al, 2004). *Bacillus licheniformis* amylase is distributed by starch hydrolysis, the size of textiles and paper size (Erickson, 1976). *Bacillus licheniformis* species are also used to produce peptide antibiotics such as bacitracin and proticin although to a number of specialty chemical such as citric acid, inosine acid (Gherna et al, 1989).

As an endospores-forming bacterium, the organism's ability to survive under a disfavorable ambient environment may increase its potential as a biocontrol agent.

2.1.2. Thermobacillus

The microbes were present in every imaginable environmental niche, from the topic to the pole and sources of hot water for submarine hydrothermal vents, especially prokaryotic communities in thermophilic habitats experienced physiological adaptation at high temperatures and chemical stress. Recently, these communities have reached the applied research center not only in terms of biotechnology prospects but also to understand the use of biomolecule primitive analogues that existed during the early environments on earth (Raddadi et al, 2015; Saxena et al, 2016; Sahay et al, 2017).

Aerobic or facultatively anaerobic thermophilic bacilli, endospore forming organisms that grow optimally over the temperature range 45 to < 70ºC have been isolated from both thermophilic and mesophilic environments. A few thermophilic bacilli have been separated from the ocean bottom. As of now, thermophilic bacilli are arranged into seven genera Bacillus, Brevibacillus, Alicyclobacillus, Aneurinibaccillus, Geobacillus, Sulfabacillus and Thermobacillus (Bae et al, 2005).

2.2. Types of Fermentation

Bacteria has been used to produce various enzymes such as amylase, xylanase, and cellulose. Previously it was considered that the best method for producing bacterial enzymes utilizes submerged fermentation (Agrawl et al, 2005). Alpha-amylase production for submerged fermentation (SmF) has been considered and depends on a kind of physical and chemical agents. Submerged fermentation (SmF) has traditionally been accepted for the production of necessary enzymes from the industrial point of view since simple parameter setting such as pH, temperature, aeration, oxygen transfer and humidity (Gangadharan et al, 2008; Subramaniyam and Vimala, 2012).

A solid state fermentation (SSF) is represented as a process using a solid substrate. Like wheat bran, rice, paper pulp. The central influence of using these substrates is that residual nutrient rich materials are easily recyclable as a substrate.

In these fermentation techniques, substrates are used so gradually and consistently, it is used for long duration of fermentation. Therefore, this system maintains a controlled release of nutrients (Subramaniyam and Vimala, 2012).

The production of a submerged fermentation (SmF) enzyme has been established for a long time over solid state fermentation (SSF). There are some advantages to using this fermentation. This system is easier to design from the researcher due to the ease of process control and sterilization. In addition, the product enzyme may be constituent or inducible and exhibit various production designs, depending on strain and culture conditions (Vidyalakshmi et al, 2009). However, the solid state fermentation (SSF) process has several advantages over the submerged fermentation (SmF) process, although this process only occurs in the laboratory (Viniegra-Gonzálezet al, 2003; Vidyalakshmi et al, 2009). This is also supported by (Wang and Chen, 2009), which has started the surface adhesion culture base as a product concentration and stability, less catabolic suppression, microorganism culture specifically for insoluble substrate and lower sterility demand due to Low water activity level (Holker et al, 2004).

2.3. Enzyme

As indicated by the International Union of Biochemistry (IUB), and depending on the nature of the reaction, the enzymes are divided into six classes: oxidoreductases, hydrolases, transferases, lyases, isomerases, and ligases. The use of enzymes in industrial applications has been of crucial importance since they can eliminate the use of high temperatures, pH values, organic solvents, and at the same time, extend high substrate specificity, low toxicity, product purity, decreased environmental impact, and ease of termination of activity. Microorganisms constitute the major source of enzymes as they produce a high concentration of extracellular enzymes. Detecting the best enzymes is simple, allowing the analysis of thousands of cultures in a short period of time. Microorganisms can be grown in high amounts over a relatively short time of time by means of established fermentation methods. The production of microbial enzymes is economical on a large scale due to the low culture medium and short fermentation cycles (Brahmachari, 2016).

2.4. Alpha-amylase

Alpha-amylase (E.C 3.2.1.1) is the catalysis enzyme that hydrolysis of internal alpha-1,4-glycosidic linkages in starch processes (Souza, 2010). Alpha-amylase is one of an essential enzyme and has a great importance to biotechnology, while the development of a class of industrial enzymes has about 25% of the world's enzymes market (Rajagopalan and Krishnan, 2008). Various species of microorganisms can be produce alpha-amylase, but for commercial applications, alpha-amylase is obtained mainly from the Bacillus genus. The alpha-amylase produces from *Bacillus licheniformis*, *Bacillus stearothermophilus*, and *Thermobacillus species* detects potential application in a wide range of industrial processes such as food, fermentation, textiles, paper, detergents and industrial pharmaceuticals (Pandey et al, 2000; Souza, 2010).

Bacterial alpha-amylases can potentially be useful in the pharmaceutical and chemical industries. However, with advances in biotechnology, applications amylase consumed in many fields such as clinical chemistry, analytical and medical as well as extensive application in starch saccharification and textile industries, food industries, beer and distillation (Kandra, 2003). Alpha-amylase is an individual of the most common and essential applications of industrial amylases and this study shows the microorganisms that produce this enzyme. Thermostability is a desirable feature of many industrial enzymes. Thermostable enzymes isolated from thermophilic organisms have noticed a number of investment applications for its stability. Since enzymatic liquefaction and starch saccharification are played at high temperature (Asgher et al, 2007; Gomes et al, 2003).

2.5. Xylan

The cell wall of the plant is a composite substance in which cellulose, hemicellulose (xylan essentially) and lignin are closely associated. Three main parts of the wood are cellulose, hemicellulose, a group of carbohydrates that form xylan main class and lignin. Heteropolysaccharide is a xylan containing substitution of acetyl,4-O-methyl-D-glucuronosyl and alpha-arabinofuranosyl appended to the back of the β-1,4-connected xylopyranose units. Xylan forms an interface between lignin and other polysaccharides.

It is restricting properties mediated by covalent and non-covalent associations with lignin, cellulose and different polymers. Lignin is linked to xylan by a residual ester present in 4-O-methyl-D-glucuronic acid as substitutes for the xylan back bone (Subramaniyan et al, 2001).

2.6. Xylanases

Microbial xylanases are an individual subunit proteins that can catalysis the endo-hydrolysis of 1, 4-β-D-xylosidic linkages in xylan. In 1961, the International Union of Biochemistry and Molecular Biology (IUBMB) named the enzyme code (EC 3.2.1.8) to xylanase (Gupta et al, 2016). The official name is endo-1,4-β-xylanase, but commonly used similar terms include xylanase, endo-xylanase, 1,4-β-D-xylan-xylanohydrolase endo-1,4-β-D-xylanase, β-1,4-xylanse, and β-xylanase.

The major sources of endo-1,4-β-xylanase (E.C 3.2.1.8) are bacteria, fungi, algae, protozoa, gastropods and anthropoids (Prada, 1995). Various xylanase organisms typically have a specific range of optimum temperatures ranging from 40°C and 85°C. Commonly, xylanases are constant in a wide range of pH 3-10 and exhibit an optimal pH range of 4 to 7. Production of xylanase, reported by various sources, indicates that amino acid aspartic acid is predominantly glutamic acid, glycine, serine and threonine (Nurizzo et al, 2002).

Xylanase has noticed its application in food and feed industries, such as clarifying fruit juices, reducing viscosity, improving liquefaction filtration of fruit juices and vegetables, macerated fruits and vegetables, fermentable barley extraction, beer, etc. (Harris and Ramalingam, 2010; Sharma and Kumar, 2013). It is also widely used in various industrial applications in regulating plant growth and biofuels as well as in paper and pulp industries (Elgharbi et al, 2015; Thomas et al, 2015).

3. MATERIAL AND METHODS

3.1. Material

3.1.1. Lab instruments

- Refrigerate (VESTEL).
- pH Meter (SARTORIUS DOCU-pH+ METER).
- Micro-centrifuge (SIGMA 1-6S).
- Micropipette (SOCOREX)
- Spectrophotometer (SHIMADAZU UV MINI-1240).
- Incubator (NÜVE EN 500).
- Shaker Incubator (JERO TECH SI-600).
- Shaker Water Bath (NÜVE ST 402).
- Safety Cabinets Hood (NÜVE LN 090).
- Bunsen Burner Flames
- Autoclave (NÜVE OT 4060V).
- Microscope (OLYMPUS SC30).

3.1.2. Media preparation

3.1.2.1. Nutrient agar (NA) preparation

The medium was used for the cultivation of a variety of microorganisms, 20 g of nutrient agar powder added to 1 L of distilled water in a flask, stirring until complete dissolution, subjected to autoclave sterilization (NÜVE OT 4060V) for 1 hr at 121°C. After that, the medium was cooled to 50°C, dispense pour 20-25 ml into Petri plate. The Petri plate has been sealed with a plastic tape cover to prevent the agar medium being dried and contaminated, store the date at 4°C for later use.

3.1.2.2. Nutrient broth (NB) preparation

Weight out 8 g of nutrient broth powder added to 1 L distilled water in a flask, the powder completely dissolve in the distilled water, dispense as required (Dispense 20-25 ml into scrawl bottles) and sterilization for 1 hr at 121°C by autoclave (NÜVE OT 4060V) after sterilization cooling and its ready for bactearial inoculation.

3.1.2.3. Starch Preparation (1%)

The 0.2 g of starch powder put in the volumetric flask containing 20 ml of distilled water pour the starch into boiling water and stir until all of the starch is dissolved. Allow the starch solution to cool to room temperature before use in alpha-amylase assay.

3.1.2.4. DNS reagent preparation

In 200 ml beaker dissolves 1 g of 3.4-dintrosalicyclic acid in 40 ml distilled water. After continuous stirring, add 30 g of potassium sodium tartrate and add slowly a solution of NaOH (1.6 g dissolved in 20 ml of distilled water). Stirring to obtain a clear solution and 100 ml the volume with distilled water is completed. Save in a dark glass bottle at a temperature below 20°C.

3.1.2.5. Xylan solution preparation (1%)

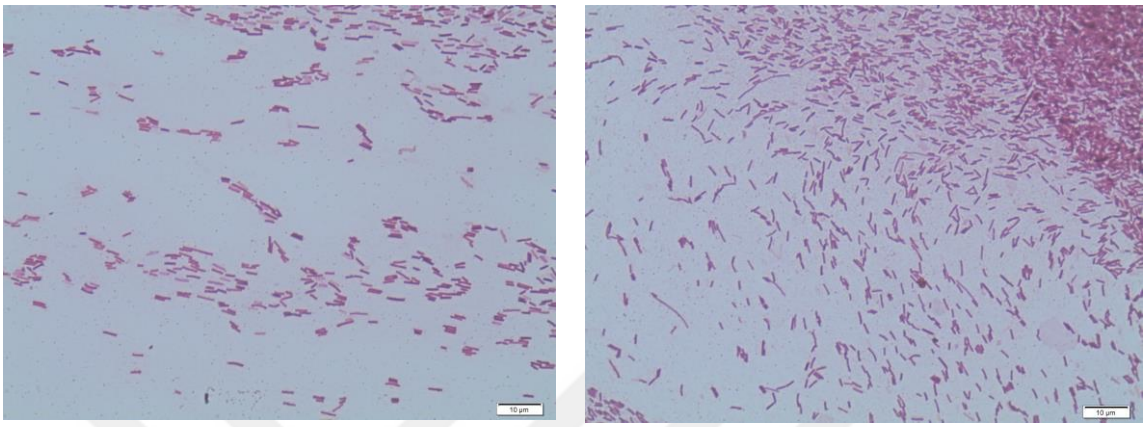
The 0.2 g of xylan powder from oat spells put in a volumetric flask containing 20 ml of distilled water pour the xylan to boiling and stir for 20 min until all of the xylan is dissolved. Centrifugation separation supernatant for use. The xylan solution saves at 4°C use to one week.

3.2. Methodes

3.2.1. Gram staining

Gram's staining methodology depends on the microorganisms ability to keep purple crystal violet. Do not decolorize gram-positive bacteria and remain purple. primary stain, crystal violet, let gram-positive microorganisms and gram-negative to become purple after staining for 30 seconds. When used gram iodine for one minute to the bacterial cells, the gram-positive and gram-negative bacteria color remains the same. The purpose is to combine stain with crystal violet to form a relatively insoluble compound gram-positive bacteria. When the 95% ethanol decolorizing agent, is added to the cell for 10-20 seconds, gram-positive remain purple. In the final, a pink safranin contrast add decolorized gram-negative bacteria without purple gram-positive bacterial color altering (Prescott at al, 2004).

The gram stained cells were examined under the microscope (OLYMPUS SC30) using the 4x to 100x (Oil immersion) objectives to observe cell shape and gram reaction.



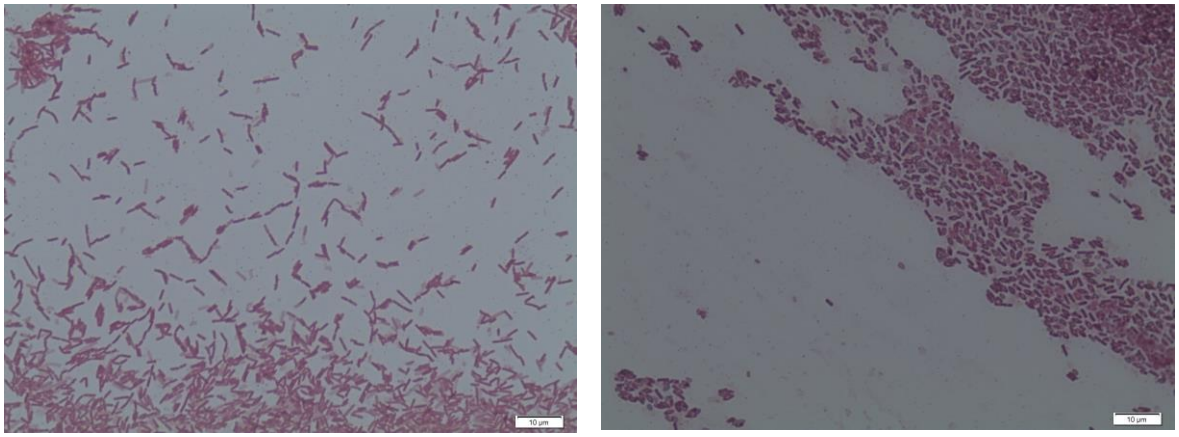
B. licheniformis *Thermobacillus sp*

Figure.3.1. Gram staining.

3.2.2. Endospore staining

Bacteria belonging to Bacillus an extremely heat-resistant structure called endospore. In the Schaeffer-Fulton method, utilized malachite green to stain the endospore and vegetative part of the cell coloration by safranin. This is an accurately stained spore will have a green endospore contained in a pink sporangium.

The smear was covered with a small piece of toweling paper and saturated it with green malachite. Heated the smear for 5 min on boiling water. After raising remove the paper and rinsed with water for 30 seconds. Safranin contrasting for about 20 seconds. For remove safranin, the smear was washed with water. Dry and observed slide under oil immersion (Benson, 1994).



Thermobacillus sp. ***B. licheniformis***

Figure.3.2. Endospore staining.

3.2.3. Phylogenetic analyzes of *Thermobacillus sp* and *B.licheniformis*

Thermobacillus sp VO15 and *B.licheniformis* VO14 were isolated from the hot-spring water of Billoris (Sağlarca), Siirt in Turkey, were used in this study. 16S rRNA gene sequences of the isolates were carried out at İontek Company (İstanbul, Turkey). The evolutionary histories were concluded utilizing the neighbor-joining process and evolutionary analyses were achieved in MEGA6.

3.2.4. Bacterial colony preparation

On the sterile surface in laminar hood inoculate each petri plate with one of the pure strain bacterial suspension samples. By micropipette, we take 1% from the pure bacterial suspension and streaked into the Petri plate that contains nutrient agar medium. All the Petri plate that incubated in an incubator at 45°C for 24 hours for growing young bacteria. After 24 hr bring all bacterial plates culture from incubator to laminar hood for transfer young bacterial colonies, by incubation loop take a loop full of the pure bacterial culture and simply dipped into the bottles that contain nutrient broth medium. Then put all the bottles that inoculated in the shaker incubator 150 rpm at 40°C for the production alpha amylase and xylanases. Each day supernatant was taken and cells were discarded and determined the specific activity of the crude and purified enzyme was using DNS assay and Lowry method (Lowry et al, 1951; Miller, 1959).

3.2.5. Solid-state fermentation (SSF)

Performed by taking 3 g of a dry substrate (particle size 1500 µm apple shell, wheat bran, rice husk, banana peel, barley husk and orange peel) in 100 ml scrawl bottles and 10 ml of distilled water were added to adjust the desired humidity level. Autoclaved at 121°C for 20 min. After cooling was inoculated with 2 ml culture broth and inoculated at 40°C for 48 hr. The suspensions were then filtered and centrifuged at 6000 rpm at 4°C for 10 min and the supernatant was utilized for the enzyme assay. NB liquid media was used as positive control.

3.2.6. Submerged fermentation (SmF)

The inoculums was prepared by adding distilled water in to nutrient broth. Inoculated 0.2 ml of bacterial suspension into 20 ml of sterilized fermentation medium pH adjusted. After inoculated at 40°C, the fermentation medium was collected by centrifugation at 6000 rpm for 10 min at 4°C. Supernatant used from enzyme assay.

3.2.7. Preparation of enzymes

B.licheniformis VO14 and *Thermobacillus sp* VO15 cells were streaked on to a nutrient agar plate supplement and at 45°C for 24 hr incubated in the incubator. Cells from this plate were incubation in a nutrient broth and at 45°C for 24 hr incubated in the shaker incubator. After incubation at 45°C for 24 hr in shaker incubator 150 rpm. Insulating the enzyme to obtain the crude enzyme, nutrient broth culture 1 ml was transferred to a centrifuge tube at 6000 rpm for 10 min centerfugation.

The supernatant of the results was used as the crude enzymatic extract. A portion of the raw extract was used to determine the enzyme productivity of the strain and the remainder was used for identifecation, characterization and partial purification of xylanase and alpha-amylase.

3.2.8. Alpha-amylase assay

Alpha-amylase activity was defined by measuring the production of the reduction of polysaccharides released in the decomposition of starch with the method of dinitrosalicylic acid (Bernfeld, 1976). According to the method of adding 0.1 ml of enzyme (crude/supernatant fermented extract) in each of the test tubes and 0.2 ml of 1% soluble starch, incubation for 30 min at 50°C. After 0.4 ml of the reagent 3, 5 dinitrosalicylic acid added the reaction was stopped, followed by heat in boiling water bath for 5 min change color. Add the final 3 ml of distilled water and absorbent reading at 489 nm in the UV/VIS spectrophotometer. The results collected were correlated with the standard maltose. Enzyme activity was expressed in units/ml.

3.3.9. Xylanase assay

The procedure of xylanase assay by 0.25 ml of supernatant (crude extract/ fermented broth supernatant), 0.25 ml of xylan supernatant solution was added, and then the solution was incubated at 50°C for 15 min. After 0.5 ml of dinitrosalicylic acid reagent added the reaction was stopped (Miller, 1956). Followed by boiling water 5 min. Each day supernatant was taken and cells were discarded and activity was measured at 540 nm. A unit of activity xylanases the amount of enzyme was defined that released 1mmol of reducing sugars as xylose under assay condition.

3.3. Factors effect on Xylanase and Alpha-amylase Production

3.3.1. Incubation period

Influence of incubation time was determined by incubating the production medium for several incubation periods (8, 16, 24, 36, 48, 72, 96, 120, 144 hr) at 40°C in shaker incubator (JERO TECH SI-600) 150 rpm.

3.3.2. Temperature

The temperature effect on the alpha-amylase and xylanase production was performed using the following temperature values (20ºC - 80ºC) for *B. licheniformis* VO14 and *Thermobacillus sp* VO15, after which and based on (DNS) assay, has been performed (Bertrand et al, 2004).

3.3.3. pH

The pH effect on the alpha-amylase and xylanase production was carried out using pH values followed by 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0 and 11.0 for both *B.licheniformis* VO14 and *Thermobacillus sp* VO15 after which it can be performed according to the dinitrosalicylic acid method.

3.3.4. Carbon sources (1%)

To study different carbon sources effect on the production of xylanase and alpha-amylase, *B. licheniformis* VO14, and *Thermobacillus sp* VO15 strains were cultured on glucose, galactose, fructose, sucrose, starch, raffinose, arabine, xylose, mannose, and lactose containing as carbon sources. The impact of carbon sources on the production of xylanase and alpha-amylase is then distinguished by dinitrosalicylic acid method.

3.3.5. Nitrogen sources (1%)

The secondary energy sources of living organisms are a source of nitrogen, which assumes an essential part in the growth of living organisms and the production enzyme. The alpha-amylase and xylanase production by used source of nitrogen. Nitrogen sources include yeast, amonium sulfat, tryptone soya broth, ammonium acetate, glycine, L-proline, D-methionine, urea, L-tyrosine and D-aspartic acid. The nitrogen sources impact on the production of xylanase and alpha-amylase is then distinguished by dinitrosalicylic acid method.

3.3.6. Surfactant

The effects of Surfactant on alpha-amylase and xylanase production were determined by incubating the bacterial suspension with a 0.5% solution of Triton X-100, Tween-20 and Tween-40 at 40°C for 24 hr in a shaker incubator (JERO TECH SI-600), 150 rpm. The impact of surfactant on the production of alpha-amylase and xylanase was defined by dinitrosalicylic method.

3.4. Standard Estimation for Xylose

Xylose calibration curve were established using known concentrations of xylose standard of different xylose concentrations, between 500 – 5000 µM, were prepared pure xylose and distilled water. Measured the absorbance of each sample according to the DNS assay. Then, the measured absorbance was 540 nm. The resulting curve was linear, and thus, was used to convert the absorbance to the xylose concentration for this experiment.

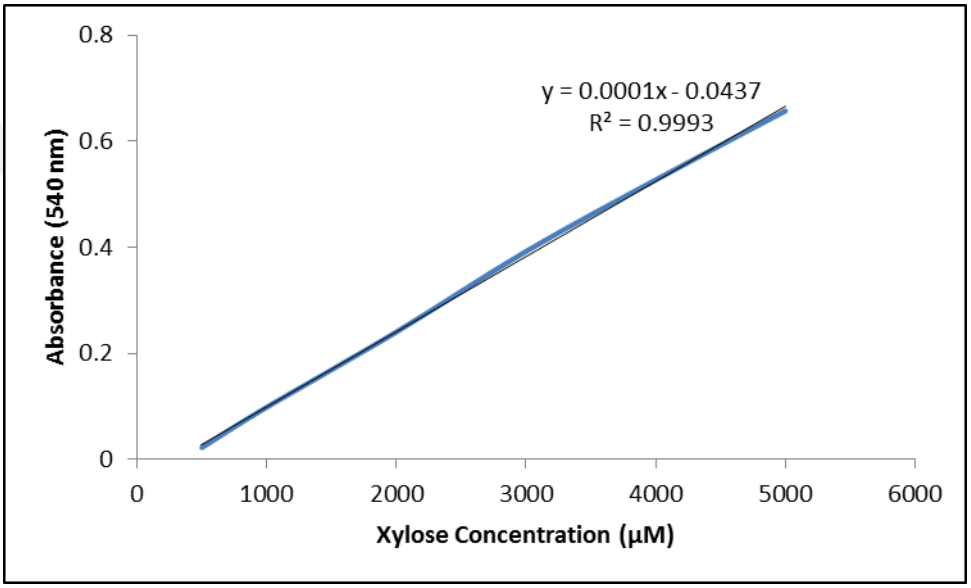


Figure 3.3. Standard curve for xylose.

