REPUBLIC OF TURKEY
SIIRT UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

PRODUCING ACTIVATED CARBON FROM SESAME STEM AND USING IT IN THE IMMOBILIZATION OF *Aspergillus niger GLUCOAMYLASE*

MASTER THESIS

Yousif Mohammed Sharif ALI
(163102009)

Department of Chemical Engineering
Thesis Supervisor: Prof. Dr. Ömer ŞAHİN

March-2018
SIIRT

THESIS ACCEPTANCE AND APPROVAL

The thesis titled "OBTAINING OF ACTIVATED CARBON FROM SEASEM STEM AND USING FOR IMMOBILIZATION OF Aspergillus niger GLUCOAMYLASE" prepared by Yousıf Mohammed Sharıf ALI, has been unanimously by majority of the following jury as a MASTER THESIS at Siirt University Institute of Science and Technolohy Depertmant of Chemical Engineering on 09/03/2018.

Jury Members	**Signature**
President Prof. Dr. Faruk SÜZERGÖZ	.....
Superviser Prof. Dr. Ömer ŞAHİN	.....
Member Assoc. Prof. Dr. Halil DEMİR	.....
Member Assist. Prof. Dr. Yakup ASLAN	.....
Member Assist. Prof. Dr. Orhan BAYTAR	.....

I confirm the above results

Assoc. Prof. Dr. Fevzi HANSU
Director of Institute of Science

This thesis study was supported by Siirt University Scientific Research Projects Coordinator ship with the 2018-SİÜFEB-28 project.

REPUBLIC OF TURKEY
SIIRT UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

PRODUCING ACTIVATED CARBON FROM SESAME STEM AND USING IT IN THE IMMOBILIZATION OF *Aspergillus niger GLUCOAMYLASE*

MASTER THESIS

Yousif Mohammed Sharif ALI
(163102009)

Department of Chemical Engineering
Thesis Supervisor: Prof. Dr. Ömer ŞAHİN

March-2018
SIIRT

DECLARATION

This thesis was prepared in accordance with the thesis writing rules, complies with the scientific code of ethics. I certify that, to the best of my knowledge, my thesis does not infringe upon anyone's copyright nor violate any proprietary rights and that any ideas, techniques, quotations, or any other material from the work of other people included in my thesis, published or otherwise, are fully acknowledged in accordance with the standard referencing practices.

Yousif Mohammed Sharif ALI

Note: In this thesis, the use of original and other source notifications, tables, figures and photographs without reference is subject to the provisions of Law No. 5846 on Intellectual and Artistic Works.

PREFACE

In this thesis, Activated carbon has been obtained from the sesam stem and activated functionalized and used for the immobilization of *Aspergillus niger* glucoamylase.

First, I would like to express my sincere gratitude and appreciation to my supervisor **PROF. Dr. ÖMER ŞAHİN,** Professor of Chemical Engineering, Vice-Rector, Dean of Faculty Engineering and Architecture, Siirt University for direct supervision, careful advice and overall assistance throughout the research study. This project would not have been possible without the support of Siirt University Scientific Research Projects Coordinator ship (BAP).

My deepest obligation and profound gratitude to **Dr. Yakup ASLAN,** Assistant Professor of Biochemistry for careful advice, encouragement, understands, excellent support throughout the research studies. He showed me different ways to approach the research problem and taught me to be persistent to accomplish my goal. Many thanks and deepest obligation to **Dr. Orhan Baytar**, Assistant Professor of Chemical Engineering for careful advice, help, and support throughout the research study.

Special thanks and sincere gratitude to **Dr. HALİL DEMİR**, Associate Professor of Chemical Engineering, Vice-Dean of the Faculty of Engineering and Architecture.

I am also grateful to many people who have helped me during my study.

Yousif Mohammed Sharif ALI
SIIRT-2018

CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

Page

ABBREVIATIONS AND SYMBOLS LISTS

Symbol	Explanation
SEM	: Scanning electron microscopy
FTIR	: Fourier transform infrared spectroscopy
TGA/TG	: Thermogravimetric analysis/thermogravimetric
BET	: Brunauer–Emmelt–Teller
CO_2	: Carbon dioxide
N_2	: Nitrogen
Ar	: Argon
Ki	: Enzyme operational stability rate constant, min^{-1}
Km	: Michaelis constant, g dm^{-3}
M-D	: Maltodextrin
R	: Reaction rate, g dm^{-3} min^{-1}
V.A	: Volume activity, g dm^{-3} min^{-1}
Vm	: Maximal reaction rate, g dm^{-3} min^{-1}
X	: Conversion, – or %
G	: Mass concentration, mg cm^{-3}
^{0}C	: Celsius
K	: Kelvin
ANGA	: Aspergillus niger glucoamylase
BSA	: Bovine Serum Albumin
g	: Gram
IU	: The International Unit of Enzyme
L	: Liter
M	: Molarity
mg	: Milligram
mL	: Milliliters
pH	: -log [H^+]
rpm	: The number of rotations per minute
UV	: Ultraviolet
UHT	: Ultra High Temperature
V	: Volume
W	: Weight
µg	: Microgram
µL	: Microliter
µmol	: Micromole
U	: Unit
nm	: Nanometer

ÖZET

YÜKSEK LİSANS TEZİ

SUSAM SAPINDAN AKTİF KARBON ELDESİ VE *Aspergillus niger* GLUKOAMİLAZIN İMMOBİLAZASYONUNDA KULLANILMASI

Yousif Mohammed Sharif ALI

Siirt Üniversitesi Fen Bilimleri Enstitüsü
Kimya Mühendisliği Anabilim Dalı

Danışman: Prof. Dr. Ömer ŞAHİN

2018, 59 Sayfa

Bu çalışmada, deneyler iki aşamada gerçekleştirilmiştir. Birinci aşamada susam çubuğundan $ZnCl_2$ aktifleştiricisi kullanılarak mikro dalga destekli kimyasal aktivasyon yöntemiyle aktif karbon sentezlenmiştir. Aktif karbon sentelenmesinde mikro dalga gücü, mikro dalga süresi, mikro dalgada farklı gazların aktivasyon süresi, aktivasyon sıcaklığı ve hammadde/ kimyasal oranı parametrelerinin etkileri incelenmiştir. Elde edilen aktif karbonların iyot sayıları belirlenip yüksek iyot sayısına sahip olanların BET yüzey alanı, SEM, TGA/ TG ve FTIR ile karkterizasyonu gerçekleştirilmiştir. BET yüzey alanı yüksek olan aktif karbonlar enzim immobilizasyonunda kullanılmıştır.

Elde edilen fonksiyonelleştirilmiş aktif karbon, ikinci aşamada Aspergillus niger glukoamilazın kovalent immobilizasyonu için kullanıldı. İmmobilizasyon koşullarını optimize ederek, % 100'lük immobilizasyon ve aktivite verimleri elde edildi. Optimum pH (5.5) ve optimum sıcaklık aralığı (55-60 °C) immobilizasyondan etkilenmez. Serbest ve immobilize enzimin Michaelis-Menten sabitleri ayrıca Linewveaver-Burk grafiği kullanılarak belirlendi. İmmobilizasyondan sonra, V_{max} değeri 1464.1'den 1342.3 μmol D-glikoz / L.dk'ya düşerken, K_m değeri 116.3'den 106.3 g maltodekstrin / L'ye düşmüştür. İmmobilize enzim, tekrarlanan yirmi kullanımdan sonra ve yirmi günlük depolamadan sonra başlangıçtaki aktivitesini kaybetmedi. Maltodekstrin, immobilize enzim kullanılarak iki saat sonra tamamen glukoza dönüştürüldü. Sonuç olarak, bu çalışmada elde edilen immobilize *Aspergillus niger* glukoamilaında glikoz şurubunun endüstriyel üretiminde ve diğer endüstriyel uygulamalarda kullanılabileceği söylenebilir.

Anahtar Kelimeler: Aktif Karbon; glukoamilaz; *Aspergillus niger*; Kovalent İmmobilizasyon; Glikoz şurubu; Maltodekstrin, Susam Kabuğu.

ABSTRACT

MASTER THESIS

PRODUCING ACTIVATED CARBON FROM SESAME STEM AND USING IT IN THE IMMOBILIZATION OF *Aspergillus niger GLUCOAMYLASE*

Yousıf Mohammed Sharıf ALI

Graduate School of Natural and Applied Science of Siirt University
The Degree of Master of Science In Chemical Engineering

Supervisor: Prof. Dr. Ömer ŞAHİN

2018, 59 Pages

In this study, the experiments have been carried out in two stages. In the first step activated carbon obtained from sesame stem and activated carbon synthesis by microwave assisted chemical activation method using $ZnCl_2$ activation. The effects of microwave power, microwave duration, different gases at microwave, activation temperature and raw material/chemical ratio parameters were examined in the activated carbon synthesis.The iodine number of the obtained activated carbon were determined and the higher ones were characterized by BET surface area, SEM, TGA/TG and FTIR. Activated carbon with high BET surface area was used for enzyme immobilization. In the second step, the covalent immobilization of *Aspergillus niger* glucoamylase on the obtained activated carbon functionalized with glutardialdehyde was studied. By optimizing the immobilization conditions, 100.00 % immobilization and activity yields obtained. Optimum pH (5.5) and optimum temperature range (55-60 ^{o}C) have not affected by immobilization. The Michaelis-Menten constants of free and immobilized enzyme were also determined by using Linewveaver -Burk plot. After immobilization, V_{max} value decreased from 1464.1 to 1342.3 μmol D-glucose / L.min, while K_m value decreased from 116.3 to 106.3 g maltodextrin / L. The immobilized enzyme didn't lose its initial activity after repeated twenty uses and after twenty days of storage. Maltodextrin was completely converted to glucose after two hours, by using an immobilized enzyme. Consequently, it can be said that immobilized *Aspergillus niger* glucoamylase obtained in the present study can be used in the industrial production of glucose syrup and in the other industrial applications.

Keywords: Activated Carbon; Glucoamylase; *Aspergillus niger;*Covalent Immobilization; Glucose Syrup; Maltodextrin, Seasem Stem.

1. INTRODUCTION

Activated carbon, AC, is a porous material got from feedstock containing carbon. The structure of the material enables the material to successfully adsorb both vaporous and fluid mixes. The different crude material can be utilized to produce activated carbon, for example, coal, wood, lignite, shells from varying fruits and nuts and few polymers (Vernersson T, 2001).

Sesame as one of the major economic crops in turkey was generated annually and usually burned in open fields, thus leading to heavy air pollution. As well known, sesame stalks possess high carbon and volatile contents, low ash, and reasonable hardness. In this study, sesame shell was used as raw materials to prepare AC. Initially, effects of parameters (activation temperature, activation time and impregnation ratio) on adsorptive property and yield of AC were investigated and optimized by iodine number. Then, the prepared AC was characterized by SEM surface area analysis.

Activated carbon is used in a number of industrial applications including separations and purifications technologies, catalytic processes, biomedical engineering, and energy storage, among other. The extensive applications of activated carbon are essential because of its wide availability high performance in adsorptions processes. Surface reactivity and the flexibility to change its physical and chemical properties for synthesizing adsorbents with very specific characteristics.

The term “immobilized enzymes” refers to “enzymes physically confined or limited in a certain defined region of space with retention of their catalytic activities, and which can be utilized repeatedly and continuously

Immobilized enzyme is important to chemical engineers, biochemists, microbiologists, and others on account of their growing use as industrial catalysts and analytical devices in this study, the activated carbon was employed in the enzyme immobilization of (*ANGA*)

The obtained activated carbon was used for immobilization of *ANGA*. The parameters such as pH and molarity of immobilization, amount of activated carbon and duration of immobilization were optimized to obtain the higher activity yield as possible. Further, the free and immobilized *ANGA* was characterized by determining optimum pH, optimum temperature, pH and thermal stabilities, kinetic parameters, operational and storage stabilities. The immobilized *ANGA* also used for the production of glucose syrup from maltodextrin.

2. LITERATURE REVIEW

2.1. Activated carbon

Activated carbon is a carbonaceous, highly porous adsorptive medium that has a complex structure composed primarily of carbon atoms. The networks of pores in activated carbons are channels created within a rigid skeleton of disordered layers of carbon atoms, linked together by chemical bonds, stacked unevenly, creating a highly porous structure of nooks, crannies, cracks, and crevices between the carbon layers (Haycarb (Pvt) Ltd. 2017).

The high surface area, large porosity, well developed internal pore structure consisting of micro-, meso- and macropores, as well as a wide spectrum of functional groups present on the surface of AC, make it a versatile material which has numerous applications in many areas (Bhatnagar A et al., 2013).

Agricultural and food industries create various waste matters that need to be utilized and convert into the value-added product. Carbonaceous materials such as coconut shell, palm shell, sawdust and tropical wood are some of the most common agricultural wastes shells used to produce activated carbon (Pragya P et al., 2013).

In the present study, the activated carbon was prepared from carbonaceous agriculture waste sesame shell by chemical activation using zinc chloride ($ZnCl_2$) at 500 °C.

Activated carbons are used in a number of industrial applications including separation and purification technologies, catalytic processes, biomedical engineering, and energy storage, among others. The extensive application of activated carbon is mainly due to its relatively low-cost with respect to other adsorbents, wide availability, high performance in adsorption processes, surface reactivity and the versatility to modify its physical and chemical properties for synthesizing adsorbents with very specific characteristics (Virgen M et al., 2012).

2.1.1. Studies related to production of activated carbon

Active carbon is a general term used to describe a family of carbon adsorbents with a large crystal form and a very large internal pore structure. Activated carbons are useful products that are harmless to human health and have a very high porosity and internal surface area. Activated carbons can attract molecules and ions in the solution towards their inner surfaces through their pores and are thus called adsorbents (Özaşık, A., 2002).

Active carbon is used for different purposes in various industries. it has a wide application area in purification industry in the food industry, purification and treatment of gases, separation of mixtures, water, and wastewater treatment, carbon contribution in metal industry, protective clothing in defense industry and health sector ; in summary, active carbon used for various purposes in everyday life is an indispensable material. Production of active carbon with strategic importance is inevitable with domestic technology (Küçükgül, 2004).

Almost every substance containing carbon is used in the production of activated carbon in order for the process to be economical, a readily available raw material should be preferred. Among these, the most commonly used raw materials are botanical originals (biomass) such as wood, coconut, hazelnut, walnut shell, fruit nuclei and fossil-based materials which are carbonized or degradable (all types of coal, lignite, coke) (Aygün, 2002).

There have been many studies on active carbon production up to now. Some of these studies are;

Girgis B and Ishak (1999) produced active carbon from the cotton stalk. In this study, the cotton stalk was first crushed and brought to the sawdust size and then impregnated with phosphoric acid by weight solutions ranging from 20 to 85 percent by weight. After the impregnation, drying at 383 K was carried out and carbonization was carried out at 773 K for 2 hours. In this study, the duration of solution impregnation was limited to 1 night. The most favorable results were obtained from the sample treated with 65% by weight of the solution and the active carbon with a surface area of 985m2/ g was obtained.

they obtained an activated carbon with a surface area of 708 m^2/ g by activation at 600 °C in the presence of water vapor, followed by activation at 800 °C - 850 °C.

Diao Y and friends (2001), In their study with questioning, they have subjected the raw material to carbonization for 15 minutes at 300 °C. they then performed chemical activation at different temperatures (400_600 °C) by adsorbing phosphoric acid to the solid product they obtained. when the obtained results are examined, it is determined that the highest surface area is 1522 m^2 / g at 500 °C.

In the study conducted by **K**öseoğlu (2005), activated carbon elution was investigated by chemical activation method at various temperature ranges using orange K_2CO_3, $ZnCl_2$, H_3BO_3, $SrCl_2.6H_2O$ compounds from the orange shell. The raw sample was subjected to activation without impregnation at temperatures of 500°C and 800°C in order to see the effect of impregnated (emulsifier). Maximum values were obtained with K_2CO_3 at 1352m^2/g at 950°C, 1215m^2/g at 500°C with $ZnCl_2$, 577m^2/g at 600°C and 290m^2/g at 600°C with H_3BO_3.

Tan I.A.W and friends (2007), they performed methylene blue decontamination experiments using activated carbon produced by physical activation with oil palm fibers in the experiments, the duration of contact, the concentration of methylene blue and the effects of temperature on the reaction efficiency were examined at pH 6.5. It was found that the equilibrium data corresponded to the Langmuir, Freundlich and Temkin isotherms and that the reaction to the reaction was endothermic and spontaneous. The temperature increase positively influenced the adsorption capacity and calculated the adsorption capacity of 384.62 mg/g at 50°C.