3.5. Standard Estimation for maltose

Maltose calibration curve were established using known concentrations of maltose standard at different maltose concentrations, between 500 – 5000 µM, were prepared pure maltose with distilled water. Measured the absorbance of each concentration according to the DNS assay. Then, the measured absorbance was 489 nm. The resulting curve was linear, and thus, was used to convert the absorbance to the maltose concentration for this experiment.

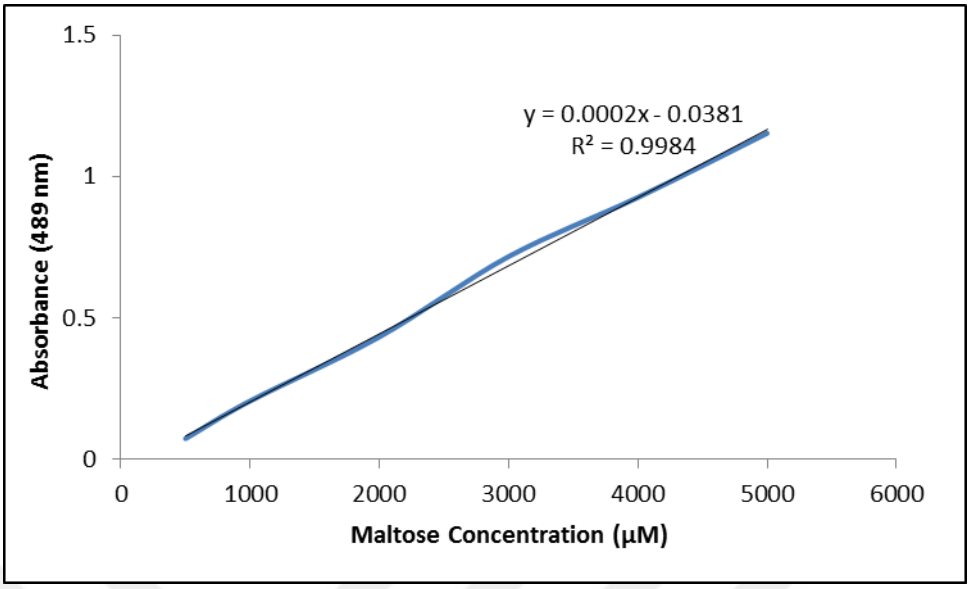


Figure 3.4. Standard curve for maltose**.**

3.6. Standard Estimation Protein

The protein was estimated by using bovine serum albumin as standard. Bovine serum albumin (BSA) calibration curve was established using known BSA concentrations at different concentrations of BSA, between 0.5-5.0 mg/ml, pure BSA were prepared with distilled water. Measured the absorbance of each concentration according to the DNA assay. Then, the absorbance measured was 660 nm. The resulting curve was linear and therefore was used to convert the concentration absorbance to the BSA for this eperiment.

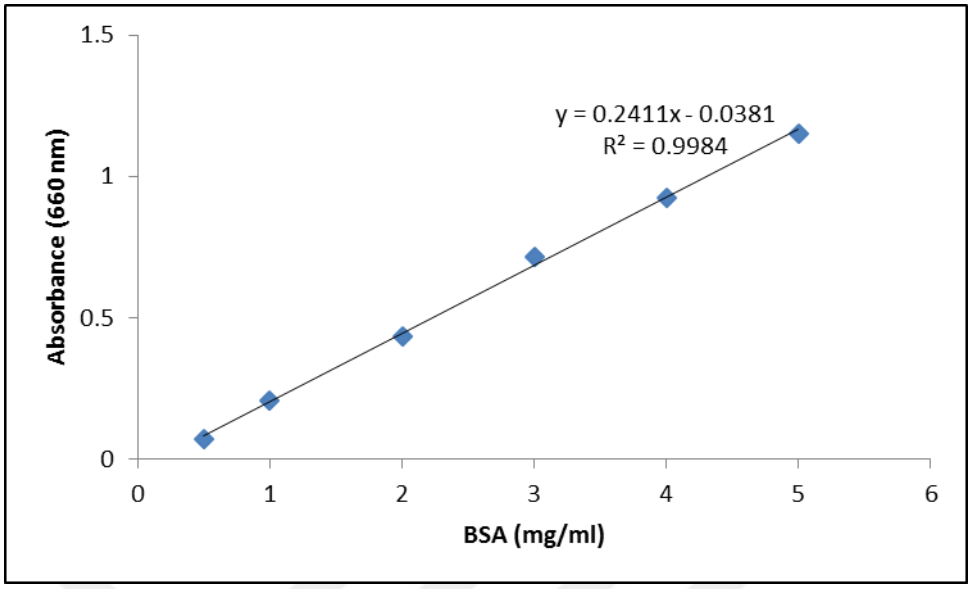


Figure 3.5. Standard curve for BSA.

3.7. Enzymes Characterization

Effect of the pH, temperature, metal ions, surfactant and detergents on the alpha-amylase and xylanase activity and stability was evaluated.

3.7.1. pH

The pH effects on alpha-amylase and xylanase activity and stability by using Sodium acetate, NaH_2PO_4, Na_2HPO_4, NaOH, and HCL were defined by the enzyme incubating in different pH 4.0 - 11.0 for the various period 30, 60, 90, 120 and 240 min at room temperature.

3.7.2. Temperatures

The effects of temperature on alpha-amylase and xylanase activity and stability were evaluated by testing a wide range of temperature 30°C - 110°C for the different period 30, 60, 90, 120 and 240 min.

3.7.3. Metal ions

The metal ions impact on xylanase and alpha-amylase activity was evaluated in the distinctive metal ions presence ($CdCl_2$, $Cu(NO_3)_2$, $Co(NO_3)_2$, $Pb(NO_3)_2$, $AlCl_3$ and $HgCl_2$). For amylase activity added 100 µl alpha-amylase enzyme supernatant and 25 µl 2 mM metal ion were incubated 40ºC for 60 min.

And for xylanase activity added 250 µl xylanase enzyme and 62.5 µl in 2 mM metal ion were incubated 40ºC for 60 min. After the incubation period, the reaction started when the substrate was added. Then, enzymatic activity was measured in standard assay conditions. Variation of absorbance recorded spectrophotometrically for alpha-amylase and xylanase at 489 nm and 540 nm, respectively, and the remaining activity was obtained.

3.7.4. Surfactants and detergent

The effect of surfactants (Triton X-100, Tween-20, Tween-40, and Tween-80) on enzyme activity was examined. 100 µl alpha-amylase enzyme and 100 µl 0.5% (v/v) of the agents were incubated 40ºC for 60 min. 250 µl xylanase and 250 µl 0.5% (v/v) of the agents were incubated 40ºC for 60 min. After the incubation period, the alpha-amylase and xylanase substrate (starch 1% and xylan 1% solution) were added respectively, the reaction was started. Then, the activity of the enzyme was measured in standard assay conditions. The changing of the absorbance was recorded spectrophotometrically alpha-amylase and xylanase at 489 nm and 540 nm respectively the residual activity was obtained.

The effect of some detergents (Alo, Omo, Ariel and Tursil) on enzyme activity was examined. 100 µl alpha-amylase enzyme and 100 µl 0.5% (v/v) of the detergents were incubated 40ºC for 60 min. 250 µl xylanase and 250 µl 0.5% (v/v) of the detergents were incubated 40ºC for 60 min. After the incubation period, the alpha-amylase and xylanase substrate (starch 1% and xylan 1% solution) were added respectively, the reaction was started. Then, enzyme activity was measured in standard assay conditions. The changing of the absorbance was recorded spectrophotometrically alpha-amylase and xylanase at 489 nm and 540 nm respectively the residual activity was obtained.

4. RESULTS

4.1 Phylogenetic Analyzes of *B.licheniformis* VO14 and *Thermobacillus sp* VO15

The sequence of 16S rRNA gene showed maximum identity for VO15, 99% and for VO14, 96% similarity with *Thermobacillus sp*. and *B.licheniformis*, respectively. Strain VO14 and VO15 gene sequences have been submitted to the National Center for Biotechnology Information (NCBI) database of Gen Bank and stored under the access number are KJ842096.1 and KJ842097.1 respectively.

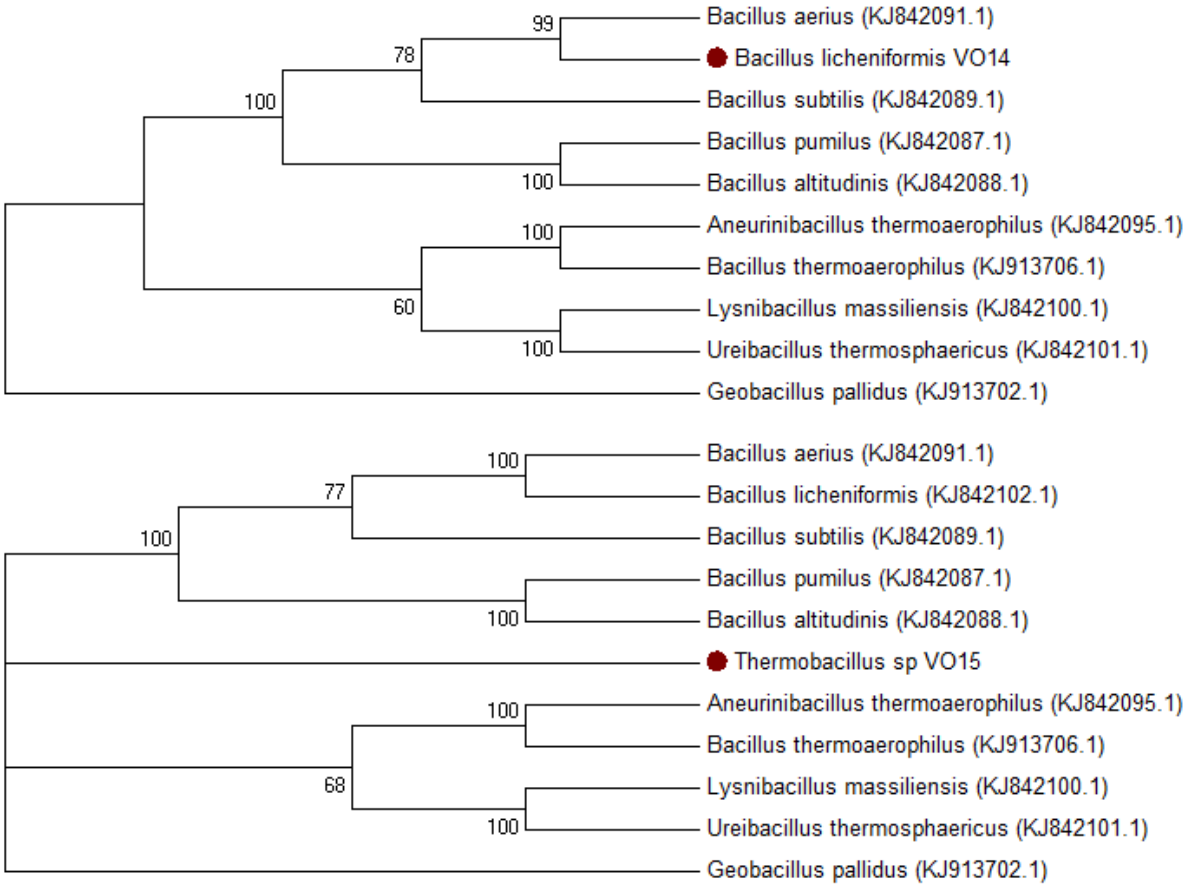


Figure 4.1. Evolutionary relationships of taxa of *B.licheniformis* VO14 and *Thermobacillus sp* VO15.

4.2. Bacterial Growth

Optimization of growth conditions of the bacteria using the incubation time, temperature and pH parameter.

4.2.1. Incubation time

Bacterial growth was affected by incubation time. Figure (4.2) shows that a maximum growth of *Bacillus licheniformis* and *Thermobacillus sp* was obtained after 4 days (96 hr) incubation, then with elongation of time, the yield of growth decreased. The optimum incubation time for *Bacillus licheniformis* at 24 hr and optimum incubation time for *Thermobacillus sp* at 20 hr.

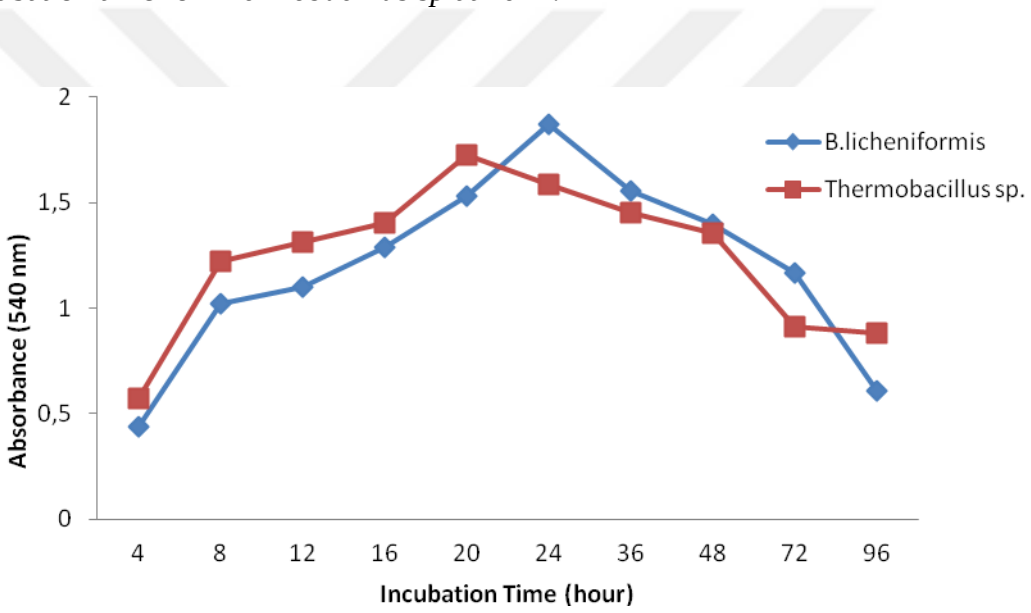


Figure 4.2. Effect incubation time on bacterial growth.

4.2.2. Temperature

Results from Figure (4.3) present the influence of different incubation temperature on the growth of *Bacillus licheniformis* and *Thermobacillus sp*. The optimum growth of *Bacillus licheniformis* at 40°C. Also, the optimum growth of *Thermobacillus sp* was obtained at 45°C.

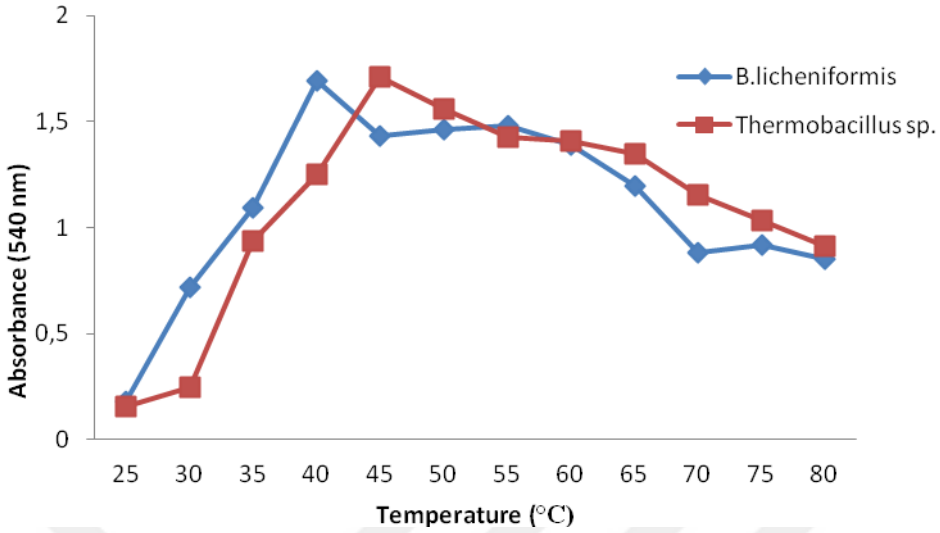


Figure 4.3. Effect of temperature on bacterial growth.

4.2.3. pH

Evaluate thr effect of pH on growth of *Bacillus licheniformis* and *Thermobacillus sp*. The optimum growth of *Bacillus licheniformis* at pH 8.0 was obtained, the optimum growth of *Thermobacillus sp* at pH 6.0.

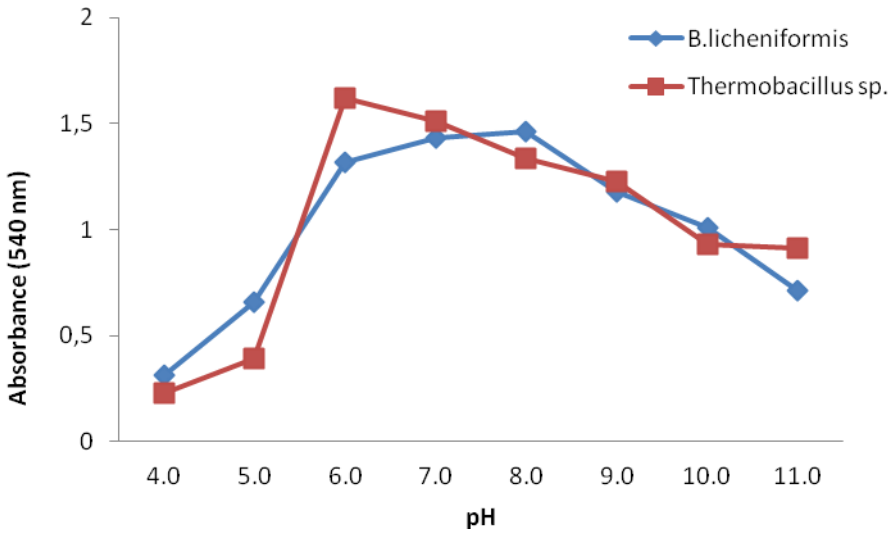


Figure 4.4. Effect of pH on bacterial growth.

4.3. Enzyme Production by SmF

4.3.1. Effect of incubation time on the production of alpha-amylase and xylanase

4.3.1.1. Effect of incubation time on the production of alpha-amylase

The produce of alpha-amylase under SmF is determined by incubating *B.licheniformis* VO14 and *Thermobacillus sp* VO15 in various time, and the fermentation condition of fermentation. Data presented in Figure (4.5) that suggest the optimum alpha-amylase production could be obtained by *B.licheniformis* VO14 at 24 hr of incubation (12.53 U/mg). The optimum alpha-amylase production by *Thermobacillus sp* VO15 could be obtained at 48 hr of incubation (15.98 U/mg).

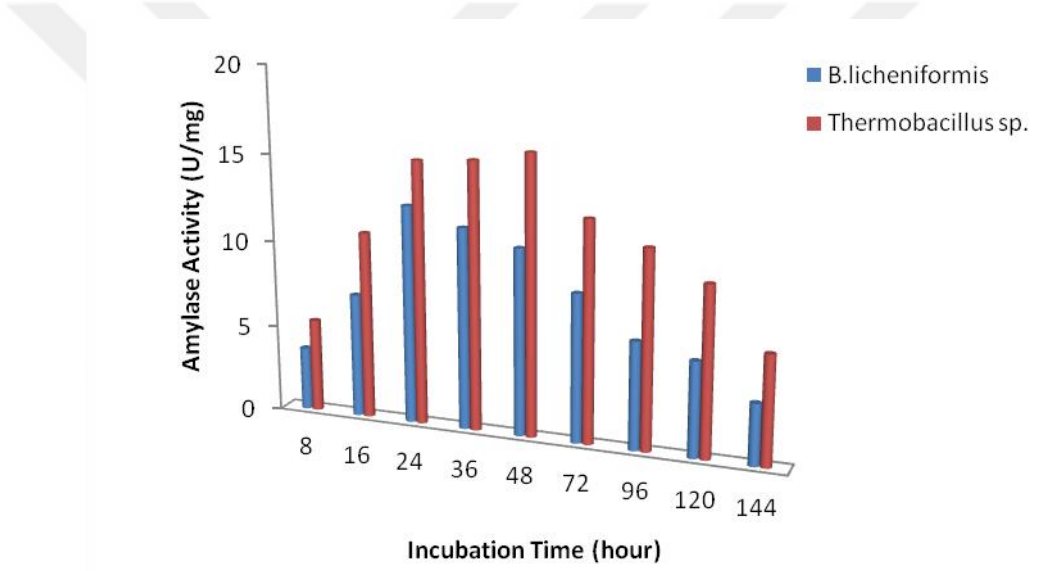


Figure 4.5. Effect of incubation time on the production of alpha-amylase.

4.3.1.2. Effect of incubation time on the production of xylanase

The xylanase production under SmF was determined by incubating the *B.licheniformis* VO14 and *Thermobacillus sp* VO15 from various time, at the optimal fermentation condition. Data presented in Figure (4.6) suggest that the maximum xylanase production by *B.licheniformis* VO14 and *Thermobacillus sp* VO15 could be obtained at 24 hr of incubation (28.24 U/mg) and (32.52 U/mg) respectively.

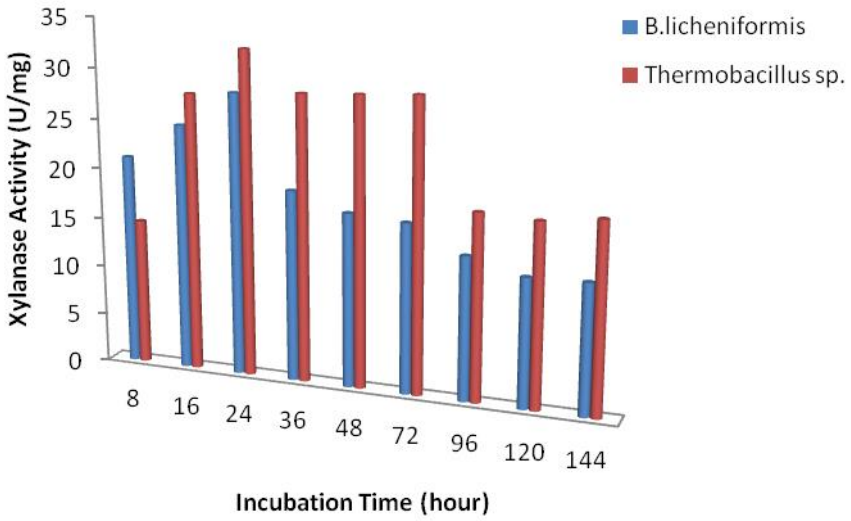


Figure 4.6. Effect of incubation time on the production of xylanase.