El-Hendawy A, Abdel-Nasser A et al. (2008), They produced activated carbon from the cotton stalk and processed impregnation solutions with a weight ratio of KOH and H_3PO_4 to the cotton stain of 0,5, 0,75 and 1 treated with cotton stalks cotton stalks treated in this manner were carburized at 500°C-700°C in the atmosphere of N_2 (g). As a result, it was seen that the ratio of activeness was better and the results were better than those treated with KOH. They produced activated carbon with an optimum surface area of 1310 m^2 /g.

Namvar-Asl M et al. (2008), they measured the capacity of capturing methane from the gas phase by producing active carbon from cellulosic-based fruit bark and stone coal. In these investigations, $ZnCl_2$, KOH, and H_3PO_4 were used as physical activators. They changed the oven temperature from 400°C to 900°C and activated it in the N_2 (g) gaseous environment. Waiting time is 30 minutes. With 120 min. They obtained their most successful results in a 60 min proline process.

Güngör C (2010) produced active carbon resulting in chemical activation of grape seed with phosphoric acid. The pore volume, BET surface area, average pore diameter and pore size of activated carbon produced by process variables such as impregnation rate (1: 1, 2: 1, 3: 1, 5: 1) and carbonation temperature (400, 500, 600 °C) Distribution of textural features. The highest surface area (1455 m^2 / g) of active carbon produced at 400°C and 5: 1 impregnation rate. He used the activated carbons produced in the solution for copper removal and examined the adsorption properties under various conditions (pH, temperature, adsorbed dose, contact sulphite, copper concentrations) and determined the optimum conditions for the adsorption process.

Aktar T (2011) determined a 2:1 impregnation rate and a carbonization temperature of 450 °C as the favorable condition for the preparation of activated carbon from agricultural byproducts and wastes by microwave pretreatment chemical method under optimal condition, the active carbon obtained from the tea waste has a BET surface area of 1236 m^2/g and a mesoprobe-rich structure (26.77% microporous, 76.23% mesoporous). The active carbon obtained from the cornelian kernel in the most favorable condition is 1647 m^2/g BET surface area and 32.90% and 67.10% of a micro and mesoporous fraction, respectively. After the adsorption processes of the activated carbons produced in said condition, both of them have an adsorption capacity of about 300mg/g in methylene blue and determined that they have an adsorption capacity of about 600mg/g in the trichloroethylene phenol and that they meet the Freundlich model.

Arulkumar M and friends (2012), they produced activated carbonium data are consistent with the shrimp shell and made Cr (VI) removal. The equilibrium with Freundlich and Langmuir isotherms. It was observed that Cr (VI) was removed in 100.6 mg / It solution from the solution with 1 g of activated carbon and this process lasted 31.4 minutes.

Bouhamed F and friends (2012) produced activated carbon using the H_3PO_4 chemical in the method of chemical activation with the Tunisia kernel. The removal of Cu (II) ions from aqueous solutions with this activated carbon provide optimum adsorption conditions: pH, initial concentration, temperature and duration of adsorption were determined. As a result, the pH=5 and the adsorption capacity of 31.25 mg / g were calculated

Kılıç M (2009) The availability of active carbon obtained from the chemical activation method of the Euphorbia rigida plant using $ZnCl_2$, K_2CO_3, NaOH and H_3PO_4 as an activator in wastewater was investigated. In the case of using K_2CO_3 as an activator, the surface area of active carbon obtained was 2613 m^2 / g, and when $ZnCl_2$ and H_3PO_4 were used, the surface area of active carbon obtained was 1115 m^2 /g and 790 m^2 / g. When NaOH is selected as an activator, a sufficiently high surface area (396 m^2 / g) is not obtained in active carbon. The resulting activated carbons were used for cadmium adsorption from aqueous solutions. For this purpose, the factors affecting the adsorption were investigated and the adsorption isotherms of Langmuir and Freundlich showed that the reaction kinetics corresponded to the so-called (pseudo) second order reaction model and determination of the thermodynamic constants, the adsorption process is performed spontaneously.

2.1.2. Production of activated carbon from sesame stem

It is showed that agricultural residues were promising raw materials for the production of carbon because of their availability at a low price and because they can be used for the production of carbon with a high adsorption capacity, considerable mechanical strength, and low ash content. Many researchers have obtained low-cost carbons or adsorbents from agricultural residues, Sesame is an important agricultural crop. To make better use of these cheap and abundant agricultural residues, these stalks could be used as a low-cost carbon source and converted into sesame stalk-based carbon (Kong H et al, 2012).

To recycle agricultural wastes and decrease activated carbon(AC) production cost, AC from sesame stalks were prepared by sodium hydroxide Activation. Activation temperature, activation time and impregnation ratio (IR) were optimized by

Response surface methodology (RSM) in AC preparation (Jiang Li, Gao Y, Liang G, 2015).

In the world, a known recycling method is activated carbon production from wastes. Activated carbon has much use of areas. Some of them are sanitation, chemical, petroleum, nuclear, automobile, pharmaceutical. in this study activated carbon produced from waste chestnut shells(Uludag O, 2016).

In this study, sesame stem was used as the starting material to prepare activated carbon. because sesame stem is the agricultural by-product and as such, it is a readily available and abundant natural material that can be considered as a raw material to produce activated carbon. There is also a lack in the mitigations of agricultural wastes so it very necessary to investigate this waste to minimize the import of commercial activated carbons.

2.2. Enzymes

Enzymes have played an important part in many parts of life since the dawn of time. Actually, they are vitally essential to the existence of life itself. Over the past few generations, science has opened the mystery of enzymes and has applied this knowledge to improve utilization of these amazing substances in an ever-growing number of applications. biotechnology is opening entryways that will further expand the utilization of enzymes into exciting new areas.

The effective catalytic properties of enzymes have already promoted their introduction into several industrial products and processes. Recent developments in biotechnology, particularly in areas such as protein engineering and directed evolution, have provided important tools for the efficient development of new enzymes. This has resulted in the development of enzymes with improved properties for established technical applications and in the production of new enzymes tailor-made for entirely new areas of application where enzymes have not previously been used (Kirk O et al., 2002).

Glucoamylases are used industrially to hydrolysis maltooligosaccharide chains of moderate length produced α-amylase hydrolysis of starch (Aehle W, 2004).

ANGA is one of the most important industrial enzymes. The in industrial conventional enzymatic reaction, a mixture of substrate and the native enzyme is incubated and after each batch of reaction, the product is recovered by denaturation of the enzyme which cannot be reused because active site is lost which is an uneconomical process. The technique of immobilization of enzyme by the various method has been developed to circumvent many difficulties associated with the use of soluble enzymes (Rani AS et al.,1999).

2.2.1. Glucoamylases

Glucoamylases (AG) (EC 3.2.1.3) are important enzymes used in the hydrolysis of starch and in many applications, on a large scale (Gupta K et al., 2015). Glucoamylases, an exo-amylase (Aiyer PV, 2005), attacks α-1,4-linkages at the non-reducing ends of starch and dextrins. When it reaches an alpha-1,6 link, it also cuts it, but at a lower rate than the α-1,4 link (Emneus J et al., 1993). During enzymatic hydrolysis, the substrates are mainly converted to D-glucose as the end product (Arıca MY et al., 1998).

2.3. Enzyme Immobilization

Enzymes have been used in the food industry since centuries as a result of the developments in biotechnology, today they have also found application areas in the pharmaceutical and chemical industries. Enzymes have extremely high catalytic activity relative to chemical catalysts and, under certain conditions, these activities are specific to a single substrate or group of substrates. For this reason, the possibility of undesired products is very low. However, the use of industrial enzymes in their natural forms has significant disadvantages. For example, the same enzyme sample is used once, and the cost of the product increases because it can be consumed with the product or reused, but can be separated from the product using expensive techniques. Another disadvantage is that significant losses occur in activity, depending on changes pH and temperature, usage conditions and storage conditions.

The first approach to overcome these disadvantages has been to add water-soluble stabilizers. The activities of the enzymes have been stabilized by adding metals, surfactants, polyols, polyethylene glycols, proteins, amino acids and some sugars. This stability contribution is the result of noncovalent interactions between the enzyme and the solvent (Drevon G, 2002). However, this method does not make any contribution to the excessive use of the same enzyme sample too often.

Another approach is enzyme immobilization. Immobilization is a water-insoluble matrix adsorption, covalent binding, or constraining the movement of the enzyme by trapping it in the matrix to maintain its enzyme activity for a long time. In enzyme immobilization, organic and inorganic structures use a large number of natural or artificial matrices. Eupergite CM and calcium alginate ($C_6H_7O_6Ca$) n are commonly used matrices. Immobilized enzymes exhibit different properties according to the structure of the matrix used and the immobilization method used, depending on the free enzymes. The optimum conditions of the immobilized enzyme, kinetic constants, and product composition may differ according to the free enzyme.

Benefits of using immobilized enzyme are shown below:

- ✓ The reaction can be separated from the medium by simple methods,
- ✓ It is more resistant to high temperature and pH,
- ✓ It makes it possible to apply continuous processes,
- ✓ I have used it many times,
- ✓ It may protect its activities for months on storage,
- ✓ Reduces product coast,
- ✓ The pure product enables the hand,
- ✓ It is possible to selectively synthesize some materials,
- ✓ Product inhibition can be avoided.

Immobilization studies began in the first half of the 1960's and more than 10,000 articles and patents were published on the subject as well as the day. The development of Immobilized Aspergillus oryzae aminophylase colonies in Japan by Tosa T et al. (1966) and the use synthetic racemic D, L-amino acids in the conversion of active enantiomers is the first industrial application of immobilized enzymes.

2.3.1. Enzyme immobilization methods

Immobilization methods are generally classified according to the type of coupling reaction. Accordingly, enzyme immobilization methods are mainly grouped into four main groups: Adsorption, entrapment (encapsulation in a polymeric gel), cross-linking covalent bonding with bifunctional reagents (Figure 2.1).

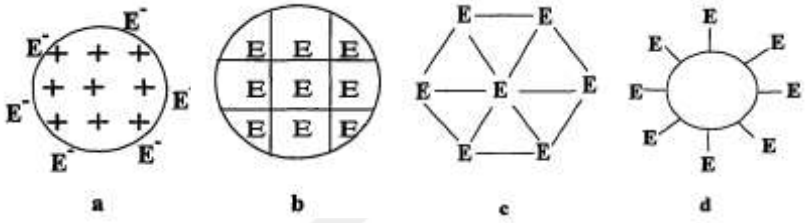

Figure 2.1. Methods of enzyme immobilization.
A.Adsorption b. Entrapment, c. Cross-linking, d. covalent bonding.

2.3.1.1. Adsorption:

The method of adsorption is to bind the enzyme physically or ionically in an inert matrix with the weakest gravitational forces (Woodward, 1985). Adsorption is a simple and economical method of immobilization. Immobilization is carried out by mixing the enzyme solution with the matrix at the appropriate pH and temperature. Weak bonds (Van der Waals and hydrogen bonds) are formed between the enzyme and the matrix, and the enzymes are easily separated from the matrix. Ion exchangers easily adsorb proteins and are widely used for enzyme immobilization. Table 2.1 shows the adsorbents used for adsorption.

Table 2.1. Adsorbents used for enzyme immobilization

INTERACTION	ADSORBENTS
Physical Adsorption	**Natural Matrices**
	Active Carbon
	Silica Gel
	alumina
	Pine
	Starch
	Modified Matrices
	Concanavaline
	Sepharose
	Tanner
	Amino hexyl cellulose
	Phenoxyacetyl cellulose
Ionic Bonding	**Cation Exchangers**
	CM-cellulose
	Dowex 50
	Amberlite CG-50
	Anion Modifiers
	DEAE-cellulose
	DEAE-sepadex
	Amberlite

2.3.1.2. Entrapment

During the formation of a polymer, entrapment of the enzyme in the forming polymer is called entrapment (O'Driscoll, 1976). One of the most commonly used polymers for this method is alginic acid.

2.3.1.3. Cross-Linking

Enzymes soluble in aqueous solutions become insoluble when covalently attached to each other using bifunctional cross-linking reagents. (Chui WK, Wan LS, 1997). Glutaraldehyde is the cross-linking reagent used for this purpose (Alexander M, 1983). The aldehyde groups in the bifunctional glutaraldehyde molecule react with the amino groups of the enzymes to cross-link the enzymes. Cross-linking can occur intermolecularly or intramolecularly and protein precipitates. The cross-linked protein can be easily separated by centrifugation.

$$E1\text{-}NH_2 + OCH\ (CH_2)_3CHO + E2\text{-}NH_2 \rightarrow E1\text{-}N{=}HC\ (CH_2)_3HC{=}N\text{-}E2$$

2.3.1.4. Covalent binding

Immobilization of proteins by methods based on the formation of covalent bonds is among the most frequently used. One advantage of these methods is that they are not released into solution upon the use of the enzyme due to the stable nature of the bonds formed between the enzyme and the matrix. However, to obtain highly bound activity, amino acid residues required for catalytic activity must not be included in the covalent linkage to the support; this can prove to be a difficult requirement to fulfill in some circumstances sometimes a simple procedure to improve activity is to perform a conjugation reaction in the presence of substrate analogs (Mattiasson B, 1991). Covalent methods for immobilization are used when there is a strict requirement for the absence of an enzyme in the urine.

A wide variety of reactions have been developed depending on the functional groups present in the matrix (Scouten W.H, 1987). Coupling methods can generally be divided into two main classes: (1) activating the matrix by adding a reactive function to a polymer, and (2) changing the polymer backbone to produce an activated group.

2.3.1.5. Studies of enzymes immobilization with activated carbon

Activated carbon is widely used in enzyme immobilization studies because it has a higher amount of enzyme binding capacity when it has a very high surface area. Adsorption and covalent binding methods were used in immobilization studies with active carbon. Adsorptive immobilized enzymes rapidly lose their activity in repeated use, with covalently bound enzymes can maintain their long-term activity. For this reason, this section mainly focuses on covalent bonding studies.

Bailey E and Cho (1981) immobilized glucoamylase and glucose oxidase enzymes (with no known organism) by adsorption with active carbon and covalent attachment methods on active carbon activated by glutaraldehyde and carbodiimide, and achieved the highest activity efficiencies with activated carbodiimide activated carbon And a 15% increase in activity yields with binding. At the end of sixty hours, only the carbodiimide significantly inhibits the initial activity of the immobilized with binding. At the end of sixty hours, only the carbodiimide significantly inhibits the initial activity of the immobilized glucoamylase. In addition, the carbodiimide-immobilized enzyme maintains 80% of its activity after thirty days in optimum storage conditions.

In another study, Ramani K et al. (2010) immobilized Pseudomonas gessardii acidic lipase on mesoporous activated carbon using adsorption method with 3570 U / g binding yield and used them in olive oil hydrolysis. The activity of the immobilized enzyme did not decrease during the repeated 5 cycles of use under optimum conditions, but the field to the end of the thirty-fifth cycle. The half-life of immobilized enzyme activity in storage conditions was determined to be approximately thirty-five days.

Ramani K and ark. (2012) have used hydrolysis of waste cooked oil by immobilizing 5440 U / g covalently bonded mesoporous activated carbon on Pseudomonas gessardii acidic lipase activated with glutaraldehyde. Immobilization resulted in an activity increase of 2.7% according to adsorption immobilization (Ramani K et al., 2010). The activity of the immobilized enzyme has been reduced to 50% in the fiftieth use, while the activity of the immobilized enzyme does not decrease during the repeated use of 20 during optimum conditions. The half-life of immobilized enzyme activity in storage conditions was determined to be fifty days.

In a different study, Jain A et al. (2016) covalently immobilized on the mesoporous activated carbon with a horseradish peroxidase supercritical fluid

immobilization method with 83.5% binding yield and 120% activity yield, demonstrating that the immobilized enzyme can be used 11 times without loss of activity.

In another study, recombinant Arthrobacter chlorophenolicus A6 Dioxygenase was immobilized on surface activated surfactant single-walled carbon nanotubes with 62% binding and 60-79% activity yields, and the immobilized enzyme activity gradually decreased to 40% at the end of seven treatments (Suma Y et al., 2016).