4.3.2 Effect of temperature on the production of alpha-amylase and xylanase

4.3.2.1 Effect of temperature on the production of alpha-amylase

The impact temperature on the production of alpha-amylase from *B.licheniformis* VO14 and *Thermobacillus sp* VO15 was studied. The maximum alpha-amylase production by *Thermobacillus sp* (18.72 U/mg) obtained at 40ºC and the production maximum alpha-amylase by *B.licheniformis* (16.34 U/mg) obtained at 45ºC.

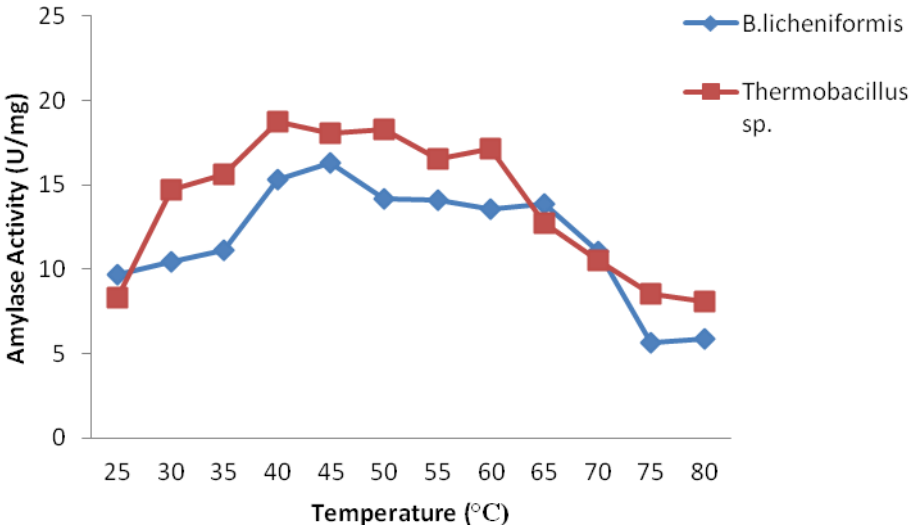


Figure 4.7. Effect of temperature on the production of alpha-amylase.

4.3.2.2. Effect of temperature on the production of xylanase

The impact temperature on the production of xylanase from *B.licheniformis* VO14 and *Thermobacillus sp* VO15 was studied. The maximum xylanase production by *B.licheniformis* (28.36 U/mg) observed at 40ºC and the maximum production of alpha amylase by *Thermobacillus sp* (33.52 U/mg) was observed at 45ºC.

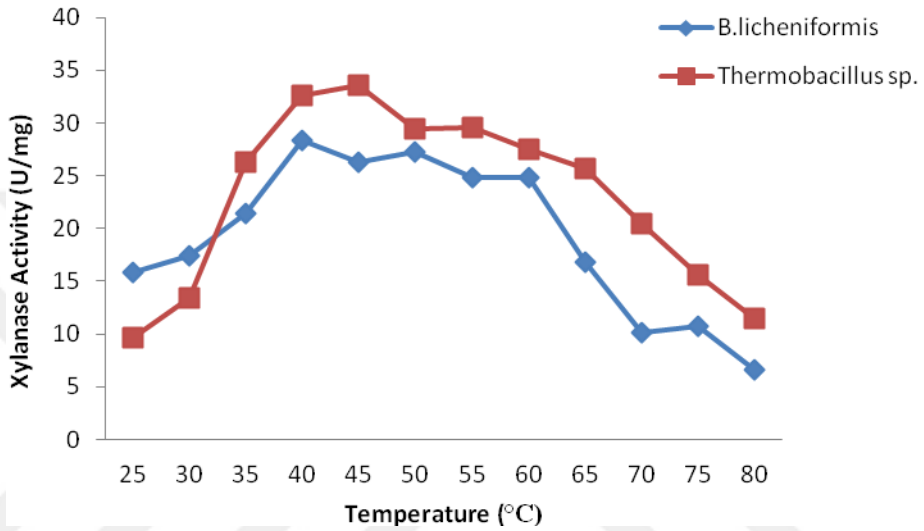


Figure 4.8 . Effect of temperature on the production of xylanase.

4.3.3. Effect of pH on the production of alpha-amylase and xylanase

4.3.3.1. Effect of pH on the production of alpha-amylase

The production alpha-amylase in this study by *B.licheniformis* VO14 and *Thermobacillus sp* VO15 It was found, the maximum production alpha-amylase from *B.licheniformis* at the range of pH 7.0 (20.32 U/mg). Also, the maximum production alpha-amylase from *Thermobacillus* sp at the range of pH 6.0 (21.36 U/mg).

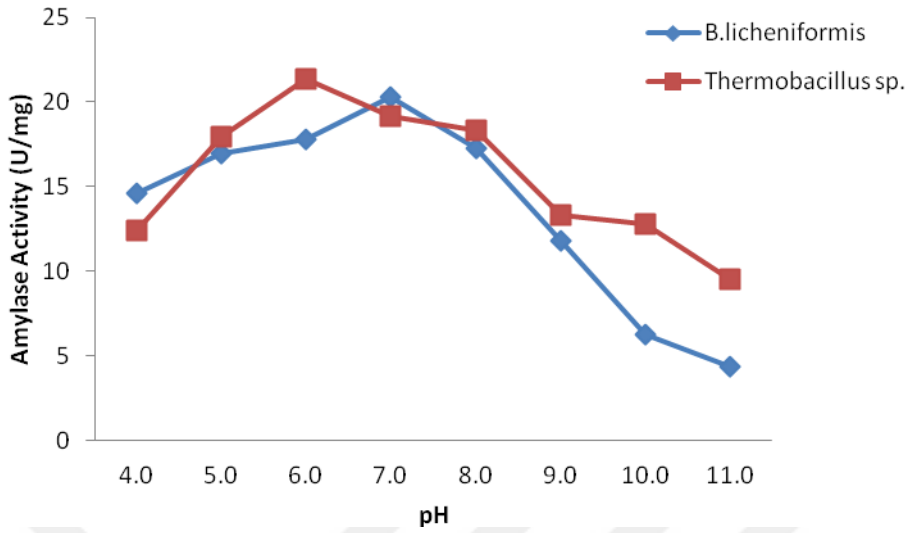


Figure 4.9. Effect of pH on the production of alpha-amylase.

4.3.3.2. Effect of pH on the production of xylanase

Xylanase production by *B.licheniformis* VO14 and *Thermobacillus sp* VO15 was found to be, the maximum xylanases production from *B.licheniformis* at the range of pH 5.0 (30.36 U/mg). And the maximum xylanases production from *Thermobacillus sp* at the range of pH 6.0 (31.23 U/mg).

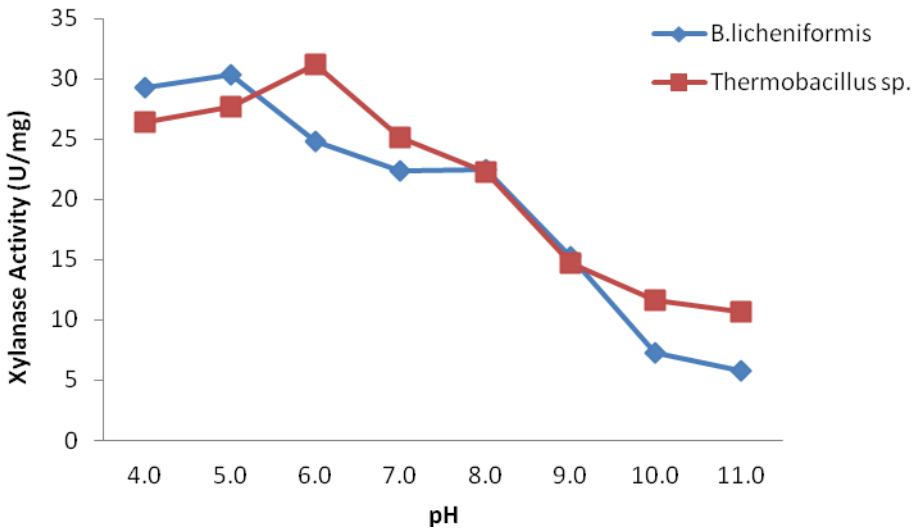


Figure 4.10. Effect of pH on the production of xylanase.

4.3.4. Effect of carbon sources on the production of alpha-amylase and xylanase

4.3.4.1 Effect of carbon sources on the production of alpha-amylase

The effect of 1% carbon sources added in the medium such glucose, galactose, starch, and lactose has been studied. As shown in Figure (4.11) for *B.licheniformis* VO14, the influence of galactose was more affected (99.7%) to alpha-amylase production than other carbon sources. Raffinose gave the lowest alpha-amylase production (18.5%). About the *Thermobacillus sp* VO15, also galactose was more affected (103.96%) to production the alpha-amylase than other carbon sources. Raffinose gave the lowest alpha-amylase production (34.73%).

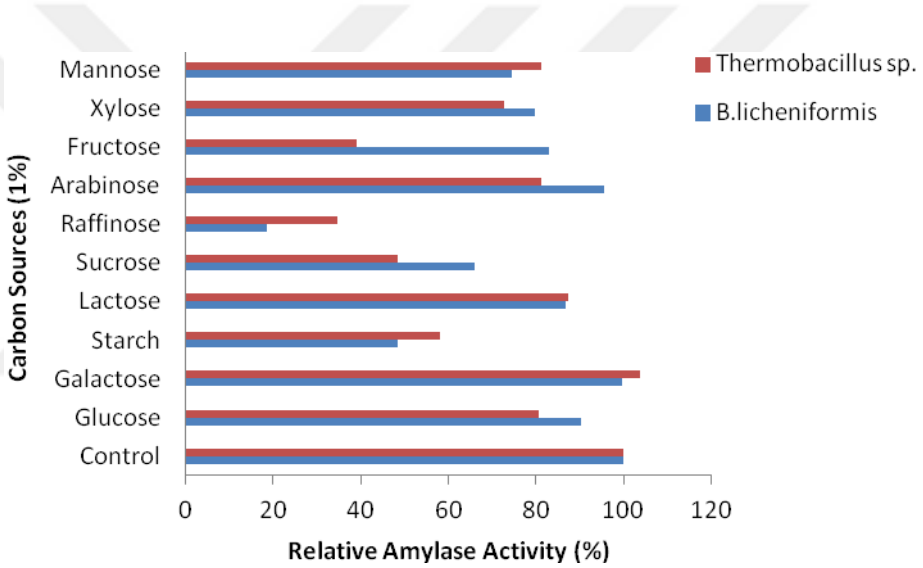


Figure 4.11. Effect of carbon sources on the production of alpha-amylase.

4.3.4.2 Effect of carbon sources on the production of xylanase

To study the effects of various carbon sources for the production of xylanase, *B.licheniformis* VO14, and *Thermobacillus sp* VO15 was grown in carbon sources 1%, such as glucose, galactose, lactose, fructose, sucrose, starch, raffinose, and arabinose. *B.licheniformis* the influence of glucose was more affected than the other carbon sources for the production of xylanase (126.28%). Raffinose gave the lowest production xylanase (37.34%) in the carbon source for production xylanase.

The *Thermobacillus sp* influence of glucose was more affected than the other carbon sources for xylanase production (109.65%). Sucrose gave the lowest production xylanase in the carbon source for production xylanase (33.04%).

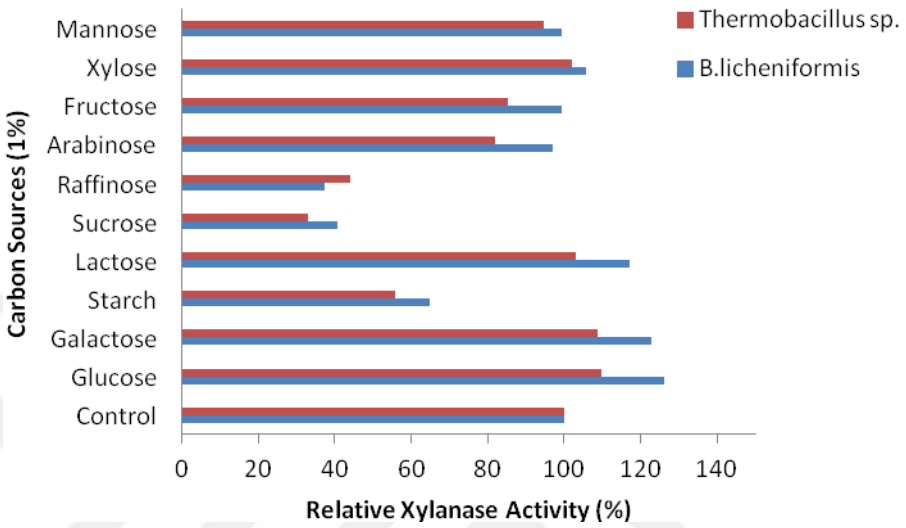


Figure 4.12. Effect of carbon sources on the production of xylanase.

4.3.5. Effect of nitrogen sources on the production of alpha-amylase and xylanase

4.3.5.1 Effect of nitrogen sources on the production of alpha-amylase

The influence of the nitrogen sources on production alpha-amylase was showing Figure (4.13). More alpha-amylase it has been reported that was produced when nitrogen compounds were used. The maximum alpha-amylase production by *B.licheniformis* VO14 was found with yeast extract (122.44%). A minimum amount of production alpha-amylase by *B.licheniformis* was seen when used ammonium sulfate (70.96%). As well as, maximum production alpha-amylase by *Thermobacillus* sp VO15 was observed with ammonium sulfate (117.6%). A minimum amount of production alpha-amylase by *Thermobacillus sp* was seen when used L-proline (52.48%).

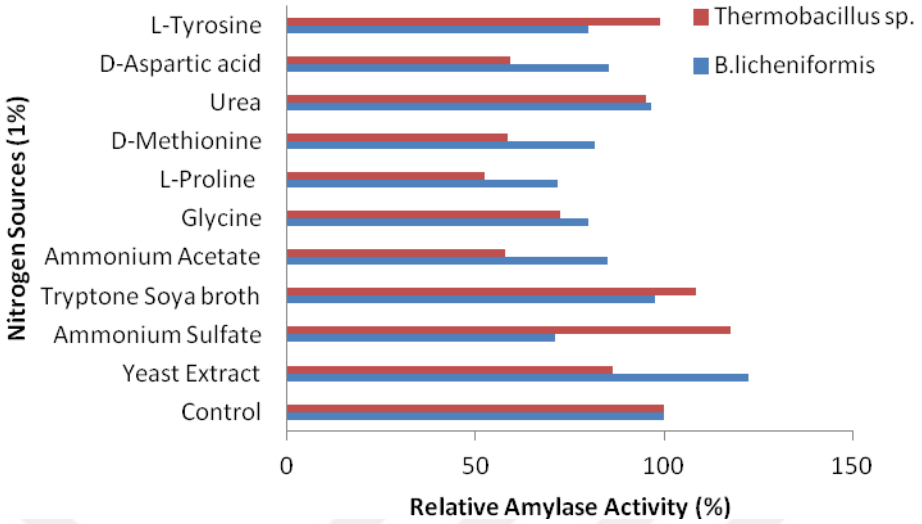


Figure 4.13. Effect of nitrogen sources on production of alpha-amylase.

4.3.5.2 Effect of nitrogen sources on the production of xylanase

Investigations have been done for the supplementation of nitrogen sources in the medium that showed effects different on xylanase production by *B.licheniformis* VO14 and *Thermobacillus sp* VO15. between various nitrogen sources, Yeast extract approved maximum production xylanase by *B.licheniformis* (124.37%). Yeast extract also supported maximum volume xylanase production by *Thermobacillus sp* (107.67%). A minimum production of xylanase from *B.licheniformis* and *Thermobacillus sp* obtained (48.15%) and (33.73%) respectively when L-proline was performed.

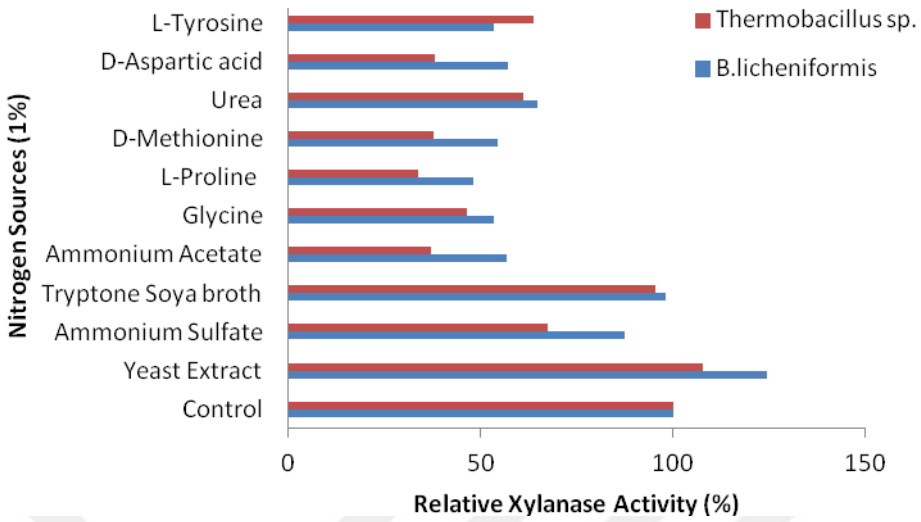


Figure 4.14. Effect of nitrogen sources on production of xylanase.

4.3.6. Effect surfactant on the production of alpha-amylase and xylanase

4.3.6.1. Effect of surfactant on production alpha-amylase

The effect of some surfactants on the production of alpha-amylase has been studied. Tween 40 causes very much effect on *B.licheniformis* VO14 and *Thermobacillus sp* VO15 for the production of alpha-amylase (135.3% and 174.6%), respectively. Tween 20 although prompting *B.licheniformis* for production (135.3%) of alpha-amylase and the Triton X100 (109.1%). Tween 20 promoting *Thermobacillus sp* for production (121.6%) alpha-amylase and Triton X100 (92.1%),

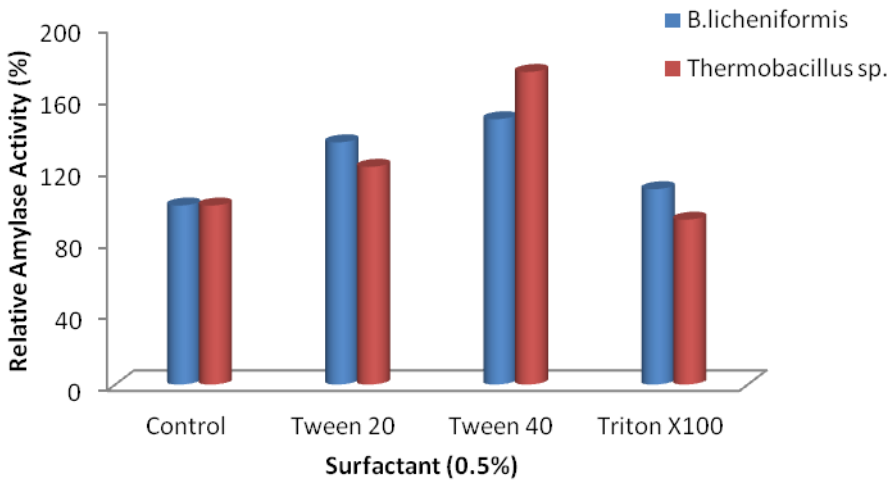


Figure 4.15. Effect of surfactant on production alpha-amylase.

4.3.6.2. Effect of surfactant on xylanase production

Perform a different type of surfactants on a production of xylanase. Tween 40 significantly increased the production of xylanase from *B.licheniformis* VO14 and *Thermobacillus sp* VO15 (114.8%) and (121.6%) respectively. The other surfactant Tween 20 and Triton X100 also have an effect on the production of xylanase but less than Tween 40 showing by Figure (4.16).

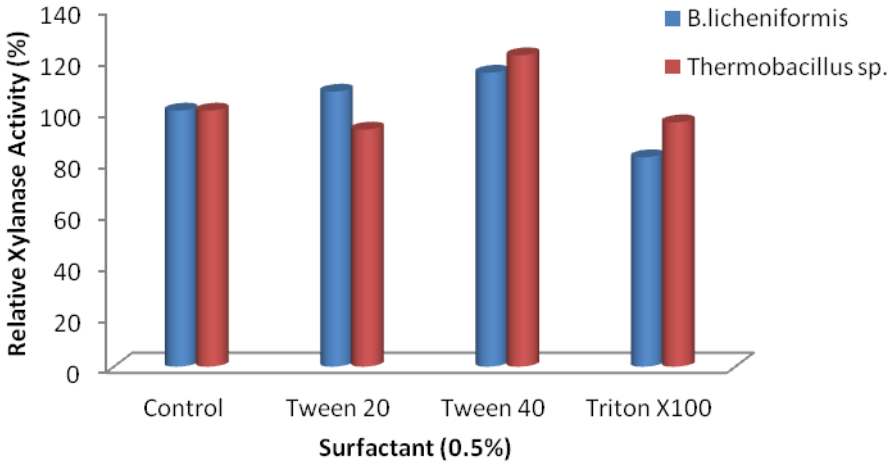


Figure 4.16. Effect of surfactant on xylanase production

4.4. Production Enzyme by (SSF)

4.4.1. Alpha-amylase production

In the production alpha-amylase by solid state fermentation, various substrates of agricultural wastes such as apple peel, wheat bran, rice flour, banana peel, orange peel and barley peel were used. Alpha-amylase production by *B.licheniformis* VO14 maximum production noted during use of orange peel (153.8%) at 48 hours incubation and maximum production of alpha-amylase from *Thermobacillus sp* VO15 noted during use of orange peel also (145.5%) at 48 hours of incubation.

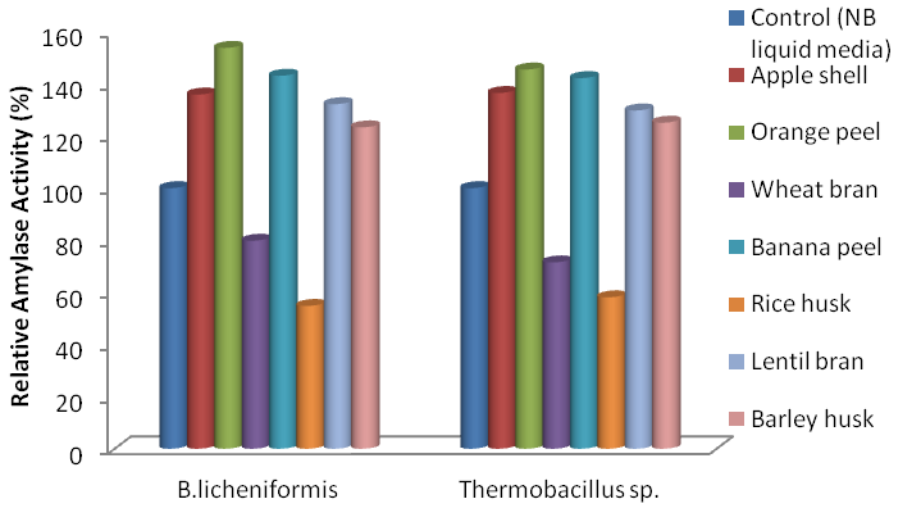


Figure 4.17. Effect of different agricultural wastes on alpha-amylase production by *B. licheniformis* and *Thermobacillus sp*.