2.3.1.6. Immobilization of *ANGA*

There are many studies in the literature about immobilization of Aspergillus niger glucoamylase (*AN*GA) using various matrices.

ANGA has been firstly immobilized on matrices containing epoxy, aldehyde, and primer amino groups and the longest activity had been obtained with a matrix containing glutaraldehyde-activated amino groups (96.6 days) (Švec F et al., 1978).

Arıca MY et al. (1998) had immobilized *ANGA* on poly (2-hydroxyethyl methacrylate) hydrogels activated by epichlorohydrin with 71% activity yield.

Rani AS et al. (1999) had immobilized *ANGA*, on the chitin without using any cross-linker reagent with 98% activity yield.

Arıca MY et al. (2000) in another study immobilized *ANGA* on spacer-arm-attached magnetic polymethylmethacrylate microspheres activated with carbodiimide and cyanogen bromide and obtained the highest activity efficiency (73%) with a cyanogen bromide activated matrix.

In their second study, Rani AS et al. (2000) had immobilized *ANGA* on active carbon by physical adsorption with 90% activity yield.

Oh JT and Kim (2000) had immobilized *ANGA* by adsorption method, but the activity yield decreased from 89.7% to 31.4% by increasing the amount of bound enzyme.

In another study, *ANGA* was immobilized in calcium alginate beads by entrapment method with 92% activity yield (Roy I and Gupta, 2004).

Sanjay G and Sugunan S (2005) had immobilized *ANGA* on montmorillonite K-10 and 3-aminopropyl triethoxy silane with adsorption method than had immobilized

with covalent bonding methods. The activity yields of 100% in adsorption and 95% in covalent adsorption had been decreased to 95% in adsorbed enzyme after 100 hours, but there was no decrease in the covalently bonded immobilized enzyme.

ANGA had been immobilized by covalent attachment onto 1,2-diaminoethane-activated poly (Glycidylmethacrylate-co-ethylene glycol dimethacrylate) and used for four weeks without any reduction in activity (Milosavic N, Prodanovic R et al., 2007).

Wang F et al. (2007) had immobilized *ANGA* on metal particles of magnetic chelate by metal affinity adsorption with 84% activity yield and when the immobilized enzyme is used 30 times, it maintains 75.7% of its initial activity.

Tanrıseven A and Ölçer Z (2008) had covalently immobilized *ANGA* onto gelatin particles activated with polyglutaraldehyde in the presence of polyethylene glycol and soluble gelatin, with of 85% activity yield, and the immobilized enzyme maintains its activity for one month.

ANGA that its Asp and Glu residues were modified with ethylenediamine had been covalently immobilized on highly active glyoxyl-agarose supports with 80% activity yield. The activity of the free enzyme had been decreased to 50% after 40 hours at 55 ° C, while the activity of the immobilized enzyme decreased to 50% (Tardioli PW et al., 2011).

Milosavic et al. (2012) had covalently immobilized *ANGA* with poly (Glycidylmethacrylate-co-ethylene glycol dimethacrylate) in the presence of periodicity with of 64.25% immobilization yield.

Gupta K et al. (2013) had immobilized cross-linked *ANGA* aggregates on magnetic nanoparticles with covalent binding with 92.8% activity efficiency, and the immobilized enzyme preserved approximately the entire activity until the end of the ten use, but then gradually decreased to 70% at the end of fiftieth use.

In another study, *ANGA* was immobilized on carbodiimide and cyanogen bromide activated mesoporous silica with 57% and 73%, activity yields, respectively but the handling and storage stability was not determined (George R and Sugunan, 2014).

Gupta et al. (2015) had immobilized *ANGA* in the form of cross-linked aggregates with a yield of 65% activity and the immobilized enzyme 25 did not lose its activity during use.

In another study, *ANGA* was immobilized on Poly (methyl methacrylate-glycidyl methacrylate) cryogel by the covalent binding method and the initial activity decreased to 93% at the end of 20 use and to 68% at the end of thirty days storage (Uygun et al. 2015). However, immobilization studies to achieve higher activity yields using more economical matrices continue today.

The most important goals of an enzyme immobilization study are obtaining high activity yield and the obtained immobilized enzyme should show a high usage and storage stabilities. However, in the previous studies in the literature related to immobilization of *ANGA* activity yields are below than 99 % and the operational and storage stabilities are poor. Therefore, the studies related to the immobilization of *ANGA* to obtain higher activity using more economical matrices are continuing today.

Therefore, this study has aimed these goals: (1) The basic goal of producing activated carbon from sesame stem, to support for immobilized enzymes (glucoamylase) then it is used for the production of glucose syrup from maltodextrin (2) the obtaining the highest activity yield as possible as by optimizing the immobilization conditions of *ANGA* on the activated acrylic supports and the showing its usability for the production of glucose syrup from maltodextrin.

3. MATERIALS AND METHODS

3.1. Materials

Sesame stem was obtained from agricultural waste used in this study, Crushing or powdering the sesame stem by grinding machine (EFLAB) manufacture in Turkey then using 3000 μm size of sieve size machine, Microwave Digestion System (MILESTONE), horizontal furnace (MAGMA THERM HEAT UNDER CONTROL, MTTF 12/75/600-BZ), Carbon dioxide Argon, nitrogen supplied from a compressed gas cylinder, was used as the activation gas for physical activation. Zinc chloride ($ZnCl_2$) was used as the activating agent. iodine, potassium iodide, sodium thiosulfate, cellulose were purchased from Sigma-Aldrich (Taufkirchen, Germany). , sesame sticks molecular sizes were 300μm.*Aspergillus Niger* Glucoamylase which has 4074 IU/g activity, is a commercial powder enzyme preparation, was provided as a gift by Bio-Cat (Troy, USA). UV-VIS Spectrometer (UV-6300PC) was purchased from VWR (Radnor, USA). pH meter (Hanna HI 2020 edge), was purchased from Hanna Instruments Ltd. (Bedfordshire, UK). A magnetic stirrer (Heidolph MR Hei-Standard) was purchased from Heidolph UK-Radleys (Shire Hill, UK). Pure water appliance (Mini Pure 1, MDM-0170) was purchased from MDM Co. Ltd. (Suwon-si, South Korea). Precision scale (Shimadzu-ATX224) was purchased from Shimadzu Corporation (Kyoto, Japan). Orbital shaking heated incubator (Mipro-MCI) was purchased from Protek Lab Group, professional laboratory solutions company (Ankara, Turkey). A vacuum pump (Biobase, GM-0.50A) was purchased from Biobase Biodustry Co., Ltd. (Shandong, China). Bovine Serum Albumin (BSA), sodium hydroxide, sodium dihydrogen phosphate, hydrochloric acid, lactose, sodium sulphite, phenol, glutardialdehyde, ethylenediamine, acetone, and D-glucose were purchased from Sigma-Aldrich (Taufkirchen, Germany). 3, 5-dinitrosalicilic acid (DNS) was purchased from Alfa Aesar (Kandel, Germany). Sodium potassium tartrate (Roechelle salt) was purchased from VWR Prolabo Chemicals (Leuven Belgium). Sodium azide was purchased from Merck Millipore (Darmstadt, Germany).

3.2. Methods

3.2.1. Production of activated carbon from sesame stem

In the first stage, 3g of (sesame stem) with the same particle size will be thoroughly mixed with the same weight of zinc chloride ($ZnCl_2$) dissolved in 2mL of purified water. After then impregnated sesame put them into the microwave at 750-watt power in different gaseous media and 20 minutes at 80 ºC microwave temperature.Activation will be performed for 45 minutes at 500 ºC in a tubular oven in the presence of nitrogen gas. The activated carbon will be cooled to room temperature in the presence of nitrogen gas. then washed activated carbon with 100 mL of 0.5 M HCl (hydrochloride acid) and then washed with 3L of hot distilled water to pH value of 6-6.5 and dried at 80 ºC. The number of iodine will be determined in the laboratory environment of the obtained activated carbon. The amount of iodine adsorbed by the activated carbon is calculated by the equation assistance given below(ASTM, 4607-94(2006)).

$$\text{Iodine number} = ((B - A) * 127 * N * 40)/(m * B) \quad (3.1)$$

A: Amount of $Na_2S_2O_3.5H_2O$ (mL) spent in titration after adsorption of activated carbon-iodine

B: Amount of $Na_2S_2O_3.5H_2O$ (mL) spent in titration for o.1 N iodine solution

N: Iodine solution concentration (N)

M: Amount of activated carbon

In the production of activated carbon from the sesame stem, the effects of the parameters given below will be examined optimize the production condition of activated carbon.

1. Effect of different gases in the microwave (N_2, CO_2, Ar)
2. Microwave duration (5, 10, 20, 30) minutes
3. Microwave power (300, 500, 750, 1000) watt
4. Activation temperature (400, 500, 600) ºC
5. Activation time (30, 45, 60) minutes
6. Raw material / activator ratio (0.5, 1, 1.5, 2)

Characterization of BET surface area, SEM, FT-IR, TGA analysis of the obtained activated carbons with high iodine numbers will be performed. The activated carbon with the highest BET surface area will be used as a matrix in the immobilization of Aspergillus Niger glucoamylase.

3.2.2. Functionalization of activated carbon obtained

The amine solution (2.5%, v/v) was prepared by mixing 2.5 ml of ethylenediamine with 97.5 ml of acetone and 30 g of dried (no moisture) active carbon was added and stirred for 10 minutes to allow amino functionalization. To facilitate strong immobilization of the enzyme, the amino-functionalized activated carbon was activated with the aldehyde group by the addition of glutaraldehyde. The aldehyde solution was prepared by mixing 100 ml of acetone with 100 ml of glutaraldehyde (25%, v / v) and then the mixture was added to the amino-functionalized active carbon and stirred gently for 30 minutes using a magnetic stirrer. The above material was dried under vacuum until the solvent had completely evaporated and heated at 150 °C for 24 hours. The prepared material was washed with distilled water to remove unbonded chemicals. The eluate was dried at 110 ºC for 6 hours to obtain the final product, which was labeled as functionalized activated carbon (FAC) for further work after knocking at 600 μm.

3.2.3. Immobilization procedure

Immobilization of *ANGA* was performed by reacting 100 mg of supports with 200 μL (40.74 IU) of *ANGA* in 5 mL of sodium phosphate buffer (0.5 M, pH 5.5) at 25 ºC for 3 h in an incubator with gentle shaked at 150 rpm. After immobilization, the beads were filtered and washed with 15 mL of sodium phosphate buffer (0.1 M, pH 5.5) and 15 mL distilled water as three aliquots respectively, on a sintered glass filter by suction under vacuum. After then, immobilized enzymes have been stored in 5 mL of sodium phosphate buffer (0.1 M, pH 5.5) in a refrigerator at +4 °C until use.

3.2.4. Optimization of immobilization procedure

Optimum conditions for immobilization were determined by changing individually the conditions, (pH from 4.0 to 7.0; buffer concentration from 0. 5 to 2.0 M; the amount of Eupergit CM from 100 to 500 mg; and duration of immobilization from 3 to 24 h).

3.2.5. Protein assay

The amounts of proteins present in the immobilization buffer before and after immobilization were determined by using Bradford Protein Assay Method (Bradford MM, 1976). Accordingly, the 0.1 mL samples were added to 3 mL Bradford reagents in 10 mL vials and incubated 45 minutes at room temperature for the completing the formations of colour and after then the absorbances were measured at 595 nm by using UV spectrophotometer. The amount of immobilized enzyme protein was assayed from the difference between the amount of protein used for immobilization minus that recovered into the supernatant plus washings (Manjon A et al., 1995).

3.2.6. Determination of *ANGA* activity

Maltodextrin solutions (1 % w/v) prepared 5 mL 25 mM sodium phosphate buffer (pH 5.5) was reacted with 200 μL of free or 0.312 g immobilized *ANGA* containing 40.74 IU activity at 55 ◦C for 60 min in an incubator with gently shaking. 200 μL of aliquots from the reaction mixture was added to 1800 μL of distilled water and boiled for 10 min to inactivate the enzyme. The amounts of D-glucose in the

diluted samples were determined by measuring its absorbance using a UV spectrophotometer at 575 nm, according to a slightly modified method of Miller (Miller GL 1959).

One IU *ANGA* activity was defined as the amount of enzyme forming 1 µmol D-Glucose from maltodextrin per minute, under optimum activity assay conditions.

3.2.7. Calculation of immobilization and activity yields

The immobilization and activity yields were calculated by using following equations.

$$Immobilization\ Yield\ (\%) = \frac{Enzyme\ used\ -\ enzyme\ in\ \ filtrate}{Enzyme\ used}\ x100 \qquad (3.2)$$

$$Activity\ Yield\ (\%) = \frac{Activity\ of\ \ immobilized\ enzyme}{Activity\ of\ \ soluble\ enzyme}\ x100 \qquad (3.3)$$

3.2.8. Characterization of free and immobilized enzyme

3.2.8.1. Effect of pH on enzyme activity

The effect of pH on enzyme activity was investigated by performing the activity assay for the free and immobilized enzymes with 1 % (w/v) buffered maltodextrin solutions, at different pHs, at 55 ^{o}C.

3.2.8.2. Effect of temperature on enzyme activity

The effect of temperature on enzyme activity was found by conducting the activity assay with 1 % (w/v) buffered maltodextrin solutions (pH 5.5) at different temperatures.

3.2.8.3. pH stability

200 µL of free or 0.312 g immobilized *ANGA* containing 40.74 IU activity were incubated in sodium dihydrogen phosphate buffer solutions at various pH ranges (3.0–8.0) at room temperature for 1 h and the retained activities were determined under standard assay conditions.

3.2.8.4. Thermal stability

200 μL of free or 0.312 g immobilized *ANGA* containing 40.74 IU activity were incubated in sodium dihydrogen phosphate buffer solutions (25 mM, pH 5.5) at temperatures from 30 to 80 ◦C for 1 h and then the retained activities were determined using the standard assay method.

3.2.8.5. Michaelis-Menten constants

Initial velocities for kinetic parameters were determined by performing the reactions between 200 μL of free or 0.312 g immobilized *ANGA* containing 40.74 IU activity and maltodextrin solutions at different concentrations (5 to 80 g/L) for 5 min. K_m and V_{max} values were calculated from Lineweaver–Burk plot.

3.2.8.6. Operational and storage stabilities of the immobilized *ANGA*

Operational and storage stabilities of the immobilized enzyme were determined by measuring the retained activities using standard activity assay method, after each use in repeated 20 batch experiments, and two days intervals for thirty days, respectively.

3.2.8.7. Production of glucose syrup from maltodextrin by using immobilized *ANGA*

0.312 g immobilized *ANGA* containing 40.74 IU activity was reacted with the buffered 5 % (w/v) maltodextrin solution (pH 5.5) at 55 °C for 6 h and D-glucose content was determined with 60 minutes intervals by using UV spectrophotometer.

3.2.8.8. Statistical Analysis

Each value in the tables and figures represent the mean for three independent experiments performed in triplicates. Data were analyzed by using Microsoft Windows Excell.

4. RESULTS AND DISCUSSIONS

4.1. Activated carbon assay

4.1.1. Production of activated carbon from sesame stem

A number of experiments were carried out to study the effect of the parameter on (Iodine number). The main parameter that affects the quality of activated carbon was impregnation ratio, gas of microwave effects, microwave power, microwave time activation time and activation temperature.

4.1.2. Effect of different gases in the microwave

Modification of sesame stem activated carbon was carried out in a microwave oven under different gases atmosphere are given in Table 4.1.

Table 4.1. Effect of microwave different gases on activated carbon

Chemical raw material ratio	using Gas	Micro-wave power (watt)	Micro-wave time (min)	Micro-wave temp (C°)	activation temp (C°)	activation time (min)	iodine number
1:1	dioxide (CO_2)	750	20	80	500	45	1199,0254
1:1	Argon (Ar)	750	20	80	500	45	1000,4145
1:1	Nitrogen (N_2)	750	20	80	500	45	1024,4146
1:1	Without microwave				500	45	852,8921

As shown in Table 4.1, it is clear that the number of iodine in activated carbon obtained in the presence of carbon dioxide is higher. This may be due to the fact that the microwave in the environment of carbon dioxide, more effective at the molecular level to decompose the structure of sesame stalk which is an agricultural waste. In the next part of the study, microwave activity was investigated in the CO_2 environment.