4.4.2. Xylanase production

In solid state fermentation for the production xylanase was performed for 48 hr with shaking. Solid state fermentation was reported for greater volumetric productivity than submerged fermentation. In this study, the influence of orange peel for maximum xylanase production from *B. licheniformis* VO14 (265.8%) and *Thermobacillus sp* VO15 (272.5%) was obtained.

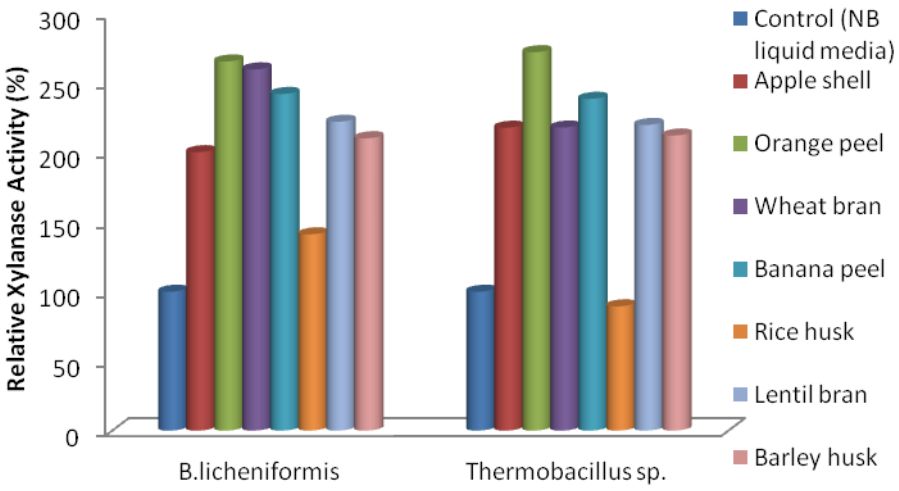


Figure 4.18. Effect different agricultural wastes on xylanase production by *B. licheniformis* and *Thermobacillus sp*.

4.5. Enzyme Characterization

4.5.1. Effect of temperature on alpha-amylase and xylanase activity

4.5.1.1. Effect of temperature on alpha-amylase activity

The effect temperature on alpha-amylase activity was assessed by measuring the enzymatic activity at different temperatures ranging from 30ºC - 80ºC. In Figure (4.19), the effect temperature showed the highest alpha-amylase activity from *B.licheniformis* VO14 at 40ºC. Furthermore, the highest alpha-amylase activity from *Thermobacillus sp* VO15 at 50ºC.

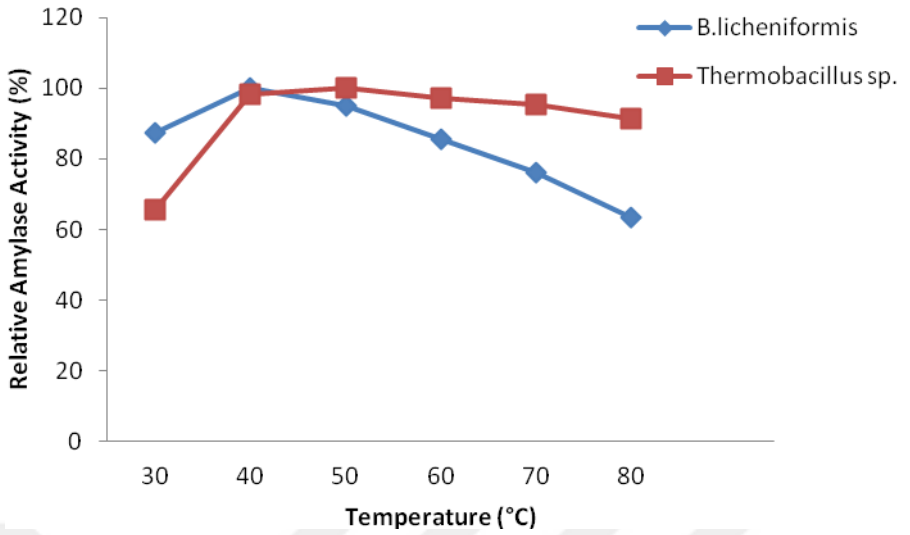


Figure 4.19. Effect of temperature on alpha-amylase activity

4.5.1.2. Effect of temperature on xylanase activity

The temperature effect on the xylanase activity was examined by measuring the enzymatic activity at different temperatures ranging from 30ºC - 80ºC. In Figure (4.20), the effect of temperature showed the highest xylanase activity from *B.licheniformis* VO14 at 40ºC. On the other hand, the highest xylanase activity from *Thermobacillus sp* VO15 at 60ºC.

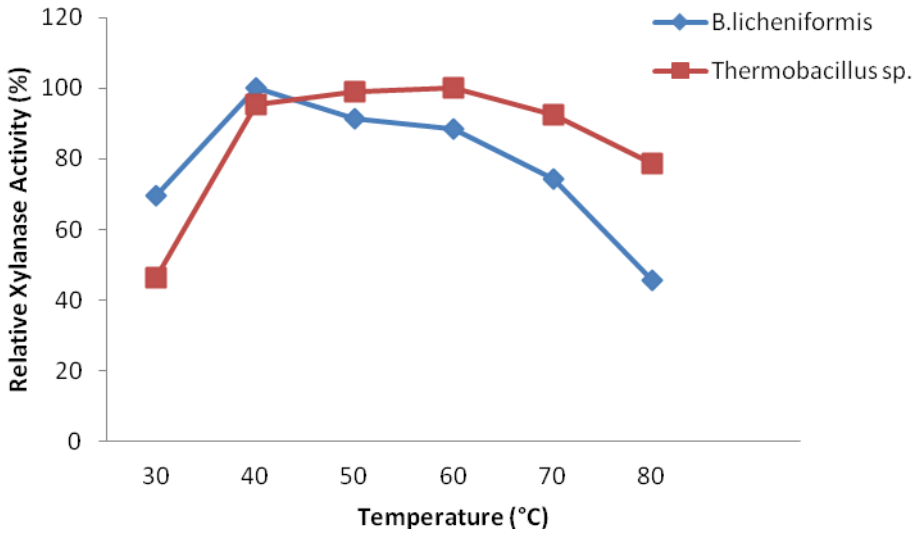


Figure 4.20. Effect of temperature on xylanase activity.

4.5.2. Effect of pH on alpha-amylase and xylanase activity

4.5.2.1. Effect of pH on alpha-amylase activity

The influence of pH on alpha-amylase activity was evaluated by measuring enzymatic activity in the presence of buffers at different pH values (pH 4.0 - 11.0). In the Figure (4.21), the effect of pH was determined the optimum alpha-amylase activity production by *B. licheniformis* VO14 at pH 6.0. In addition, the optimal pH of the alpha-amylase activity isolated from *Thermobacillus sp* VO15. It was pH 7.0. The alpha-amylase from *B.licheniformis* lost about 40% of its activity at pH 4.0 and it lost about 70% of its activity at pH 11.0. While the alpha-amylase from *Thermobacillus sp* lost about 51% of its activity at pH 4.0 and it lost about 57% of its activity at pH 11.0.

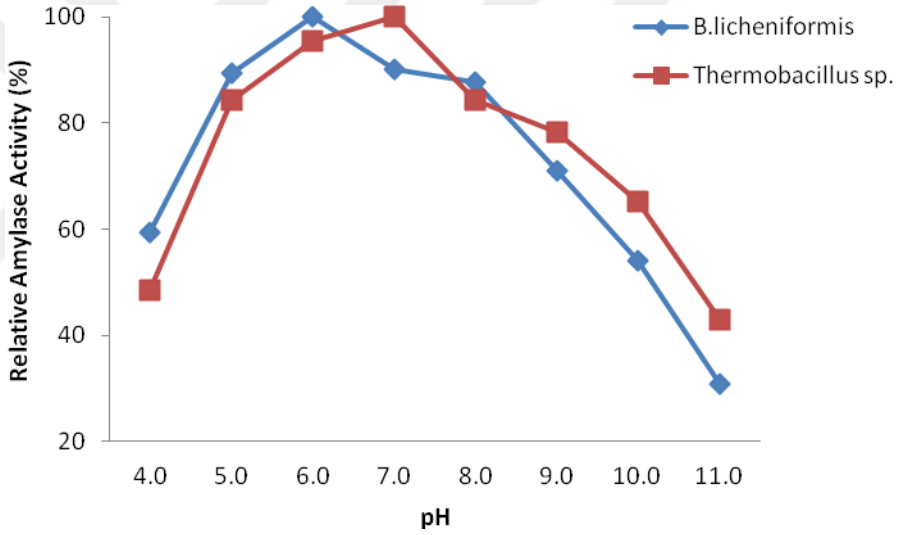


Figure 4.21. Effect of pH on alpha-amylase activity

4.5.2.2. Effect of pH on xylanase activity

The influence of pH on xylanase activity was evaluated by measuring enzymatic activity in the presence of buffers at different pH values (pH 4.0 - 11.0). In the Figure (4.22), the effect of pH showed the optimum xylanase activity production by *B.licheniformis* VO14 at pH 5.0. In addition, the optimal pH of the xylanase enzyme isolated from *Thermobacillus sp* VO15. It was pH 6.0. Xylanase from *B. licheniformis* lost about 39% of its activity at pH 4.0 and it lost about 65% of its activity at pH 11.0.

While the xylanase from *Thermobacillus sp* lost about 25% of its activity at pH 4.0 and it lost about 60% of its activity at pH 11.0.

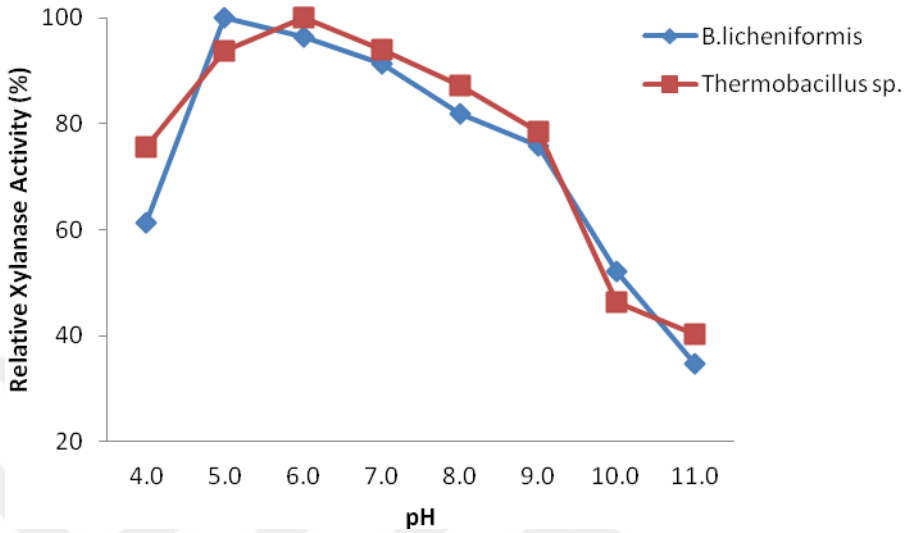


Figure 4.22. Effect of pH on xylanase activity.

4.5.3. Effect of metal ions on alpha-amylase and xylanase activity

4.5.3.1. Effect of metal ions on alpha-amylase activity

The effect of the metal ions on the activity of alpha-amylase was measured in the different metal ions presence such as ($CdCl_2$, $Cu(NO_3)_2$, $Co(NO_3)_2$, $Pb(NO_3)_2$, $AlCl_3$, $CaCl_2$, $ZnCl_2$ and $HgCl_2$). The alpha-amylase supernatant and metal ion 2 mM were incubated for 60 min at 40ºC. After that incubation added the substrate and the reaction were initiated. Then, measured the activity of alpha-amylase under the standard assay conditions. The results in the Figure (4.23), showed *B.licheniformis* VO14 alpha-amylase increase the optimum activity when added $Co(NO_3)_2$. And *Thermobacillus sp* VO15 alpha-amylase enzyme obtained the maximum activity during added $Co(NO_3)_2$.

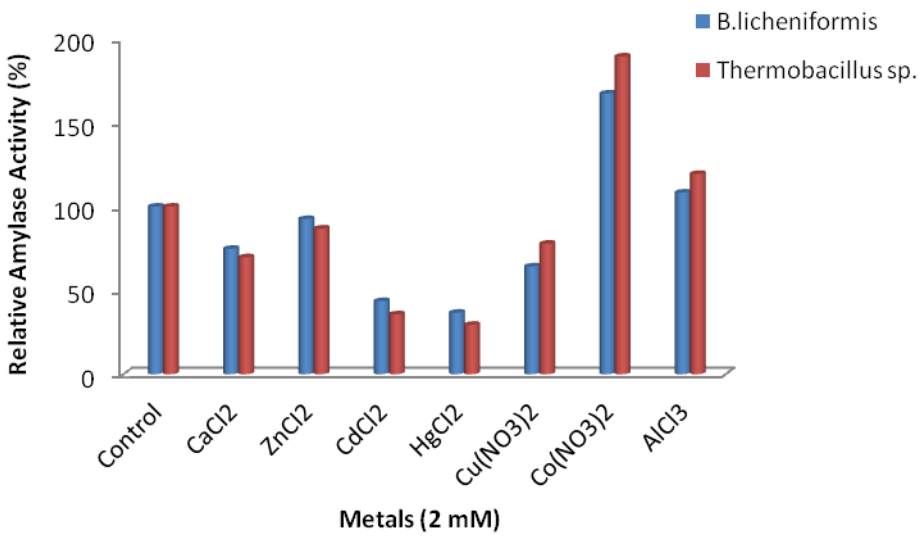


Figure 4.23. Effect of metal ions on alpha-amylase activity

4.5.3.2. Effect of metal ions on xylanase activity

The effect of the metal ions on the activity of xylanase was measured in the different metal ions presence such as ($CdCl_2$, $Cu(NO_3)_2$, $Co(NO_3)_2$, $Pb(NO_3)_2$, $AlCl_3$, $CaCl_2$, $ZnCl_2$ and $HgCl_2$). The xylanase supernatant and metal ion 2 mM were incubated for 60 min at 40ºC. After that incubation added the substrate and the reaction were initiated. Then, measured the activity of xylanase under the standard assay conditions. The results in the Figure (4.24), showed *B.licheniformis* VO14 xylanase the maximum activity obtained during added $Co(NO_3)_2$. And *Thermobacillus sp* VO15 xylanase obtained the maximum activity during added $Co(NO_3)_2$.

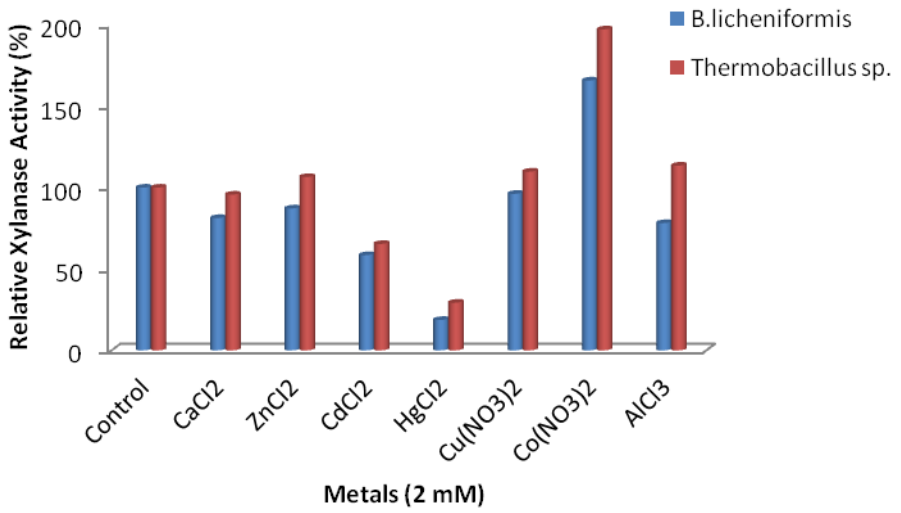


Figure 4.24. Effect of metal ions on xylanase activity

4.5.4. Effect of NaCl on alpha-amylase and xylanase activity

4.5.4.1. Effect of NaCl on alpha-amylase activity

Determined the effect different NaCl concentration (1.0%, 5.0%, 10.0% and 15.0%) on the activity of the alpha-amylase. In generally NaCl did not cause much effect on the activity the *B. licheniformis* VO14 alpha-amylase showed nearly 98% and *Thermobacillus sp* VO15 alpha-amylase showed nearly 93% of its activity in the presence of 1% NaCl. Effect other concentration its lower than 1% NaCl.

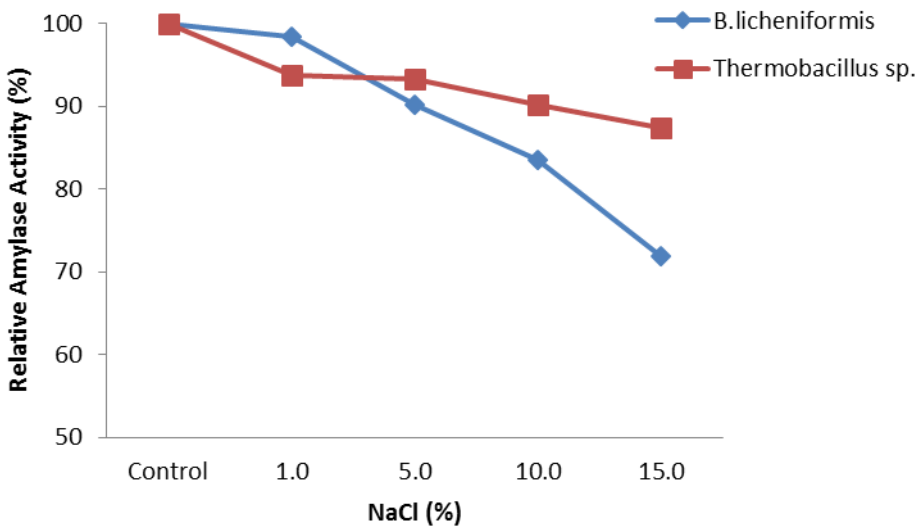


Figure 4.25. Effect of NaCl on alpha-amylase activity.

4.5.4.2. Effect of NaCl on xylanase activity

effect different concentration (1.0%, 5.0%, 10.0% and 15.0%) NaCl on the xylanase activity. In generally NaCl did not cause much effect on the activity of xylanase same alpha-amylase. The *B. licheniformis* VO14 xylanase showed nearly 93% and *Thermobacillus sp* VO15 xylanas showed nearly 97.8% of its activity in the presence of 1% NaCl. Effect other concentration its lower than 1% NaCl.

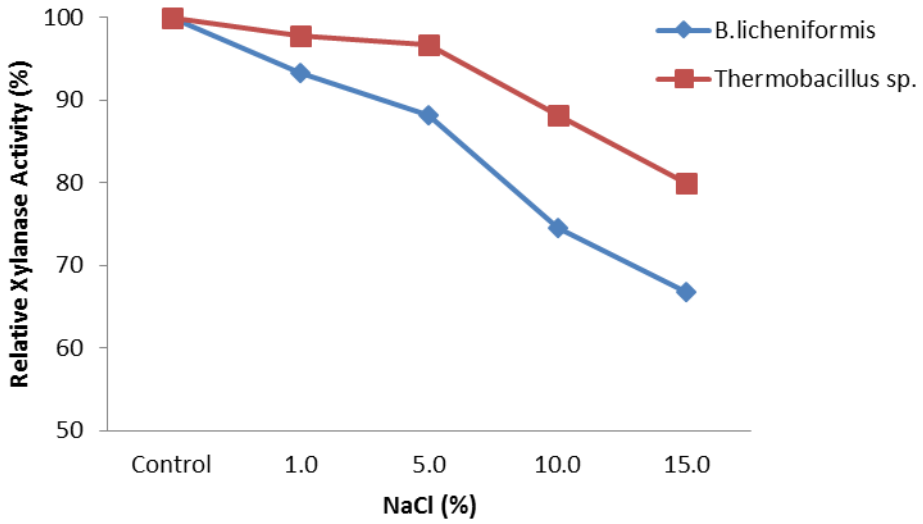


Figure 4.26. Effect of NaCl on xylanase activity.

4.5.5. Effect of surfactant and detergent on alpha-amylase and xylanase activity

4.5.5.1. Effect of surfactant and detergent on alpha-amylase activity

The influence of surfactants and detergents on alpha-amylase activity was evaluated by measuring enzymatic activity in the presence of 0.5% surfactants or detergents. Types of surfactant used such as (Tween20, Tween40, Tween80, and Triton X100). In the Figure (4.27), the effect of surfactants showed the optimum alpha-amylase activity produced by *B. Licheniformis* VO14 and *Thermobacillus sp* VO15 were obtained at used Tween40 (137.3% and 145.8%) respectively.

While types of detergents used such as (Alo, Omo, Tursil, and Ariel). The effect of detergent showed the optimum alpha-amylase activity production by *B. licheniformis* VO14 were obtained at used Tursil (96.4%). In addition, the optimal activity of the alpha-amylase enzyme isolated from *Thermobacillus sp* VO15. It was Omo (105.3%).

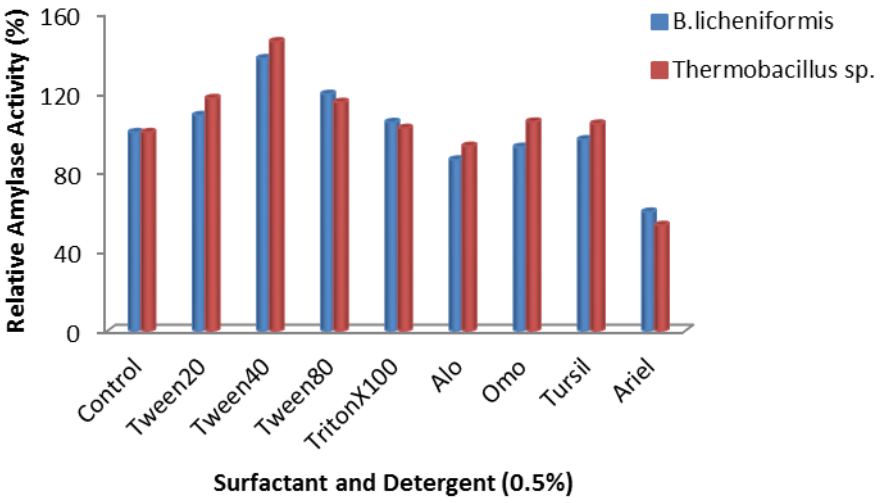


Figure 4.27. Effect of surfactant and detergent on alpha-amylase activity.

4.5.5.2. Effect of surfactant and detergent on xylanase activity

The influence of surfactants and detergents on xylanase activity was evaluated by measuring enzymatic activity in the presence of 0.5% surfactants or detergents. Types of surfactants used such as (Tween20, Tween40, Tween80, and Triton X100). In the Figure (4.28), the effect of surfactants showed the optimum xylanase activity produced by *B. Licheniformis* VO14 and *Thermobacillus sp* VO15 were obtained at used Tween40 (145.1% and 131.2%) respectively.