4.1.3. Microwave duration

Experimental studies showing the best microwave duration in Fig. 4.1 are given in the number of iodine from the activated carbonate microwaves obtained 750 W power, 500 °C temperature, and 45 activation times at CO_2 atmospheres.

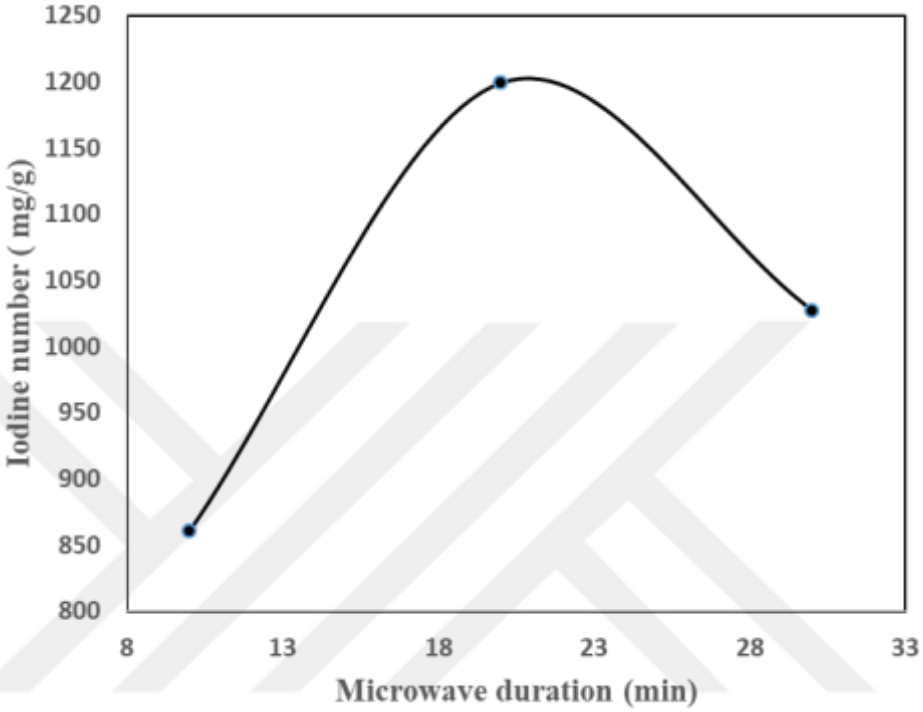

Figure 4.1. Effect of activation time on iodine value of activated carbon (Microwave duration)

Fig. 4.1 shows the microwave time, increase the iodine number at a time from 10 to 20 minutes and decrease at 30 minutes. This is thought to be due to the fact, that the pores of the active carbon obtained are not fully formed because the activator is not sufficient for the impregnation of raw material when the microwave time is at 10 min. When the microwave time is 30 minutes, it is estimated that the pore structure of the activator is deteriorated in the microwave environment. It is also understood from the iodine number that the 20 min activation of the microwave duration is sufficient time for the impregnation of the raw material. In the rest of the work, the microwave duration time was worked for 20 minutes.

4.1.4. Microwave power

After the best microwave time has been determined, the effect of the microwave power on the sesame stalk, the sample after the application of CO_2 and different microwave power, has been subjected to activation for 45 minutes in nitrogen atmosphere at 500 °C and the change of the iodine numbers of the obtained samples with microwave power is given in Fig. 4.2.

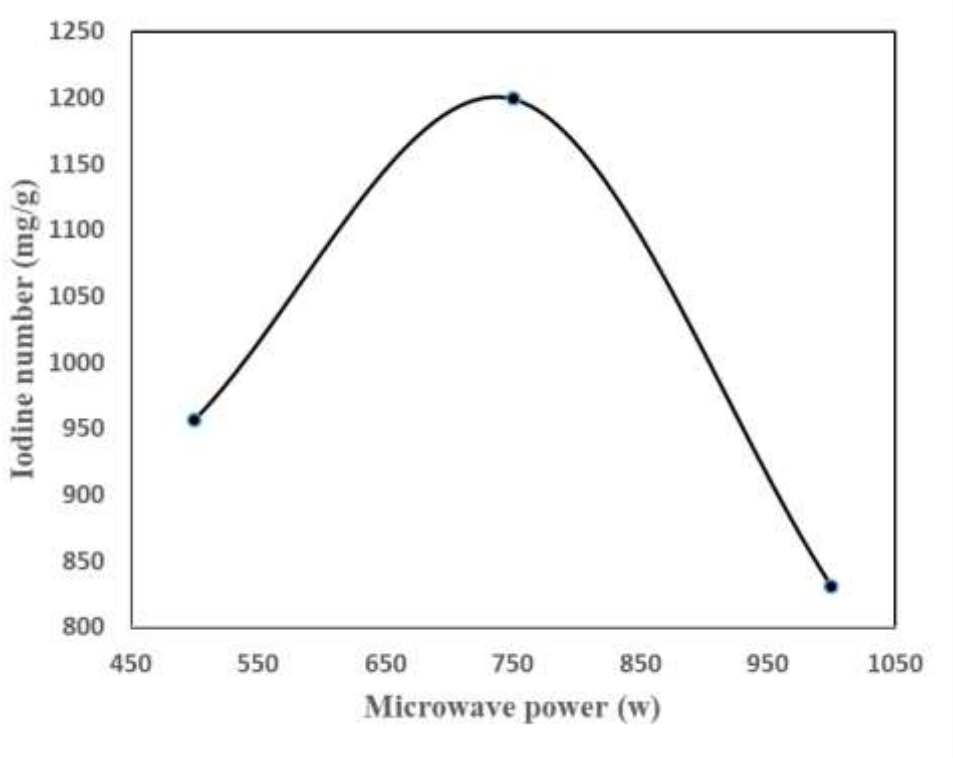

Figure 4.2. The graph of changing of microwave power with iodine number

As seen in Fig. 4.2. The iodine numbers increase with increasing microwave power from 500 W to 750 W and decreases by increasing microwave power to 1000 W. The probable cause is that when the microwave power is 500 W, the activating chemical is not sufficient to penetrate the inner parts of the sesame stem and when the microwave power is 1000 W activating chemical, the activator over crows and the active carbon pores that can be obtained may cause macro structure. The best microwave power was determined to be 750 W, and the rest of the experiments were performed in the presence of 750 W microwave power

4.1.5. Activation temperature

The changing of iodine numbers of activated carbons obtained from the activation time of the 45 minutes in a nitrogen atmosphere at different temperatures of sesame stems pretreated with 750 W microwave power and 20 min microwave time and the CO_2 gas environment is given in Fig .4.3.

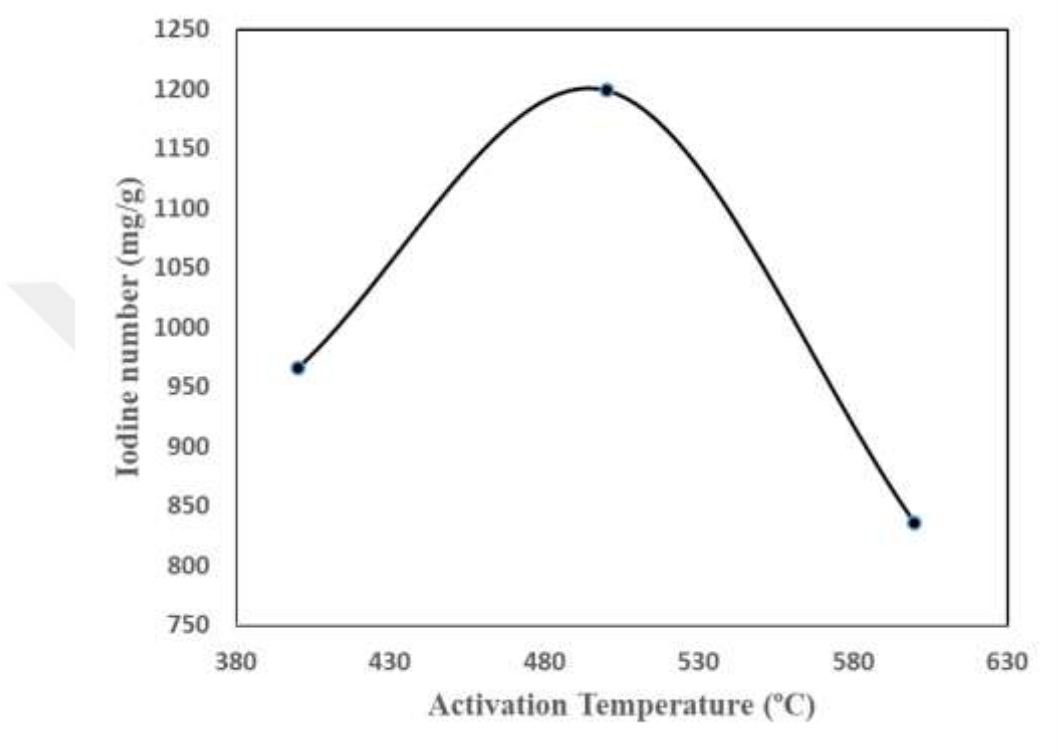

Figure 4.3: Effect of activation temperature on iodine value of the activated carbon