While types of detergent used such as (Alo, Omo, Tursil, and Ariel). The effect of detergents showed the optimum xylanase activity production by *B. Licheniformis* VO14 were obtained at used Tursil (121.3%). In addition, the optimal activity of the xylanase enzyme isolated from *Thermobacillus sp* VO15. It was Tursil (128.9%).

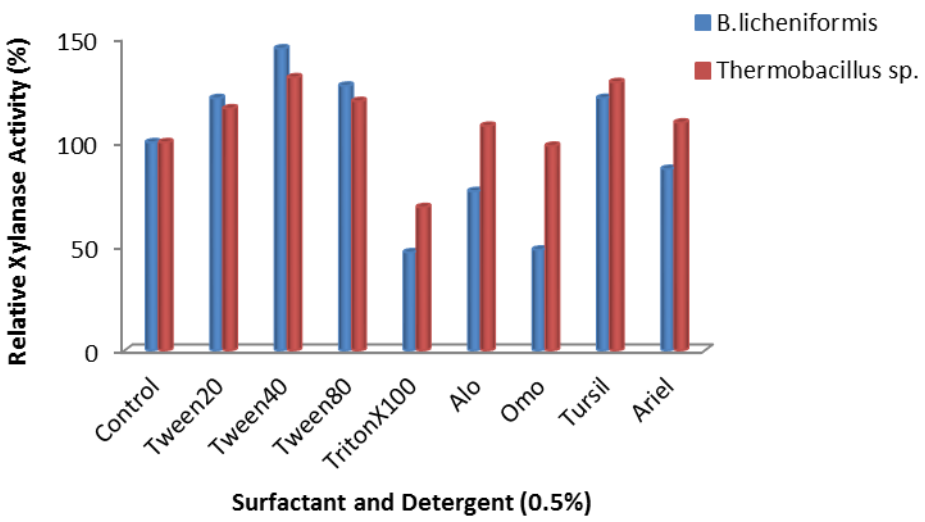


Figure 4.28. Effect of surfactant and detergent on xylanase activity.

4.5.6. Degradation of different starch by alpha-amylase

Detection the degradation different type of starch such as (wheat starch, potato starch, rice starch, and corn starch). By used 0.2 ml from 0.5% of starch solution with 0.1ml alpha-amylase supernatant were incubation for 30 min at 50ºC. The absorbance of each starch solution was measured according to the DNS assay. In the results the wheat starch degradation 100% by *B. Licheniformis* VO14 and *Thermobacillus sp* VO15 alpha-amylase.

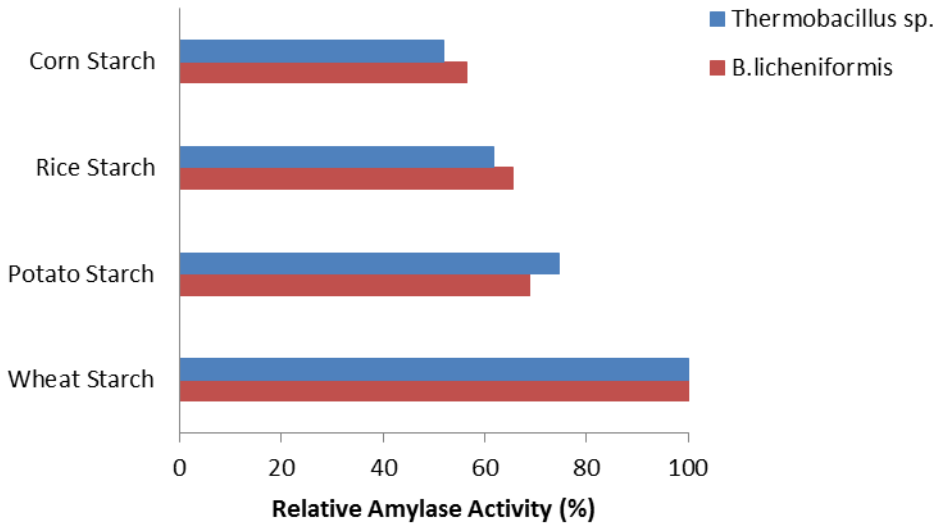


Figure 4.29. Degradation of different starches by alpha-amylase.

4.5.7. Effect of temperature on alpha-amylase and xylanase stability

4.5.7.1. Alpha-amylase stability produced by *B. licheniformis* VO14

The temperature stability of the alpha-amylase was studied by incubating the enzme at different temperatures (50ºC - 110ºC) during 240 min. After the incubation time the remaining activities were measured spectrophotometrically under standard assay conditions. They were calculated and outlined against temperature values as in Figure (4.30). Although preparing the control, the temperature treatment was not applied to the enzyme and the activity of the alpha-amylase was observed as hundred percent. Temperature values (50ºC - 110ºC) were examined to compare the differences of the stabilities.

- The enzyme at 50ºC keep more than 95% of its activity after 90 min and lost about 16% of its activity after 240 min incubation time.
- The enzyme at 110ºC keep more than 95% of its activity after 30 min and lost about 50% of its activity after 240 min incubation time.

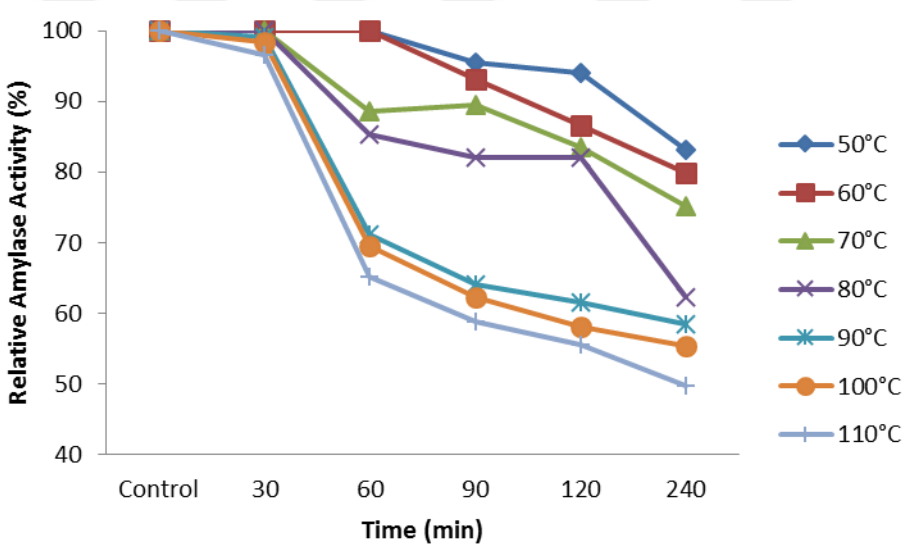


Figure 4.30. Thermal stability of alpha-amylase produced by *B. licheniformis.*

4.5.7.2. Alpha-amylase stability produced by *Thermobacillus sp* VO15

The temperature stability of the alpha-amylase was studied by incubating the enzyme at different temperatures (50ºC - 110ºC) during 240 min. After the incubation time the remaining activities were measured spectrophotometrically under standard assay conditions. They were calculated and outlined against temperature values as in Figure (4.31) Although preparing the control, the temperature treatment was not applied to the enzyme and the activity of the alpha-amylase enzyme was observed as hundred percent. Temperature values (50ºC - 110ºC) were examined to compare the differences of the stabilities.

- The enzyme at 50ºC keep more than 95% of its activity after 120 min and lost about 3.9% of its activity after 240 min incubation time.
- The enzyme at 110ºC keep more than 95% of its activity after 30 min and lost about 20% of its activity after 240 min incubation time.

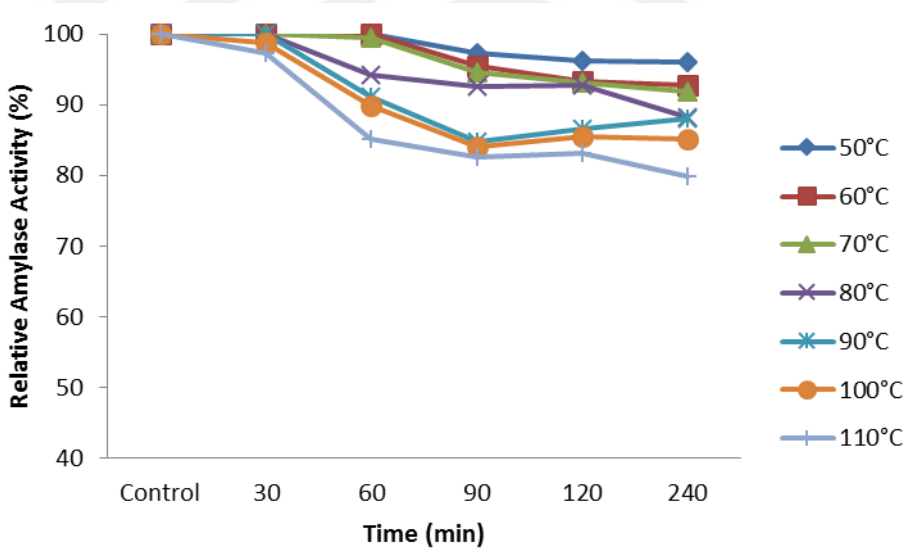


Figure 4.31. Thermal stability of alpha-amylase produced by *Thermobacillus sp.*

4.5.7.3. Xylanase stability produced by *B. licheniformis* VO14

The temperature stability of the xylanase enzyme was studied by incubating the enzyme at several different temperatures (50ºC -110ºC) through 240 min. After the incubation time the remaining activities were estimated spectrophotometrically under standard assay conditions.

They were determined temperature values as in Figure (4.32). While preparing the control, the temperature operation was not applied to the enzyme and the activity of the xylanase enzyme was observed as hundred percent. Two temperature values (50ºC - 110ºC) were examined to distinguish the differences of the xylanase enzyme stabilities.

- The xylanase enzyme at 50ºC maintained 100% of its activity after 60 min and lost about 16% of its activity after 240 min incubation time.
- The enzyme at 110ºC maintained more than 98% of its activity after 30 min and lost about 57% of its activity after 240 min incubation time.

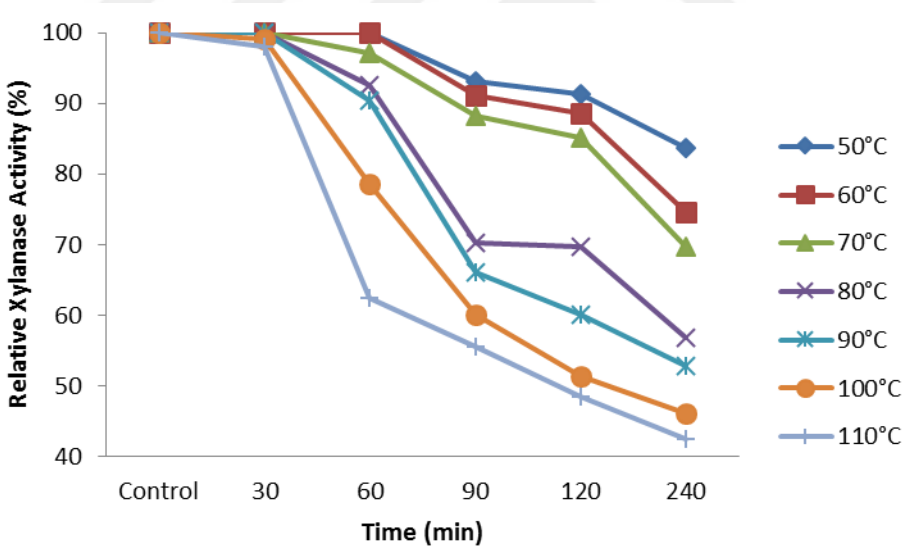


Figure 4.32. Thermal stability of xylanase produced by *Bacillus licheniformis*.

4.5.7.4. Xylanase stability produced by *Thermobacillus sp* VO15

The temperature stability of the xylanase was studied by incubating the enzyme at several different temperatures (50ºC - 110ºC) through 240 min. Following the incubation time the remaining activities were estimated spectrophotometrically under standard assay conditions. They were determined temperature values as in Figure (4.33). While preparing the control, the temperature operation was not applied to the enzyme and the activity of the xylanase enzyme was observed as hundred percent. Two temperature values (50ºC - 110ºC) were examined to distinguish the differences of the xylanase enzyme stabilities.

- The xylanase at 50ºC maintained 98% of its activity after 240 min and lost about 2% of its activity after 240 min incubation time.
- The enzyme at 110ºC maintained more than 97% of its activity after 30 min and lost about 20% of its activity after 240 min incubation time.

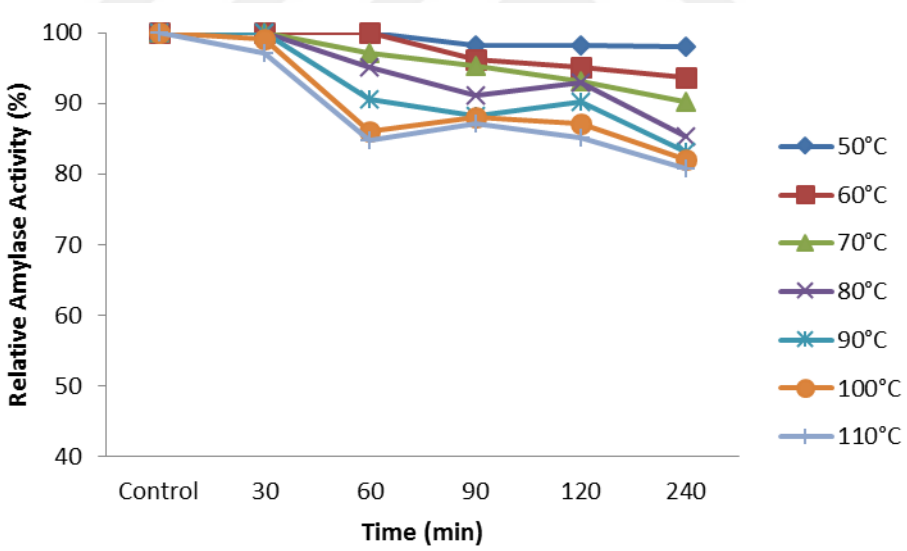


Figure 4.33. Thermal stability of xylanase produced by *Thermobacillus sp.*

4.5.8. Effect of pH on alpha-amylase and xylanase stability

4.5.8.1. Alpha-amylase stability produced by *B. licheniformis* VO14

The pH stability of the alpha-amylase produced by *Bacillus licheniformis* was investigated by incubating the enzyme in different pH (4.0 - 11.0) during 240 min. After the incubation time the remaining activity was estimated spectrophotometrically under standard test conditions. They have measured pH values as in Figure (4.34). While preparing the control, the pH treatment was not applied to the enzyme and the activity of the alpha-amylase enzyme was observed as hundred percent. Two pH values (4.0 and 11.0) were analyzed to compare the differences of the stabilities.

- The enzyme at pH 4.0 retained 94% of its activity after 60 min and lost 20% of its activity after 240 min incubation time.
- The enzyme at pH 11.0 retained about 75% of its activity after 30 min and lost 68.5% of its activity after 240 min incubation time.

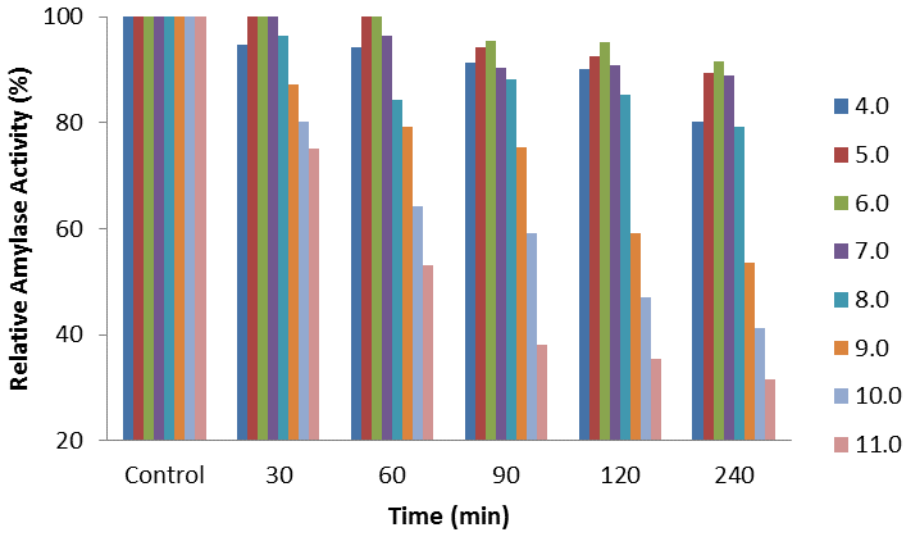


Figure 4.34. pH stability of alpha-amylase produced by *Bacillus licheniformis.*

4.5.8.2. Alpha-amylase stability produced by *Thermobacillus sp* VO15

The pH stability of the alpha-amylase produced by *Thermobacillus sp* was investigated by incubating the enzyme in different pH (4.0 - 11.0) during 240 min. After the incubation time the remaining activity was estimated spectrophotometrically under standard test conditions. They have measured pH values as in Figure (4.35).

While preparing the control, the pH treatment was not applied to the enzyme and the activity of the alpha-amylase was observed as hundred percent. Two pH values (4.0 and 11.0) were analyzed to compare the differences of the stabilities.

- The enzyme at pH 4.0 retained 96% of its activity after 60 min and lost 20% of its activity after 240 min incubation time.
- The enzyme at pH 11.0 retained about 85% of its activity after 30 min and lost 71.4% of its activity after 240 min incubation time.

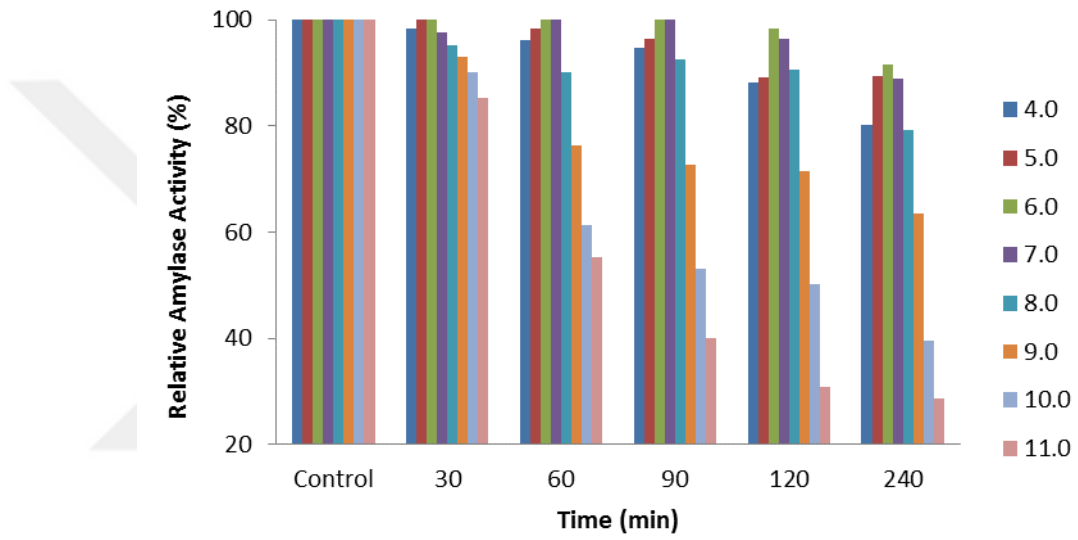


Figure 4.35. pH stability of alpha-amylase produced by *Thermobacillus sp*

4.5.8.3. Xylanase stability produced by *B. licheniformis* VO14

The pH stability of the xylanase produced by *Bacillus licheniformis* was investigated by incubating the enzyme in different pH (4.0 - 11.0) during 240 min. After the incubation time the remaining activity was estimated spectrophotometrically under standard test conditions. They have measured pH values as in Figure (4.36). While preparing the control, the pH treatment was not applied to the enzyme and the activity of the xylanase was observed as hundred percent. Two pH values (4.0 and 11.0) were analyzed to compare the differences of the stabilities.

- The enzyme at pH 4.0 retained 98% of its activity after 30 min and lost 27.8% of its activity after 240 min incubation time.

- The enzyme at pH 11.0 retained about 70% of its activity after 30 min and lost 65.4% of its activity after 240 min incubation time.

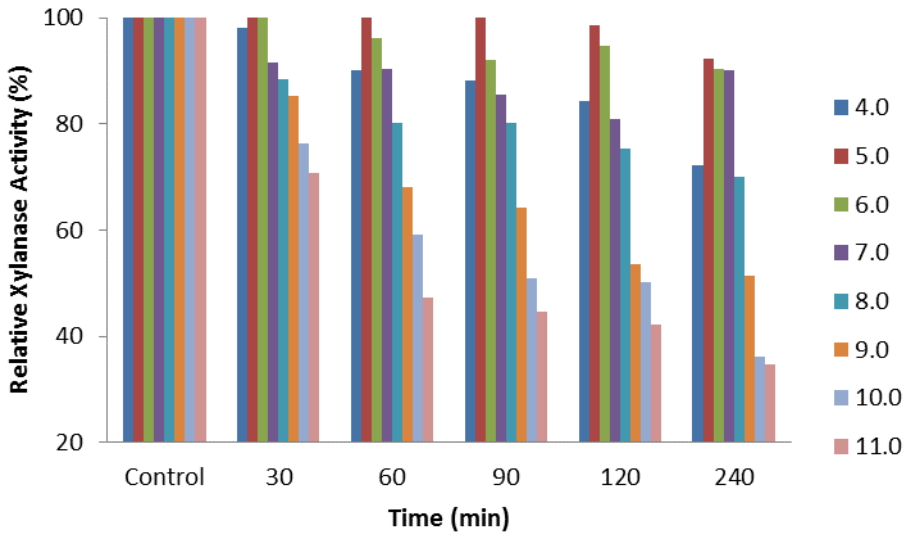


Figure 4.36. pH stability of xylanase produced by *Bacillus licheniformis*

4.5.8.4. Xylanase stability produced by *Thermobacillus sp* VO15

The pH stability of the xylanase produced by *Thermobacillus sp* was investigated by incubating the enzyme in different pH (4.0 - 11.0) during 240 min. After the incubation time the remaining activity was estimated spectrophotometrically under standard test conditions. They have measured pH values as in Figure (4.37). While preparing the control, the pH treatment was not applied to the enzyme and the activity of the xylanase was observed as hundred percent. Two pH values (4.0 and 11.0) were analyzed to compare the differences of the stabilities.

- The enzyme at pH 4.0 retained 94% of its activity after 60 min and lost 34.7% of its activity after 240 min incubation time.
- The enzyme at pH 11.0 retained about 76% of its activity after 30 min and lost 69.3% of its activity after 240 min incubation time.

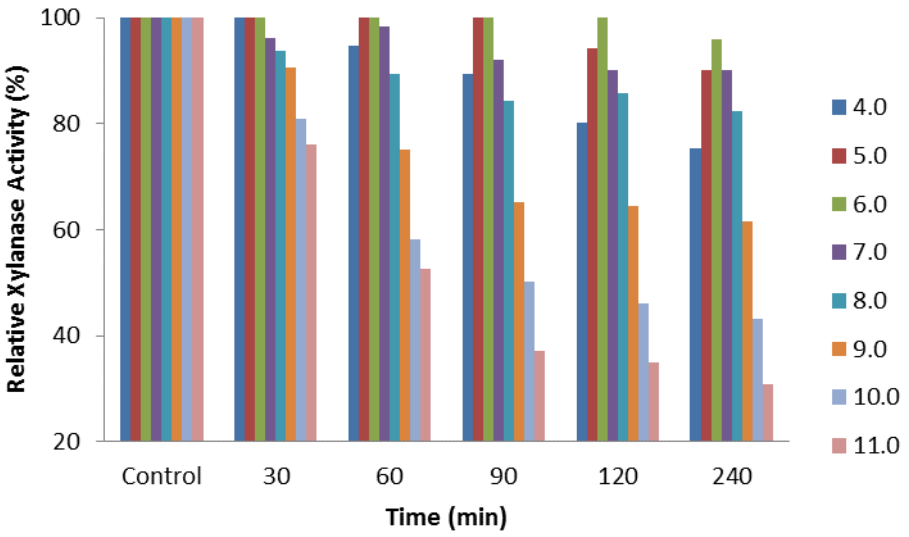


Figure 4.37. pH stability of xylanase produced by *Thermobacillus sp.*

4.6. Antibiotic Sensitivity Test (AST)

Once we have identified the bacterium which is causing the infection we need to find out the antibiotics that would be effective against it. This is done by antibiotic sensitivity testing. The Kirby-Bauer (K-B) disc-spread test is the most common method for testing resistance/susceptibility to antibiotics. The results of these tests help the doctor choose which antibiotic to use when treating a patient (Hudziki, 2009). The presence or absence of growth around the disk is an indirect measure of antibiotic capacity to inhibit bacteria. A clear zone appears around the disk where growth has been inhibited, the size of the inhibition zone depends on the sensitivity of bacteria to antibiotics.

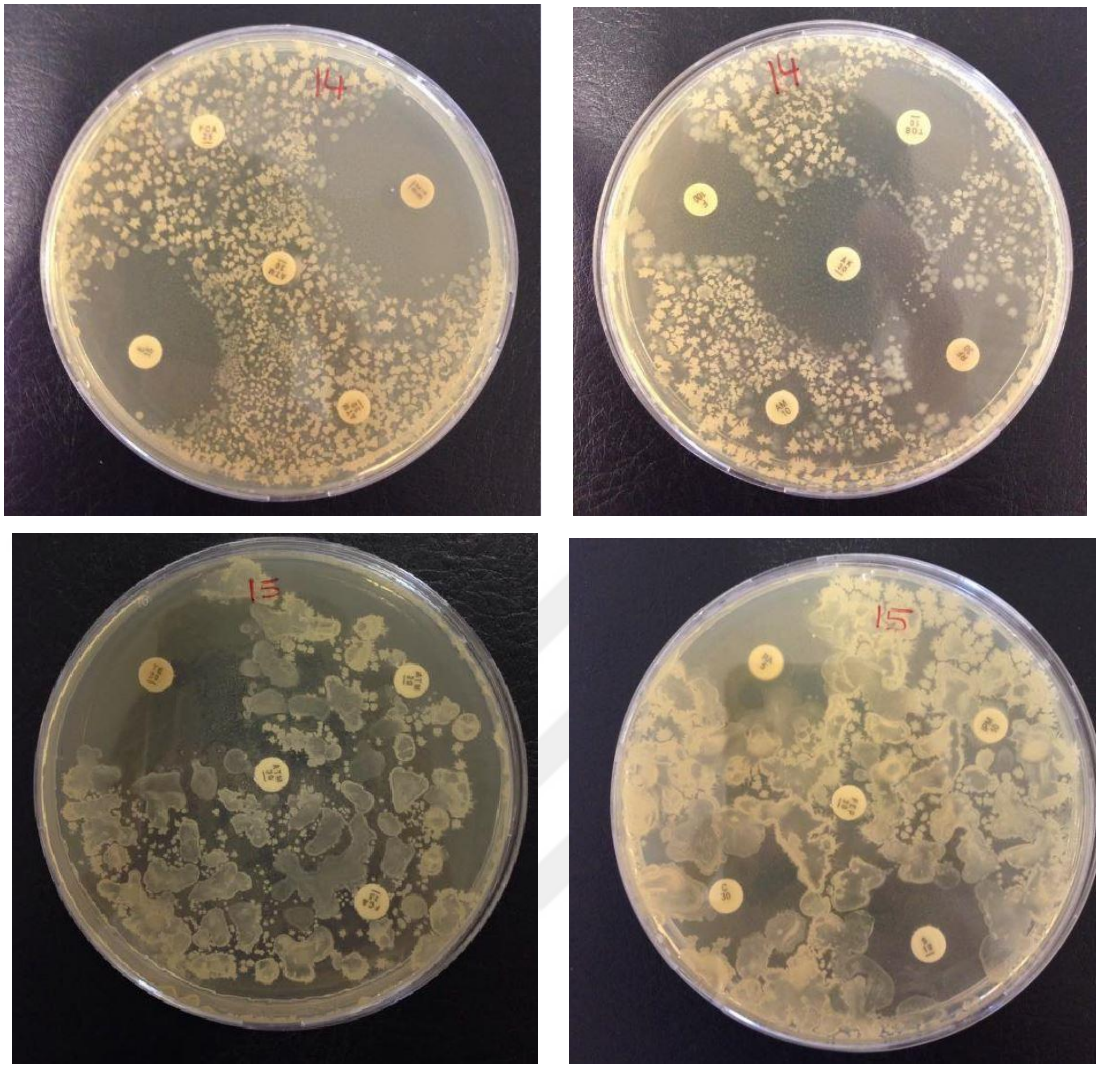


Figure 4.38. The samples of antibiotic test

Table 4.1. The results of antibiotic resistances test

Antibiotics	*B.licheniformis*	*Thermobacillus sp*
Amikacin (AK 30)	15 mm	12 mm
Rifamycin (RF 30)	10 mm	10 m
Tobramycin (TOB 10)	10 mm	-
Nitrofuration (F 100)	10 mm	-
Ampicillin (AM 10)	3 mm	-
Cefepime (FEP 30)	-	-
Chloramphenicol (C 30)	15 m	-
Imipenem (IP 10)	-	-
Rifampin (RA 5)	7 mm	10 mm
Streptomycin (S 10)	10 mm	10 mm
Aztronam (ATM 30)	-	-
Tetracycline (TE 30)	15 mm	17 mm
Fluconazole (FCA 25)	-	-

Resistance (-)

5. DISCUSSION AND RECOMMENDATION

5.1. Discussion

Solid Substrate Fermentation (SSF) is described as a process that controls the growth of microorganisms on moist solid substrates can also be sources of energy. Fermentation takes place in the absence or almost absence of free flowing water, therefore, closes the natural environment for which the appropriate organism (Pandey et al, 2000; Gabiatti et al, 2006). Besides, submerged fermentation (SmF) is a process of growth of a microorganism in a liquid medium. They have conducted many investigations in both processes for the production of enzymes (Blandino et al, 2002). The production of a submerged fermentation (SmF) enzyme has been established for a long time over solid state fermentation (SSF). There are some advantages to using this fermentation. This system is easier to design from researchers due to the ease of process control and sterilization. In addition, enzymatic products may be constituent or inducible and exhibit different production patterns, depending on strain and culture conditions(Vidyalakshmi et al, 2009). However, SSF process maintains several advantages over SmF process, even though this process present only at laboratory level (Noe Aguilar et al., 2008; Vidyalakshmi et al, 2009). This is also supported by Wang and Chen (2009), which stated that the surface adhesion-based culture are productive than SmF. Several advantages are higher productivity of SSF fermentation, final concentration, higher product stability and less catabolism repression, specifically for the culture of insoluble substrate microorganisms and lower sterility demand due to the low water activity used (Holker et al, 2004).

Bacteria grow well and metabolize various substrates in a certain set of environmental conditions. In nature, several strains of the same species often differ in their carbon and nitrogen requirement sources, optimum temperature, pH, and other factors to grow and produce maximal amount of enzymes. Therefore, the variation in the optimal investigation for different Bacillus species.

Incubation time has a great effect on the production of enzymes. The microorganism production of enzymes is directly related to the incubation period (Smitt et al, 1996). As incubation period increase till the optimum period, enzyme production also increases, but beyond the optimum, production declines.

This is because the cells can reach the phase of decline and show a low synthesis of alpha-amylase (Mulimani and Ramalingam, 2000; Sivakumar et al, 2012). The optimum incubation period for maximum alpha amylase production from experimental isolate. The optimum incubation period was 24 hr for *B. licheniformis* V014, on the other hand, the optimum temperature was 48 hr for *Thermobacillus sp* VO15 (Souza, 2010).

Temperature is a critical environmental factor controlling the growth and production of metabolites by microorganisms and this generally varies from one organism to another (Banagree and Bhattacharya, 1992; Kumar and Takagi, 1999). It is a highly sensitive parameter for alpha-amylase production (Zar et al, 2012). The effect of temperature on the production of amylase is related to the growth of the microbes (Kanthiresan and Manivannan, 2006). Low temperature and high can affect the metabolism of the microorganism and, hence, the production of the enzyme. The higher temperature, the enzyme production decreases, which could be due to reduced growth and enzyme inactivation or suppression of cell viability (Ellaiah et al, 2002). Conversely, low temperature values may decrease the metabolism of the microorganism and enzymatic synthesis, therefore (Mazutti et al, 2007). *Bacillus spp*. Produce amylase at a much wider range of temperature. Several studies have shown the *Bacillus spp.* The optimal temperature is 45°C for *Bacillus licheniformis*, on the other hand, the optimum temperature for *Thermobacillus sp* was 45°C (Souza, 2010). From this thesis the maximum alpha-amylase production by *Thermobacillus sp* VO15 (18.72U/mg) obtained at 40°C and the maximum alpha-amylase production by *B. licheniformis* VO14 (16.34U/mg) obtained at 45°C.The production decreased when the temperature increases or decrease.

Including the physical parameters, the pH of the growth medium is important to induce morphological changes in the organism and excretion of the organism enzyme. Most Bacillus strains used commercially for the production of alpha-amylase by submerged fermentation (SmF) having an optimal pH between 6 and 8 for the growth and production of enzymes (Senthilkumer et al, 2012). pH 7 was found optimum for alpha-amylase synthesis for many *Bacillus spp* (Teodoro and Martin, 2000; Oyeleke et al, 2010; Singh et al, 2012). The optimum pH was 7 for *B. licheniformis* VO14, on the other hand, the optimum pH was 6 for *Thermobacillus sp* VO15 (Ikram et al, 2003). Increase or decrease from this pH showed the production of alpha-amylase decrease.

The characteristics and volume of carbon source in the culture medium are necessary for the production of bacteria extracellular alpha-amylase (Akcan, 2011). The production of amylase is influenced by the type of carbon sources used in the media, however, the effect of carbon changes with the production strain other condition (Meenakshi et al, 2009; Ashwini et al, 2011). The effect of carbon sources on alpha-amylase production was previous studies using, the nature of the compound employed or the concentration that could stimulate or decrease the production of amylase (Sivakumar et al, 2012).

Alpha-amylase is an inducible enzyme, usually in the occupation of starch or induces hydrolysis. In this test, various carbon sources such as glucose, lactose, maltose, and starch were supplemented in fermentation medium for the alpha-amylase production. Carbon sources have been added to the average at 1% level. The increase in alpha-amylase production by *B. licheniformis* VO14 was noted in the medium supplemented with galactose (99.7%) followed by arabinose (95.52%). In the presence of other carbon sources, however, the enzyme production has been reduced. Furthermore, increase in alpha-amylase production by *Thermobacillus sp* VO15 was noted in the medium supplemented with galactose (103.96%) followed by lactose (95.52%). In the presence of other carbon sources, however, the enzyme production has been reduced. In the present study, lowest alpha-amylase enzyme yield *B. licheniformis* VO14 and *Thermobacillus sp* VO15 was concerned when raffinose was used as an additional carbon source.

The source and application of nitrogen in the fermentation medium have an influence on the microbial production of alpha-amylase. Production of alpha-amylase from *B. licheniformis* VO14 and *Thermobacillus sp* VO15 was examined by addition of different sources of nitrogen on a basal medium were supplied yeast, ammonium and sulphate, tryptone soybean ammonium acetate broth, glycine, L-proline , D-methionine, urea, L-tyrosine and D-aspartic acid at a level of 1% in the medium fermentation. A high level of alpha-amylase (122.44%) by *B. licheniformis* VO14 produced when yeast extract was used as nitrogen source followed by tryptone soya broth (97.73%) and Compared to all the nitrogen sources was used , alpha-amylase production by *B.licheniformis* VO14 was found lowest (70.96%) in media containing ammonium sulfate.

High level of alpha-amylase (117.6%) by *Thermobacillus sp* V015 produced when ammonium sulfate was used as nitrogen source followed by tryptone soya broth (108.52%). Compared to all the organic nitrogen sources was used, alpha-amylase production by *Thermobacillus sp* VO15 was found lowest (52.48%) in media containing L-proline.

The effect of temperature on alpha-amylase activity was assessed by measuring the enzymatic activity at different temperatures ranging from 30ºC - 80ºC. Effect of temperature showed the highest alpha-amylase activity from *B. licheniformis* VO14 at 40ºC. On the other hand, the highest alpha-amylase activity from *Thermobacillus sp* at 50ºC. The alpha-amylase from *B. licheniformis* VO15 at temperatures below 40ºC lost 12.8% of its activity and also the alpha-amylase at temperatures above 40ºC lost 4.9% of its activity. In addition, the alpha-amylase from *Thermobacillus sp* at temperatures below 50ºC lost 1.6% of its activity and also the alpha-amylase at temperatures above 50ºC lost 2.8% of its activity. When the activities at various temperatures were examined, it was noted that the alpha-amylase activity from *B. licheniformis* VO14 at 40ºC and the highest alpha-amylase activity from *Thermobacillus sp* VO15 at 50ºC was approximately high activity in these conditions.

The influence of pH on alpha-amylase activity was evaluated by measuring enzymatic activity in the presence of buffers at different pH values (4.0 - 11.0). The effect of pH determined the optimum alpha-amylase activity production by *B. licheniformis* VO14 at pH 6.0. In addition, the optimal pH of the alpha-amylase enzyme isolated from *Thermobacillus sp* VO15. It was pH 7.0.

The *B. licheniformis* alpha-amylase showed a slightly lower activity than the alpha-amylase from *Thermobacillus sp*. The alpha-amylase from *B. licheniformis* lost about 40% of its activity at pH 4.0 and it lost about 70% of its activity at pH 11.0. While the alpha-amylase from *Thermobacillus sp* lost about 51% of its activity at pH 4.0 and it lost about 57% of its activity at pH 11.0.

The effect of the metal ions on the activity of alpha-amylase was measured in the different metal ions presence such as ($CdCl_2$, $Cu(NO_3)_2$, $Co(NO_3)_2$, $Pb(NO_3)_2$, $AlCl_3$, $CaCl_2$, Z_nCl_2 and $HgCl_2$). The alpha-amylase supernatant and metal ion 2 mM were incubated for 60 min at 40ºC.

After that incubation added the substrate and the reaction were initiated. Then, measured the activity of alpha-amylase under the standard assay conditions. While preparing the control, the metal ion was not added into the enzyme supernatant and its activity was regarded as hundred percent. The results showed *B. licheniformis* VO14 alpha-amylase the optimum activity (167.5 %) when used ($Co(NO_3)_2$), and ($AlCl_3$) the second metal ions increased the activity of alpha-amylase for (108.4 %). *B.licheniformis* alpha-amylase decreases the activity (36.7% and 43.6%), when the used of ($HgCl_2$) and ($CdCl_2$), respectively. While *Thermobacillus sp* VO15 alpha-amylase the optimum activity (189.6%) when used ($Co(NO3)_2$), and ($AlCl_3$) the second metal ions increased the activity of alpha-amylase for (119.5%). *Thermobacillus sp* alpha-amylase decrease the activity (29.5% and 35.7%), when the used of ($HgCl_2$) and ($CdCl_2$), respectively. but (Z_nCl_2) did not affect the activity of the alpha-amylase so much.

The impact of surfactants and detergents on alpha-amylase activity was estimated by measuring enzymatic activity in the presence of 0.5% surfactants or detergents. Types of surfactant used such as (Tween20, Tween40, Tween80, and Triton X100). The effect of surfactants determined the optimum activity of alpha-amylase produced by *B. licheniformis* VO14 and *Thermobacillus sp* VO15 were concerned at used tween40 (137.3% and 145.8%) respectively. Tween80 is an another surfactants that effect on Alpha-amylase activity (119.3%) to *B. licheniformis* alpha-amylase also Tween20 is an another surfactants that effect on alpha-amylase activity (117.2%) to *Thermobacillus sp* alpha-amylase. the lower *B. licheniformis* alpha-amylase activity observed through the use of Triton x100 (105.1%) and *Thermobacillus sp* alpha-amylase activity recognized through the use of Triton x100 (102.1%).

While types of detergents used such as (Alo, Omo, Tursil, and Ariel). The effect of detergents confirmed the optimum alpha-amylase activity production by *B.licheniformis* VO14 were obtained at used Tursil (96.4%). In addition, the optimal activity of the alpha-amylase isolated from *Thermobacillus sp* VO15. It was Omo (105.3%). The lower impact noticed of Ariel (59.9%) to alpha-amylase produced by *B.licheniformis.* The lower effect noticed of Ariel (53.2%) to alpha-amylase produced by *Thermobacillus sp.*

Studied temperature effect on alpha-amylase stability produced by *B.licheniformis* VO14 by incubating the enzyme at different temperatures (50ºC -110ºC) during 240 min. After the incubation time the remaining activities were measured spectrophotometrically under standard assay conditions. Temperature values analyzed to compare the differences of the stabilities. The enzyme at 50ºC keep more than 95% of its activity after 90 min and lost about 16% of its activity after 240 min incubation time. Enzyme at 60ºC keep more than 95% of its activity after 60 min and lost about 20% of its activity after 240 min incubation time . The enzyme at 70ºC keep more than 95% of its activity after 90 min and lost about 25% of its activity after 240 min incubation time. The enzyme at 80ºC keep more than 95% of its activity after 60 min and lost about 38% of its activity after 240 min incubation time. The enzyme at 90ºC keep more than 95% of its activity after 30 min and lost about 35% of its activity after 90 min incubation time and lost about 41% of its activity after 240 min incubation time. The enzyme at 100ºC keep more than 95% of its activity after 30 min and lost about 38% of its activity after 90 min incubation time and lost about 45% of its activity after 240 min incubation time. The enzyme at 110ºC keep more than 95% of its activity after 30 min and lost about 41% of its activity after 90 min incubation time and lost about 50% of its activity after 240 min incubation time. In the results determined that the alpha-amylase isolated from *B. licheniformis*. It was stable at temperature 50ºC of the other temperature value.

Temperature effect on alpha-amylase stability produced by *Thermobacillus sp VO15*. Studied by incubating the enzyme at different temperatures (50ºC -110ºC) during 240 min. After the incubation time the remaining activities were measured spectrophotometrically under standard assay conditions. The temperature values analyzed to compare the differences of the stabilities. The enzyme at 50ºC keep more than 95% of its activity after 120 min and lost about 3.9% of its activity after 240 min incubation time. Enzyme at 60ºC keep more than 95% of its activity after 90 min and lost about 7% of its activity after 240 min incubation time . The enzyme at 70ºC keep more than 95% of its activity after 60 min and lost about 8% of its activity after 240 min incubation time. Enzyme at 80ºC keep more than 95% of its activity after 30 min and lost about 12% of its activity after 240 min incubation time.

The enzyme at 90ºC keep more than 95% of its activity after 30 min and lost about 12% of its activity after 240 min incubation time. Enzyme at 100ºC keep more than 95% of its activity after 30 min and lost about 15% of its activity after 240 min incubation time. The enzyme at 110ºC keep more than 95% of its activity after 30 min and lost about 17.5% of its activity after 90 min incubation time and lost about 20% of its activity after 240 min incubation time. In the results determined that the alpha-amylase isolated from *Thermobacillus sp*. It was stable at temperature 50ºC of the other temperature value.

The pH stability of the alpha-amylase produced by *B. licheniformis* VO14 was investigated by incubating the enzyme in different pH (4.0 - 11.0) during 240 min. After the incubation time the remaining activity was estimated spectrophotometrically under standard test conditions. The pH values were analyzed to compare the differences of the stabilities. The enzyme at pH 4.0 retained 94% of its activity after 60 min and lost 20% of its activity after 240 min incubation time. Alpha-amylas at pH 5.0 retained 100% of its activity after 60 min and retained about 94% of its activity after 90 min and lost 20% of its activity after 240 min incubation time. Enzyme at pH 6.0 retained 100% of its activity after 60 min and retained about 95% of its activity after 120 min and lost nearly 19% of its activity after 240 min incubation time. Enzyme at pH 7.0 retained 96% of its activity after 60 min and lost 11% of its activity after 240 min incubation time. Alpha-amylase at pH 8.0 retained 96% of its activity after 30 min and lost 20% of its activity after 240 min incubation time. Enzyme at pH 9.0 retained about 87% of its activity after 30 min and lost 55.5% of its activity after 240 min incubation time. Enzyme at pH 10.0 retained about 80% of its activity after 30 min and lost 59% of its activity after 240 min incubation time. And the alpha-amylase at pH 11.0 retained about 75% of its activity after 30 min and lost 68.5% of its activity after 240 min incubation time. In the results determined that the alpha-amylase isolated from *Bacillus licheniformis*. It was stable at pH 7.0 of the other pH value.

The pH stability of the alpha-amylase produced by *Thermobacillus sp* V015 was investigated by incubating the enzyme in different pH (4.0 - 11.0) during 240 min. After the incubation time the remaining activity was estimated spectrophotometrically under standard test conditions. The pH values were analyzed to compare the differences of the stabilities. The enzyme at pH 4.0 retained 96% of its activity after 60 min and lost 20% of its activity after 240 min incubation time.

Alpha-amylase at pH 5.0 retained 96% of its activity after 60 min and lost 10.5% of its activity after 240 min incubation time. Enzyme at pH 6.0 retained 100% of its activity after 90 min and retained about 98% of its activity after 120 min and lost nearly 8.4% of its activity after 240 min incubation time. Enzyme at pH 7.0 retained 100% of its activity after 90 min and lost 11% of its activity after 240 min incubation time. Enzyme at pH 8.0 retained 90% of its activity after 120 min and lost 20.7% of its activity after 240 min incubation time. The enzyme at pH 9.0 retained about 93% of its activity after 30 min and lost 36.5% of its activity after 240 min incubation time. Enzyme at pH 10.0 retained about 90% of its activity after 30 min and lost 60% of its activity after 240 min incubation time. And the enzyme at pH 11.0 retained about 85% of its activity after 30min and lost 71.4% of its activity after 240 min incubation time. In the results determined that the alpha-amylase isolated from *Thermobacillus sp*. It was stable at pH 6.0 of the other pH value.

Enzymatic technology has become increasingly common in industrial applications a wide range of research has been done on those executed in the processes of production of food enzymes. One of these enzymes, which has attracted more and more consideration in recent years is a xylanase. At present, the most common application of xylanase can be seen in the bleaching of kraft pulp. By xylanase, you can decrease the number of bleaching levels, and the consumption of chemicals (Techapun et al, 2002). The second vital utilization of xylanase is in the baking sector. Xylanases are of extraordinary significance since preparing has been found to enhance the versatility and quality of the mixture, in this manner making it less demanding to deal with a bigger volume of bread and enhanced piece structure (Collins et al, 2005; Butt et al, 2008). Xylanases with advantages of being environmentally friendly and effective in reducing chemicals consumption in the bleach plant have been thoroughly investigated as a means to improve product quality and reduce the production cost in the pulping industry. a suitable xylanase needs to be identified and the appropriate application conditions need to be determined. Treatment conditions such as temperature, pH, pulp consistency, xylanase dosage and reaction time, are all important factors that could significantly affect the efficiency of xylan from industrial biotechnology.

This research was performed for the production of xylanase in different fermentation periods. Reduction of enzyme performance after optimal levels might be due to denaturing and breakdown of xylanase due to interplay with another component of the statement or substrate inhibition. The optimum incubation period for maximum xylanase production from experimental isolate. *B. licheniformis* VO14 and *Thermobacillus sp* VO15 could be obtained at 24 hr of incubation (28.24 U/mg) and (32.52 U/mg) respectively. Incubation *B. licheniformis* VO14 and *Thermobacillus sp* VO15 beyond 24 hr led to declining in the xylanase production.

Temperature is a significant parameter that needs to be regulated and this generally varies from one organism to another (Sivakumaret al, 2011). The influence of temperature on enzyme in two ways. First-way is an influence direct on the reaction constant rate, Second-way thermal enzyme denaturation at a temperature promoted (Demirkan et al, 2017). Therefore, whether the microorganism is mesophilic or thermophilic required for detection the optimal incubation temperature fermentation. the growth of the microorganisms is closely related to the production xylanase in the optimum temperature. Generally, enzymatic activity progressively increased with optimum temperatures after it started to decrease with the further rise in temperature. The xylanase activity of the isolates in this study was the optimal temperature range of 40°C for the production of xylanase from *B. licheniformis* VO14 and 45°C for the production of xylanase from *Thermobacillus sp* VO15. If the incubation temperature *B.licheniformis* VO14 and *Thermobacillus sp* VO15 beyond 40°C and 45°C respectively, led to declining in the xylanase production.

Initial pH is one of the critical parameters that correlate with microbial growth since the hydrogen ion concentration focus assumes an imperative part in prompting morphological changes in the life form and in emission enzyme. In the present study, the impact of pH was investigated for the production of xylanase. Maximum production of xylanase from *B. licheniformis* VO14 and *Thermobacillus sp* VO15 It was observed when the pH of the initial medium was 5.0 and 6.0 which gave (30.36 U/mg) and (33.23U/mg), respectively. A gradual decrease in enzyme yiled pH performance of 7.0 to 11.0 was achieved because the pH increase of the medium beyond 6.0 non-preferred secretion of the enzyme from the bacterium.

Growth and enzyme production of any organism are strongly influenced by the nutrients available in the medium of growth. Xylanase is an inducible enzyme. It should be noted that carbon sources effect on the behavior of the xylanase production in the medium. Some carbon sources maintain good growth with low production of xylanase while others support good growth and secretion of xylanase. This research has been conducted to optimize fermentation medium with carbon sources for the production of industrial enzymes. This research was carried out on production and supplementation with various carbon sources, in particular, glucose, galactose, lactose, fructose, sucrose, starch, raffinose and arabinose at a concentration of 1% (w/v). The production medium was then fermented using *B. licheniformis* VO14 and *Thermobacillus sp* VO15 for the production of xylanase. In this study, there is a significant increase in the xylanase yield in the case of glucose supplementation (126.28% and 109.65%), respectively, while the production of xylanase from *B. licheniformis* was eliminated when the bacterium was cultivated in a production medium with raffinose (37.34%). But the production of xylanase from *Thermobacillus sp* suppressed when bacteria were cultivated in the production medium with sucrose (33.04%).

It has been recorded that nitrogen sources have added producing an effect on the production of different enzymes in the submerged fermentation method. The addition of various nitrogen sources, particularly yeast, amonium sulfat, tryptone soya broth, ammonium acetate, glycine, L-proline, D-methionine, urea, L-tyrosine and D-aspartic acid. was examined in this study. Regarding control, there was a significant increase in the performance of xylanase in the case of supplementation of yeast extract. Among the various sources of nitrogen experimented for xylanase production from *B. licheniformis* VO14 and *Thermobacillus sp* VO15, 1% yeast extract recorded a maximum yield of xylanase (124.37%) and (107.67%) followed by tryptone soya broth (98.08%) and (95.37%), respectively. The nitrogen sources experimented, yeast extract was the best inducer for the production of xylanase, while L-proline was a poor inducer.

The effect of temperature on xylanase activity was examined by measuring the enzymatic activity at different temperatures ranging from 30ºC, 40ºC, 50ºC, 60ºC, 70ºC, and 80ºC. Effect of temperature showed the highest xylanase activity from *B.licheniformis* VO14 at 40ºC.

On the other hand, the highest xylanase activity from *Thermobacillus sp* VO15 at 60ºC. The xylanase enzyme from *B. licheniformis* at temperatures below 40ºC lost 30.5% of its activity and also the xylanase enzyme at temperatures above 40ºC lost 8.5% of its activity. In addition, the xylanase enzyme from *Thermobacillus sp* at temperatures below 60ºC lost 0.9% of its activity and also the xylanase enzyme at temperatures above 60ºC lost 7.5% of its activity. When the activities at various temperatures were examined, it was noted that the xylanase activity from *B. licheniformis* VO14 at 40ºC and xylanase activity from *Thermobacillus sp* VO15 at 60ºC was approximately constant in these conditions.

Influence of pH on xylanase activity was evaluated by measuring enzymatic activity in the presence of buffers at different pH values (4.0 - 11.0). The effect of pH showed the optimum xylanase activity production by *B. licheniformis* VO14 at pH 5.0. In addition, the optimal pH of the xylanase enzyme isolated from *Thermobacillus sp* V015. It was pH 6.0. The *B.licheniformis* xylanase enzyme showed a slightly lower activity than the xylanase enzyme from *Thermobacillus sp*. Xylanase enzyme from *B.licheniformis* lost about 39% of its activity at pH 4.0 and it lost about 65% of its activity at pH 11.0. While the xylanase enzyme from *Thermobacillus sp* lost about 25% of its activity at pH 4.0 and it lost about 60% of its activity at pH 11.0.

Effect of the metal ions on the activity of xylanase was measured in the different metal ions presence such as ($CdCl_2$, $Cu(NO_3)_2$, $Co(NO_3)_2$, $Pb(NO_3)_2$, $AlCl_3$, $CaCl_2$, Z_nCl_2 and $HgCl_2$). The xylanase supernatant and metal ion 2 mM were incubated for 60 min at 40ºC. After that incubation added the substrate and the reaction were initiated. Then, measured the activity of xylanase under the standard assay conditions. The results showed *B. licheniformis* VO14 xylanase enzyme the optimum activity (165.8%) when used ($Co(NO_3)_2$), and ($Cu(NO_3)_2$) the second metal ions increased the activity of xylanase (96.2%). *B.licheniformis* xylanase enzyme decreases the activity (18.7% and 58.5%), when the used of ($HgCl_2$) and ($CdCl_2$), respectively. While *Thermobacillus sp* xylanase enzyme the optimum activity (197.2%) when used ($Co(NO3)_2$), and ($AlCl_3$) the second metal ions increased the activity of xylanase for (113.5%).

Thermobacillus sp xylanase enzyme decrease the activity (29.3% and 65.4%), when the used of ($HgCl_2$) and ($CdCl_2$), respectively.

The impact of surfactants and detergents on xylanase activity was estimated by measuring enzymatic activity in the presence of 0.5% surfactant or detergent. Types of surfactant used such as (Tween20, Tween40, Tween80, and Triton X100). The influence of surfactant confirmed the optimum activity of xylanase produced by *B.licheniformis* VO14 and *Thermobacillus sp* VO15 were concerned at used Tween40 (145.1% and 131.2%) respectively. Xylanase activity (127.2% and 119.8%) for xylanase produced by *B.licheniformis* and *Thermobacillus sp*. the lower *B. licheniformis* xylanase activity observed through the use of TritonX100 (47.1%) and *Thermobacillus-sp* xylanase lower activity observed through the use of TritonX100 (68.8%).

While types of detergents used such as (Alo, Omo, Tursil, and Ariel). The influence of detergent confirmed the optimum xylanase activity production by *B.licheniformis* VO14 were concerned at used Tursil (121.3%). In addition, the optimal activity of the xylanase enzyme isolated from *Thermobacillus sp* VO15. It was Tursil (128.9%). The lower influence noticed of Omo (48.4%) to xylanase produced by *B.licheniformis.* The lower influence noticed of Omo (98.3%) to xylanase produced by *Thermobacillus sp.*

Since the xylanase thermal stability is very important because they are potential applications in various processes of industrial, the strain isolated from utilizing could be a good candidate for biotechnological applications. Temperature effect on xylanase stability produced by *B. Licheniformis* VO14. By incubating the enzyme at several different temperatures (50ºC -110ºC) through 240 min. Following the incubation time the remaining activities were estimated spectrophotometrically under standard assay requirements. While temperature values analyzed to compare the differences of the stabilities. The enzyme at 50ºC keep more than 95% of its activity after 60 min and lost about 16% of its activity after 240 min incubation time. Enzyme at 60ºC keep more than 95% of its activity after 60 min and lost about 25% of its activity after 240 min incubation time . The enzyme at 70ºC keep more than 95% of its activity after 60 min and lost about 30% of its activity after 240 min incubation time. The enzyme at 80ºC keep more than 95% of its activity after 30 min and lost about 43% of its activity after 240 min incubation time. The enzyme at 90ºC keep more than 95% of its activity after 30 min and lost about 47% of its activity after 240 min incubation time.

The enzyme at 100ºC keep more than 95% of its activity after 30 min and lost about 54% of its activity after 240 min incubation time. And the enzyme at 110ºC keep more than 95% of its activity after 30 min and lost about 45% of its activity after 90 min incubation time and lost about 58% of its activity after 240 min incubation time. In the results determined that the xylanase stability isolated from *B. licheniformis*, it was stable at temperature 50ºC of the other temperature value.

Temperature effect on xylanase stability produced by *Thermobacillus sp* VO15. By incubating the enzyme at several different temperatures (50ºC -110ºC) through 240 min. Following the incubation time the remaining activities were estimated spectrophotometrically under standard assay requirements. Temperature values analyzed to compare the differences of the stabilities. The xylanase enzyme at 50ºC maintained 98% of its activity after 240 min and lost about 2% of its activity after 240 min incubation time. Enzyme at 60ºC keep more than 95% of its activity after 120 min and lost about 6.4% of its activity after 240 min incubation time . The enzyme at 70ºC keep more than 95% of its activity after 90 min and lost about 10% of its activity after 240 min incubation time. The enzyme at 80ºC keep more than 95% of its activity after 60 min and lost about 14.7% of its activity after 240 min incubation time. The enzyme at 90ºC keep more than 95% of its activity after 30 min and lost about 17.8% of its activity after 240 min incubation time. The enzyme at 100ºC keep more than 95% of its activity after 30 min and lost about 18% of its activity after 240 min incubation time. And the enzyme at 110ºC maintained more than 97% of its activity after 30 min and lost about 20% of its activity after 240 min incubation time. In the results determined that the xylanase stability isolated from *Thermobacillus sp*, it was stable at temperature 50ºC of the other temperature value.

The pH stability of the xylanase enzyme produced by *B. licheniformis* VO14 was investigated by incubating the enzyme in different pH (4.0 - 11.0) during 240 min. After the incubation time the remaining activity was estimated spectrophotometrically under standard test conditions. The pH values were analyzed to compare the differences of the stabilities. The enzyme at pH 4.0 retained 98% of its activity after 30 min and lost 27.8% of its activity after 240 min incubation time. Enzyme at pH 5.0 retained 100% of its activity after 120 min and lost 7.6% of its activity after 240 min incubation time.

Enzyme at pH 6.0 retained 96% of its activity after 60 and lost nearly 10% of its activity after 240 min incubation time. The enzyme at pH 7.0 retained 90% of its activity after 60 min and lost 10% of its activity after 240 min incubation time. Enzyme at pH 8.0 retained 88% of its activity after 30 min and lost 30% of its activity after 240 min incubation time. Enzyme at pH 9.0 retained about 85% of its activity after 30 min and lost 49% of its activity after 240 min incubation time. The enzyme at pH 10.0 retained about 76.2% of its activity after 30 min and lost 63.8% of its activity after 240 min incubation time. And the enzyme at pH 11.0 retained about 70% of its activity after 30 min and lost 65.4% of its activity after 240 min incubation time. In the results determined that the xylanase stability from *Bacillus licheniformis*, it was stable at pH 5.0 of the other pH value.

The pH stability of the xylanase enzyme produced by *Thermobacillus sp* VO15 was investigated by incubating the enzyme in different pH (4.0 - 11.0) during 240 min. After the incubation time the remaining activity was estimated spectrophotometrically under standard test conditions. The pH values were analyzed to compare the differences of the stabilities. The enzyme at pH 4.0 retained 94% of its activity after 60 min and lost 34.7% of its activity after 240 min incubation time. Enzyme at pH 5.0 retained 100% of its activity after 90 min and lost 10% of its activity after 240 min incubation time. Enzyme at pH 6.0 retained 100% of its activity after 120 and lost nearly 4.2% of its activity after 240 min incubation time. The enzyme at pH 7.0 retained 98% of its activity after 60 min and lost 10% of its activity after 240 min incubation time. Enzyme at pH 8.0 retained 89% of its activity after 60 min and lost 17.7% of its activity after 240 min incubation time. Enzyme at pH 9.0 retained about 90% of its activity after 30 min and lost 38.5% of its activity after 240 min incubation time. The enzyme at pH 10.0 retained about 80% of its activity after 30 min and lost 56.8% of its activity after 240 min incubation time. And the enzyme at pH 11.0 retained about 76% of its activity after 30 min and lost 69.3% of its activity after 240 min incubation time. In the results determined that the xylanase stability from *Thermobacillus sp*, it was stable at pH 6.0 of the other pH value.

5.2. Recommendation

In light of the finding of this study, the accompanying suggestions were made:

- Optimization methods such as factorial design could give best interaction of the factor for maximum enzymes production. Therefore, if enough materials and equipment are there, the factorial design would be recommended.
- For bacterial cultures cultivating in liquid media, continuous shaking of the growth media is necessary. Therefore, fermentation of cultures in an incubation shakers would improve the performance of the isolates in the producing alpha-amylase and xylanases.
- Amylase and xylanases of the different biochemical properties are needed when they are used at large scale applications. Therefore, characterization of their activity and stability with respect to temperature, pH, carbon and nitrogen sources ect. Is necessary.
- Identification of the type of the amylase and xylanases that the isolate produced would give full information of the both amylase and xylanases for which application could be used. Therefore, introduction of method of purification equipment's and identification material to the laboratory would be necessary.

6. REFERENCE

Abdel-Naby, 1993: Amylases.In: Enzyme Technology, A. Pandey, C. Webb, C.R. Soccol,C. Larroche (Eds.), Asiatech Publishers Inc., New Delhi,India (2005) pp. 189 220.

Aehle, W., & Misset, O. (1999). Enzymes for industrial applications. Biotechnology Set, Second Edition, 189-216.

Akcan, N. (2011). High level production of extracellular α-amylase from Bacillus licheniformis ATCC 12759 in submerged fermentation. Rom Biotechnol Lett, 16(6), 6833-6840.

Anbu, Periasamy, et al. "Microbial Enzymes and Their Applications in Industries and Medicine 2016." BioMed research international 2017 (2017).

Anthea M, Hopkins J, McLaughlin CW, Johnson S, Warner MQ, LaHart D, Wright JD, Human Biology and Health, Englewood Cliffs, New Jersey, USA, 76-1, (1993).

Arican B, Highly thermostable, thermophilic, alkaline, SDS and chelator resistant amylase from a thermophilic Bacillus sp. isolate A3-15. Bioresource Technology, 99: 3071–3076, (2008).

Ashwini, K., Gaurav, K., Karthik, L., & Bhaskara Rao, K. V. (2011). Optimization, production and partial purification of extracellular α-amylase from Bacillus sp. marini. Arch Appl Sci Res, 3(1), 33-42.

Aubel, D. Morris, R.P. Rimann, M. Kaufmann, H. Thompson, C.J. Bailey, J.E. Fussenegger, M. 2001. Design of a novel mammalian screening system for the detection of bioavailable, non-cytotoxic streptogramin antibiotics. J. Antibiot, 54:44–54.

Bae, S. S., Lee, J. H., & Kim, S. J. (2005). Bacillus alveayuensis sp. nov., a thermophilic bacterium isolated from deep-sea sediments of the Ayu Trough. *International journal of systematic and evolutionary microbiology*, *55*(3), 1211-1215.

Banerjee, R., & Bhattacharyya, B. C. (1992). Extracellular alkaline protease of newly isolatedRhizopus oryzae. Biotechnology letters, 14(4), 301-304.

Barbesgaard, P., Heldt-Hansen, H. P., & Diderichsen, B. (1992). On the safety of Aspergillus oryzae: a review. Applied Microbiology and Biotechnology, 36(5), 569-572.

Bajpai, P., Bhardwaj, N. K., Bajpai, P. K., & Jauhari, M. B. (1994). The impact of xylanases on bleaching of eucalyptus kraft pulp. Journal of biotechnology, 38(1), 1-6.

Beg, Q., Kapoor, M., Mahajan, L., & Hoondal, G. S. (2001). Microbial xylanases and their industrial applications: a review. Applied microbiology and biotechnology, 56(3-4), 326-338.

Bennett, T.P. and Frieden, E. 1969. Modern Topics in Biochemistry, Macmillan: London, pp. 43–45.

Blandino, A., Iqbalsyah, T., Pandiella, S., Cantero, D., & Webb, C. (2002). Polygalacturonase production by Aspergillus awamori on wheat in solid-state fermentation. *Applied Microbiology and Biotechnology*, *58*(2), 164.

Brahmachari, G. (2016). Biotechnology of Microbial Enzymes: Production, Biocatalysis and Industrial Applications. Academic Press.634 (1), 1-4.

Brayer, G.D.; Luo, Y.; Withers, S.G. (1995). The structure of human pancreatic alpha-amylase at 1.8 A resolution and comparisons with related enzymes. Protein Sci 4, 1730-1742.

Bruinenberg P.M, Hulst A.C, Faber A, Voogd R.H. 1996. A process for surface sizing or coating of paper. European Patent Application, EP 0 690 170 A1.

Burhan, A., Nisa, U., Gokhan, C., Omer, C., Ashabil, A., & Osman, G. (2003). Enzymatic properties of a novel thermophilic, alkaline and chelator resistant amylase from an alkalophilic Bacillus sp. Isolate ANT-6. Process Biochemistry, 38, 1397–1403.

Busch JE and Stutzenberger FJ, Amyloytic activity of Thermomonospora fusca. World J. Microbiol. Biotechnol, 13(6): 637-642, (1997).

Butt, M.S.,Tahir-Nadeem, M., Ahmad, Z. and Tauseef Sultan, M.(2008). "Xylanases and their applications in baking industry" Foodtechnol.Biotechnol. 46(1):22-31.

Collins, T., Gerday, Ch. and Feller, G. (2005). "Xylanases, xylanase families and extremophilic xylanases." FEMS Microbiol. Reviews 29:3-23.

Chi, Z. Chi, Z. Liu, G. Wang, F. Ju, L. Zhang, T. 2009. Saccharomycopsis fibuligera and its applications in biotechnology. Biotechnol Adv, 27:423-43.

Della Torre, C. L., & Kadowaki, M. K. (2017). Thermostable xylanase from thermophilic fungi: Biochemical properties and industrial applications. *African Journal of Microbiology Research*, *11*(2), 28-37.

Demirkan, E., Sevgi, T., & Başkurt, M. (2017). Optimization of physical factors affecting the production of the α-amylase from a newly isolated Bacillus sp. M10 strain. Karaelmas Fen ve Mühendislik Dergisi, 7(1), 23-30.

Drauz, K., Gröger, H., & May, O. (Eds.) Enzyme catalysis in organic synthesis: a comprehensive Handbook, John Wiley & Sons, (2012).

Dumoulina, Y. Cartiliera, L. Mateescub, M. 1999. Cross-linked amylose tablets containing a-amylase: an enzymatically-controlled drug release system, Journal of Controlled Release, 60:161–167.

Ehrlich, H. L., Newman, D. K., & Kappler, A. (Eds.). (2015). Ehrlich's Geomicrobiology. CRC press.

Ellaiah, P., Adinarayana, K., Bhavani, Y., Padmaja, P. and Srinivasulu, B. 2002. Optimization of process parameters for glucoamylase production under solid state fermentation by a newly isolated *Aspergillus sps. Process Biochem*. 38: 615-620.

Emmanuel L, Stephan J, Bernard H and Abdel B, Thermophilic archeal amylolytic enzymes. Enzyme Microb. Technol, 26: 3- 14, (2000).

Fadel-M, Production of Thermostable amylolytic enzyme by Aspergillus niger F- 909 under solid state fermentation. Egyptian J. Microbial, 35: 487-505, (2000).

Feitkenhauer, H. 2003. Anaerobic digestion of desizing wastewater: influence of pretreatment and anionic surfactant on degradation and intermediate accumulation. Enzyme Microb. Technol. 33:250–258.

Fogarty W.M, Kelly C.T. 1980. In Economic Microbiology, Microbial Enzymes and Bioconversions. Academic Press, pp. 115–170.

Francis, F.; Sabu, A.; Nampoothiri, K.M.; Ramachandran, S.; Ghosh, S.; Szakacs, G.; Pandey, A. (2003). Use of response surface methodology for optimizing process parameters for the production of α-amylase by Aspergillus oryzae. Biochem. Eng. J. 15, 107–115.

Fryer, P.J. Asteriadou, K.A. 2009. Prototype cleaning map: a classification of industrial cleaning processes. Trends in Food Science and Technology, 20:255-262.

G.L.Miller,"Use of dinitrosalisylic acid reagent for determination of reducing sugar,"AnalyticalChemistry,vol.31,no.3,pp. 426–429,1959.

Gabiatti Jr, C., Vendruscolo, F., Piaia, J. C. Z., Rodrigues, R. C., Durrant, L. R., & Costa, J. A. V. (2006). Radial growth rate as a tool for the selection of filamentous fungi for use in bioremediation. Braz Arch Biol Technol special, (49), 29-34.

Gangadharan, D.; Sivaramakrishnan, S.; Nampoothiri, K.M.; Sukumaran, R.K.; Pandey, A. (2008). Response surface methodology for the optimization of alpha amylase production by Bacillus amyloliquefaciens. Bioresour Technol 99, 4597-4602.

Gopinath, S. C., Anbu, P., Arshad, M. K., Lakshmipriya, T., Voon, C. H., Hashim, U., & Chinni, S. V. (2017). Biotechnological Processes in Microbial Amylase Production. *BioMed research international*, *2017*.

Guha S, Bhutty S, Khurana S.M.P, Kohli UK. Optimization of cultural conditions for production of thermo-alkali tolerant xylanase from Bacillus sp. Inter J Res Pure and Appl Microbiol. 2013:3:116–120.

Gupta, R.; Gigras, P.; Mohapatra, H.; Goswami, V.K.; Chauhan, B. (2003). Microbial α-amylase: a biotechnological perspective. Process Biochem 38, 1599 - 1616.

Haq, I. Ali, S. Javed, M.M. Hameed, U. Saleem, A. Adnanf-Qadeer, M.A. 2010. Production of alpha amylase from a randomly induced mutant strain of bacillus amyloliqefaciens and its application as a desizer in textile industry. Pak. J. Bot. 42(1): 473- 484.

Hasan, F., Shah, A.A. and Hameed, A. 2006. Industrial application of microbial lipase. Enzyme and Microbial Technology, 39(2): 235–251.

Haseltine C, Rolfsmeier M and Blum P, The glucose effect and regulation of α-amylase synthesis in the hyperthermophilic archaeon Sulfataricus solfataricus. J. Bacteriol, 178, 945-950, (1996).

Hmidet, N. Bayoudh, A. Berrin, J.G. Kanoun, S. Juge, N. Nasri, M. 2008. Purification and biochemical characterization of a novel alpha-amylase from Bacillus licheniformis. NH1: cloning, nucleotide sequence and expression of amyN gene in Escherichia coli. Process Biochemistry, 43:499-510.

Hölker, U., Höfer, M., & Lenz, J. (2004). Biotechnological advantages of laboratory-scale solid-state fermentation with fungi. *Applied Microbiology and Biotechnology*, *64*(2), 175-186.

Hudzicki, J. (2009). Kirby-Bauer disk diffusion susceptibility test protocol.

Ikram ul, H.; Ashraf, H.; Iqbal, J.; Qadeer, M.A. (2003). Production of alpha amylase by *Bacillus licheniformis* using an economical medium. *Bioresour Technol* 87, 57-61.

Iulek, J.; Franco, O.L.; Silva, M.; Slivinski, C.T.; Bloch, C., Jr.; Rigden, D.J.; Grossi deSa, M.F. (2000). Purification, biochemical characterisation and partial primary structure of a new alpha-amylase inhibitor from Secale cereale (rye). Int J Biochem Cell Biol 32, 1195- 1204.

Jurado-Alameda, E. Bravo-Rodriguez, V. Bailón-Moreno, R. Nuñez Olea, J. Altmajer-Vaz, D. 2003. Bath-substrate-flow method for evaluating the detersive and dispersant performance of hard-surface detergents. Industrial & Engineering Chemistry Research, 42:4303-4310.

Kamal KB, Balakrishnan H, Rele MV (2004). Compatibility of alkaline xylanases from an alkaliphilic Bacillus NCL (87-6-10) w ith commercial detergents and proteases. J. Ind. Microbiol. Biotechnol. 31(2):83-87.

Kandra, L. (2003). α-amylase of medical and industrial importance. Journal of Molecular Structure (Theochem) 666–667, 487–498.

Kandra, L. (2003). α-amylase of medical and industrial importance. Journal of Molecular Structure (Theochem) 666–667, 487–498.

Kathiresan, K. and Manivannan, S. 2006. α-amylase production by Penicillium fellutanum isolated from mangrove rhizospheric soil. Afr. J. Biotechnol. 5: 829-832.

Kiddinamoorthy, J., Anceno, A. J., Haki, G. D., & Rakshit, S. K. (2008). Production, purification and characterization of Bacillus sp. GRE7 xylanase and its application in eucalyptus Kraft pulp biobleaching. *World Journal of Microbiology and Biotechnology*, *24*(5), 605-612.

Kirk, O., Borchert, T. V., & Fuglsang, C. C. (2002). Industrial enzyme applications. Current opinion in biotechnology, 13(4), 345-351.

Konsoula, Z, Liakopoulou-Kyriakides M, "Co-production of alpha-amylase and beta-galactosidase by Bacillus subtilis in complex organic substrates." Bioresource Technology, 98, 150- 157, (2007).

Kost, J., & Shefer, S. (1990). Chemically-modified polysaccharides for enzymatically-controlled oral drug delivery. Biomaterials, 11(9), 695-698.

Kuddus, M. (2014). Bio-statistical approach for optimization of cold-active α-amylase production by novel psychrotolerant M. foliorum GA2 in solid state fermentation. Biocatalysis and Agricultural Biotechnology, 3(2), 175-181.

Kuhad RC, Singh A (1993) Lignocellulosic biotechnology: current and future prospects. Crit Rev Biotechnol 13:151–172.

Kumar, C.G. and Takagi, H. 1999. Microbial alkaline protease: from a bio industrial viewpoint. *Biotechnol. Adv*. 17(7): 561-594.

Kumari, A., Singh, K., & M Kayastha, A. (2012). α-Amylase: General Properties, Mechanism and Biotechnological Applications-A Review. Current Biotechnology, 1(1), 98-107.

Lonsane BK and Ramesh MV, Production of bacterial thermostable α-amylase by solid state fermentation: a potential tool for achieving economy in enzyme production and starch hydrolysis. Adv. Appl. Microbial, 35: 1-56, (1990).

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the folin phenol reagent. Journal of Biological Chemistry, 193, 265–275.

Maheshwari, R., Bharadwaj, G., Bhat, M.K., 2000. Thermophilic fungi: their physiology and enzymes. Microbiol. Mol. Biol. Rev. 64, 461–488.

Mazutti, M., Ceni, G., DiLuccio, M. and Treichel, H. 2007. Production of insulinase by solid state fermentation: effect of process parameters on production and preliminary characterization of enzyme preparations. *Bioprocess Biosyst. Eng*. 30: 297-304.

Meenakshi, C., Narender, K. Vikrant, A., Karupothula, S., Shobhana, B. and Sushma, S. 2009. Detection of Alpha Amylase Producing Bacteria (Bacillus sp. 5250) from soil: Isolation and Optimization of Various Conditions of Growth. Research Journal of Biotechnology. 4 (1): 50-56.

Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 1956; 31: 426–8.

Mitidieri, S. Martinelli, A.H.S. Schrank, A. Vainstein, M.H. 2006. Enzymatic detergent formulation containing amylase from Aspergillus niger: a comparative study with commercial detergent formulations, Bioresour. Technol, 97:1217–1224.

Mobini-Dehkordi, M., & Javan, F. A. (2012). Application of alpha-amylase in biotechnology. Journal of Biology and today's world, 1(1), 15-20.

Moraes, L.M.P. Filho, S.A. Ulhoa, C.J. 1999. Purification and some properties of an α-amylase glucoamylase fusion protein from Saccharomyces cerevisiae. World J. Microbiol. Biotechnol, 15:561-564.

Mulimani, V.H. and Ramalingam, G.N.P. 2000. α-amylase production by solid state fermentation: anew practical; approach to biotechnology courses. Biochem. Educ. 28: 161-163.

Nair, S.G.; Sindhu, R.; Shashidhar, S. (2008). Purification and biochemical characterization of two xylanases from *Aspergillus sydowii* SBS 45. *Appl. Biochem. Biotechnol.,* 149: 229-243.

Niehaus F, Bertoldo C, Kahler M, Antranikian G (1999) Extremophiles as a source of novel enzymes for industrial applications. Appl Microbiol Biotechnol 51:711–729.

Nielsen, J.E.; Borchert, T.V. (2000). Protein engineering of bacterial alpha-amylases. Biochim Biophys Acta 1543, 253-274.

Ninawe, S.; Kapoor, M.; Kuhad. R.C. (2007). Purification and characterization of extracellular xylanase from *Streptomyces cyaneus* SN32. *Bioresour. Technol*., 99: 1252-1258.

Nkohla, A., Okaiyeto, K., Olaniran, A., Nwodo, U., Mabinya, L., & Okoh, A. (2017). Optimization of growth parameters for cellulase and xylanase production by Bacillus species isolated from decaying biomass. *Journal of Biotech Research [ISSN: 1944-3285]*, *8*, 33-47.

Oboh, G. "Isolation and characterization of amylase from fermented cassava (Manihot esculenta Crantz) wastewater". African journal of biotechnology, 4 (10).October (2005).

OGASAHARA, K., IMANISHI, A., & ISEMURA, T. (1970). Studies on Thermophilic α-Amylase from Bacillus stearothermophilus: II. Thermal Stability of Thermophilic α-Amylase. The Journal of Biochemistry, 67(1), 77-82.

Onosakponome, I. (2017). Production, Partial Purification and Characterization of Cellulase by Aspergillus Niger from Submerged Fermentation of Grape Bagasse.

Oyeleke, S.B., Auta, S.H. and Egwim, E.C. 2010. Production and characterization of amylase produced by Bacillus megaterium isolated from a local yam peel dumpsite in Minna, Niger State. Journal of Microbiology and Antimicrobial. 2(7): 88-92.

Öner, E.T. 2006. Optimization of ethanol production from starch by an amylolytic nuclear petite Saccharomyces cerevisiae strain. Yeast, 23:849-856.

Pandey, A., Benjamin, S., Soccol, C. R., Nigam, P., Krieger, N., & Soccol, V. T. (1999). The realm of microbial lipases in biotechnology. Biotechnology and applied biochemistry, 29(2), 119-131.

Pandey, A., Soccol, C. R., Nigam, P., & Soccol, V. T. (2000). Biotechnological potential of agro-industrial residues. I: sugarcane bagasse. Bioresource technology, 74(1), 69-80.

Panneerselvam, T., & Elavarasi, S. (2015). Isolation of-Amylase Producing Bacillus subtilis from Soil. *Int. J. Curr. Microbiol. App. Sci*, *4*(2), 543-552.

Polizeli, M. L. T. M., Rizzatti, A. C. S., Monti, R., Terenzi, H. F., Jorge, J. A., & Amorim, D. S. (2005). Xylanases from fungi: properties and industrial applications. *Applied microbiology and biotechnology*, *67*(5), 577-591.

Prescott, Lansing M. Harley, John P. Klein, Donald A. Lansing M. Prescott, John P. Harley, and Donald A. Klein. Microbiología. McGraw-Hill,, 2004.

Raddadi N, Cherif A, Daffonchio D, Neifar M, Fava F (2015) Biotechnological applications of extremophiles, extremozymes and extremolytes. Appl Microbiol Biotechnol 99:7907–7913.

Rajagopalan, G.; Krishnan, C. (2008). Alpha-amylase production from catabolite derepressed Bacillus subtilis KCC103 utilizing sugarcane bagasse hydrolysate. Bioresour Technol 99, 3044-3050.

Ramasesh N, Sreekantiah K.R, Murthy K.R, Ramasesh V.S. 1982. Purification and characterization of thermophilic alpha-amylase of Aspergillus niger. Van Thieghem Starke, 34, 274–279.

Rani, S., & Nand, K. (1996). Development of cellulase-free xylanase-producing anaerobic consortia for the use of lignocellulosic wastes. *Enzyme and microbial technology*, *18*(1), 23-28.

Rao MB, Tanksale AM, Gathe MS, Deshpande VV, Molecular and Biotechnological aspects of microbial proteases. Microbial. Microbial. Rev, 62: 597-635, (1998).

Ratna, S., & Mustafa, B. (2011). Solid substrate fermentation (SSF) and submerged fermentation (SmF) of aspergillus versicolor A6 using pineapple waste as substrate for pectinase production.

Reda, 2007; Hmidet ,2010, Production of a thermo stable a-amylase from Bacillus sp. PS-7 by solid-state fermentation and its synergistic use in the hydrolysisof malt starch for alcohol production, Process Biochem.40 (2005) 525 534.

Reddy, N.S.; Nimmagadda, A.; Sambasiva Rao, K.R.S. (2003). An overview of the microbial α-amylase family. Afr. J. Biotechnol. 2, 645- 648.

Regulapati, R. Malav, PN. Gummadi, SN. 2007. Production of thermostable alpha amylase by solid state fermentation- A review, 2(1):1- 11.

Robinson T, Mchnlan G, Marchant R and Nigam P, Remediation of dyes in textile effluent: a critical review on current treatment technologies with a proposed alternative. Biosensor. Technol, 77: 247- 255, (2001).

S. Ahmed, S. Riaz, A. Jamil, Molecular cloning of fungal xylanases: anoverview, Appl. Microbiol. Biotechnol. 84 (2009) 19–35.

Sahay, H., Yadav, A. N., Singh, A. K., Singh, S., Kaushik, R., & Saxena, A. K. (2017). Hot springs of Indian Himalayas: potential sources of microbial diversity and thermostable hydrolytic enzymes. *3 Biotech*, *7*(2), 118.

Sadhukhan RK, Manns S, Roy SK and Chakraborty SL, Thermostable amyloytic amylase enzyme from a celluolytic fungus Myceliophthora thermophilla D14 ATCC 48104. Appl. Microbioal. Biotechnol., 1990, 33: 692-696.

Sajedi, R.H.; Naderi-Manesh, H.; Khajeh, K.; Ahmadvand, R.; Ranjbar, B.; Asoodeh, A.; Moradian, F. (2005). A Ca-independent α-amylase that is active and stable at low ph from the Bacillus sp. KR-8104. Enzyme Microb. Technol. 36, 666–671.

Salis, A., Monduzzi, M., Solinas, V., Polaina, J., &MacCabe, A. P. (2007). Industrial Enzymes: Structure Function and Applications.

Sanchez, O.J. Cardona, C.A. 2008. Trends in biotechnological production of fuel ethanol from different feedstocks. Bioresour Technol, 99:5270-5295.

Sanghi, A., Garg, N., Gupta, V. K., Mittal, A., & Kuhad, R. C. (2010). One-step purification and characterization of cellulase-free xylanase produced by alkalophilic Bacillus subtilis ash. *Brazilian Journal of Microbiology*, *41*(2), 467-476.

Saxena AK, Yadav AN, Rajawat M, Kaushik R, Kumar R, Kumar M, Prasanna R, Shukla L (2016) Microbial diversity of extreme regions: an unseen heritage and wealth. Indian J Plant Genet Resour 29:246–248.

Saxena, R.K. Dutt, K. Agarwal, L. Nayyar, P. A. 2007. Highly thermostable and alkaline amylase from a Bacillus sp. PN5. Bioresour Technol, 98:260-265.

Segel, I. H. (1975). Enzyme kinetics (Vol. 360). Wiley, New York.

Senthilkumar, P.K., Uma, C. and Saranraj, P. 2012. Amylase Production by Bacillus sp. Using Cassava as Substrate. International Journal of Pharmaceutical & Biological Archives, 3(2): 300-306.

Sharma M, Kumar A (2013). Xylanases: an overview. Br. Biotechnol. J.3 (1):1-28.

Senthilkumar, P.K., Uma, C. and Saranraj, P. 2012. Amylase Production by Bacillus sp. Using Cassava as Substrate. International Journal of Pharmaceutical & Biological Archives, 3(2): 300-306.

Singh, P., Gupta, P., Singh, R. and Sharma, R. 2012. Factors affecting α- Amylase Production on Submerged Fermentation by Bacillus sp. International Journal of Pharmacy & Life sciences. 3(12): 2243-2246.

Singh, R. (2007). Development of N-heterocyclic Carbenes as Organic Catalysts and Efficient Ligands in Palladium Mediated Transformations. ProQuest.

Singh, S., Madlala, A.M., Prior, B.A., 2003. Thermomyces lanuginosus: properties of strains and their hemicellulases. FEMS Microbiol. Rev.27, 3–16.

Singh, S., Sharma, V., Soni, M. L., & Das, S. (2011). Biotechnological applications of industrially important amylase enzyme. *International Journal of Pharma and Bio Sciences, 2*, 486-496.

Singhania, R.R.; Patel, A.K.; Soccol, C.R.; Pandey, A. (2009). Recent advances in solid-state fermentation. Biochem. Eng. J. 44, 13–18.

Sivakumar, T., Shankar, T., Vijayabaskar, P., Muthukumar, J. and Nagendrakannan, E. 2012. Amylase Production Using Bacillus cereus Isolated from a Vermicompost Site. *International Journal of Microbiological Research* 3 (2): 117-123.

Sivakumar, T., Ramasubramanian, V., Shankar, T., Vijayabaskar, P., Anandapandian, K. 2011. Screening of keratinolytic bacteria Bacillus cereus from the feather dumping soil of sivakasi. J. Basic Appl. Bio. 5: 305-314.

Smitt, J.P., Rinzema, J., Tramper, H., Van, M. and Knol, W. 1996. Solid state fermentation of wheat bran by *Trichoderma reesei* QMQ414. *Appl. Microbial. Biotechnol*. 46: 489-496.

Sodhi HK, Sharma K, Gupta JK and Soni SK, Production of a thermostable α- amylase from Bacillus sp. PS-7 by solid- state fermentation and its synergistic use in the hydrolysis of malt starch for alcohol production. Process Biochem, 40(2): 525-534, (2005).

Souza, P. M. D. (2010). Application of microbial α-amylase in industry-A review. *Brazilian Journal of Microbiology*, *41*(4), 850-861.

Srinivasan MC, Rele MV (1999) Microbial xylanases for paper industry. Curr Sci 77:137–142.

Subramaniyam, R. and Vimala, R. (2012) solid state and submerged fermentation for the production of bioactive substances: a comparative study. *International Journal of Science and Nature*, **3**(3): 480-486.

Subramaniyan, S., & Prema, P. (2002). Biotechnology of microbial xylanases: enzymology, molecular biology, and application. *Critical reviews in biotechnology*, *22*(1), 33-64.

Subramaniyan, S., & Prema, P. (2000). Studies on production of bacterial xylanases (Doctoral dissertation, Regional Research Laboratory).

Sumner, J. B., & Somers, G. F. (2014). *Chemistry and methods of enzymes*. Academic Press.

Tangphatsornruang, S.; Naconsie, M.; Thammarongtham, C.; Narangajavana, J. (2005). Isolation and characterization of an alpha- amylase gene in cassava (Manihot esculenta). Plant Physiol Biochem 43, 821-827.

Tanyildizi, M.S.; Ozer, D.; Elibol, M. (2005). Optimization of α-amylase production by Bacillus sp. using response surface methodology Process Biochem 40, 2291–2296.

Techapun, C., Charoenrat, T., Watanabe, M., Sasaki, K., & Poosaran, N. (2002). Optimization of thermostable and alkaline-tolerant cellulase-free xylanase production from agricultural waste by thermotolerant Streptomyces sp. Ab106, using the central composite experimental design. *Biochemical Engineering Journal*, *12*(2), 99-105.

Teodoro, C.E.deS. and Martins, M.L.L. 2000. Culture conditions for the production of thermostable amylase by Bacillus sp. Brazilian Journal of microbiology. 32:298-302.

Thomas, L., Sindhu, R., Binod, P., & Pandey, A. (2015). Production of an alkaline xylanase from recombinant Kluyveromyces lactis (KY1) by submerged fermentation and its application in bio-bleaching. Biochemical Engineering Journal, 102, 24-30.

Uday, U. S. P., Choudhury, P., Bandyopadhyay, T. K., & Bhunia, B. (2016). Classification, mode of action and production strategy of xylanase and its application for biofuel production from water hyacinth. *International journal of biological macromolecules*, *82*, 1041-1054.

Van der Maarel, M.J.; van der Veen, B.; Uitdehaag, J.C.; Leemhuis, H.; Dijkhuizen, L. (2002). Properties and applications of starch-converting enzymes of the alpha-amylase family. J Biotechnol 94, 137-155.

Veille, C. Zeikus, G.J. 2001. Hyperthermophilic enzymes: sources, use, and molecular mechanisms for thermostability. Microbiol. Mol. Biol. Rev, 65:1–43.

Vidyalakshmi, R., Paranthaman, R., & Indhumathi, J. (2009). Amylase production on submerged fermentation by Bacillus spp. World Journal of Chemistry, 4(1), 89-91.

Viniegra-González, G., Favela-Torres, E., Aguilar, C. N., de Jesus Rómero-Gomez, S., Dıaz-Godınez, G., & Augur, C. (2003). Advantages of fungal enzyme production in solid state over liquid fermentation systems. *Biochemical Engineering Journal*, *13*(2), 157-167.

Wang, Z., Chen, L., & Huang, X. (2009). Research on Advanced Materials for Li-ion Batteries. Advanced Materials, 21(45), 4593-4607.

Whitcomb, D.C.; Lowe, M.E. (2007). Human pancreatic digestive enzymes. Dig Dis Sci 52, 1-17.

Whitehurst, R. J., & Van Oort, M. (Eds.). (2010). *Enzymes in food technology (Vol. 388)*. Chichester: Wiley-Blackwell.

Wong, K. K., Tan, L. U., & Saddler, J. N. (1988). Multiplicity of beta-1, 4-xylanase in microorganisms: functions and applications. Microbiological reviews, 52(3), 305.

Wu, H. Mulchandani, A. Chen, W. 2008. Versatile microbial surface-display for environmental remediation and biofuels production. Trends in Microbiology, 14, 4:181-188.

Zar, M.S., Ali, S. and Haq, I. 2012. Optimization of the alpha amylase production from Bacillus amyloliquefaciens IIB-14 via parameter significance analysis and response surface methodology. African Journal of Microbiology Research. 6(17):3845-3855.

Zeman, N. McCrea, J. 1985. Alpha-amylase Production Using a Recombinant DNA Organism. Cereal food world, 30:777-780.

Zhan FX, Wang QH, Jiang SJ, Zhou YL, Zhang GM, Ma YH (2014). Developing a xylanase XYNZG from *Plectosphaerella cucumerina* for baking by heterologously expressed in *Kluyveromyces lactis*. BMC Biotechnol. 14(1):107.

SKILLS

- Immunology
- Virology
- Medical Bacteriology
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- Molecular biology
- Biochemistry
- Food microbiology
- Industrial microbiology
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- **Kurdish:** Mother Language
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MSC. RESEARCH:

Production and Characterization of Thermostable α-amylase and Xylanase from *Thermobacillus sp* VO15 and *B. licheniformis* VO14.

EXPERIENCE

DIAGNOSIS DISEASES • PRIVATE LABORATORY • TWO YEARS

From private laboratory work every day's at (2 pm – 8 pm)
Without Friday.

DIAGNOSIS DISEASES • W.H.O • SIX MONTH

Work with W.H.O in hospital laboratory for help migration people, I'm worked on diagnosis TB infection every day's at (8pm-12pm) except Friday.

VOLUNTEER EXPERIENCE OR LEDERSHIP

Three month work by volunteer in hospital laboratory government in the central laboratory for diagnosis diseases from Sulaimani city at (8pm-12pm) except Friday.