Fig.4.3. shows that when the activation temperature increases from 400 °C to 500 °C, the number of iodine increases and when the activation temperature reaches 600 °C, the number of iodine decreases. The probable cause of this is that when the activation temperature is 400 °C, the pores of the active carbon obtained are not fully formed and when the temperature is 600 °C, the pores of the activated carbon structure are collapsed. It can be said that the 500 °C activation temperature is ideal for the pores of the active carbon obtained. In the following sections of the study, the activation temperature was set at 500 °C.

4.1.6. Activation time

The variation of the iodine numbers obtained for the activation time of 750 W microwave power, 20 min microwave time, sesame stems pretreated with CO_2 gas at 500 °C activation temperature with different activation times is given in Figure 4.4.

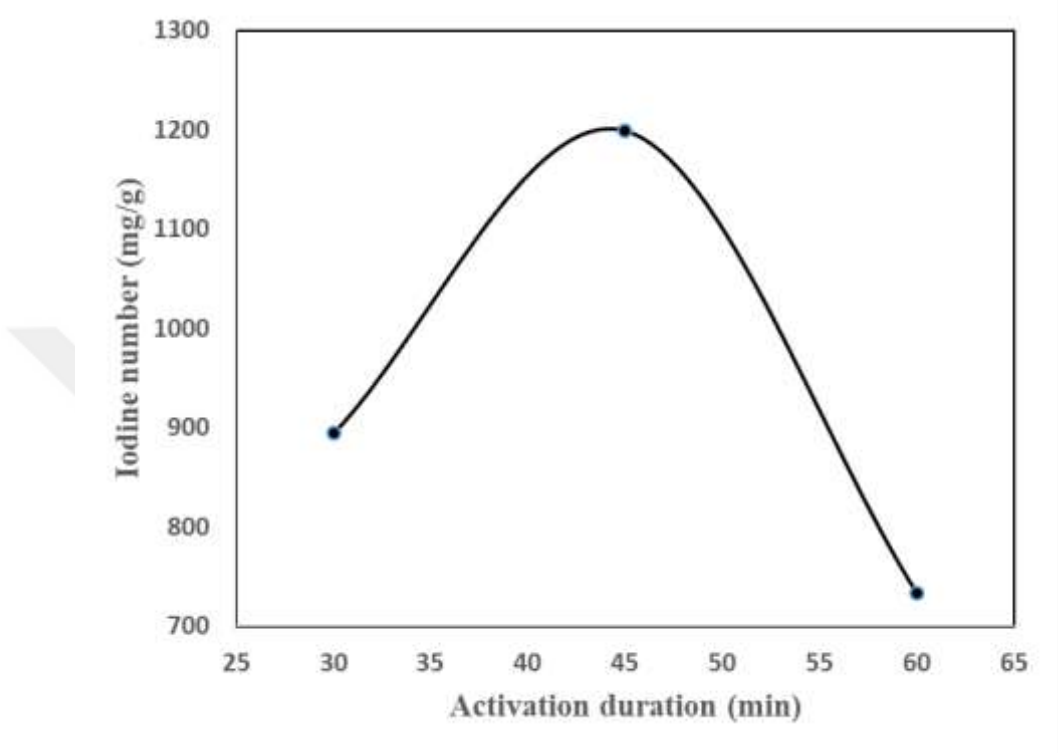

Figure.4.4. Effect of activation time on iodine value of activated carbon

From Figure 4.4. It is seen that when the activation temperature increases from 30 min to 45 min, the number of iodine increases and decreases when it is increased to 60 min. It is likely that this would be sufficient to extend the activation period to 45 minutes, during which the 30-minute activation period was insufficient for the development of new pores and existing pores. When the activation period is 60 minutes, it can be said that the micro pores have turned into macro pores and some pores have collapsed.

4.1.7. Raw material / activator ratio

In this part of the study show in figure 4.5, involved different activator of $ZnCl_2$ (3, 1.5, 4.5 g) mixed with 3g of sesame stem and 2 mL of distilled water put in microwave at (750 watt, 80 °C for 20 min) under CO_2 the maximum Iodine number shown at in 3g of $ZnCl_2$.

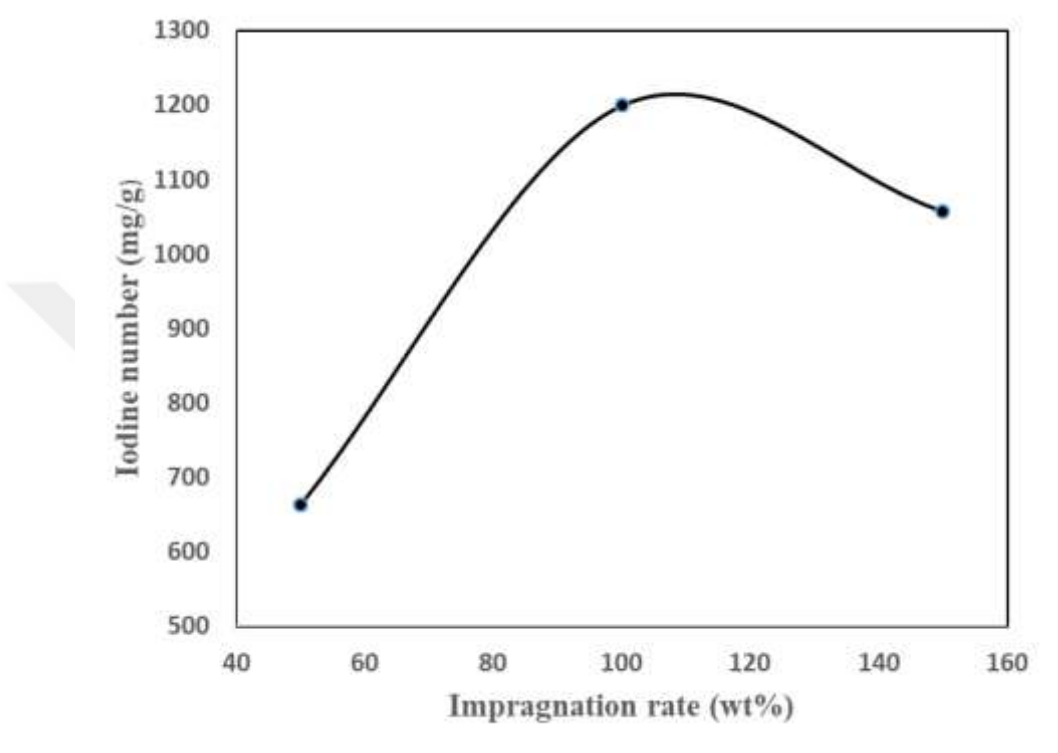

Figure 4.5. Effect of activation concentration Zinc chloride percent and iodine value of activated carbon

As seen in Figure 4.5, when the ratio of raw material/chemical substance is increased from 50% to 100%, the number of iodine increases and when it is increased to 150%, the number of iodine decreases. It can be said that micropores are formed in the structure of active carbon obtained from 50% to 100% of the possible activator amount, and when it is increased to 150%, micropores are converted into mesopores. In the following parts of the study, the impregnation is taken as 100%.

4.1.8. Scanning electron microscopy

Scanning electron microscopy was used to watch the morphology of crude sesame stem carbonized in figure 4.6, SEM micrographs of raw sesame stem can be seen. The surface of the sesame stem was heterogeneous, harsh and nonporous before any physical or chemical treatment. The micrographs of raw material can be useful to observe the structural changes happened after impregnation, carbonization and activation processes.

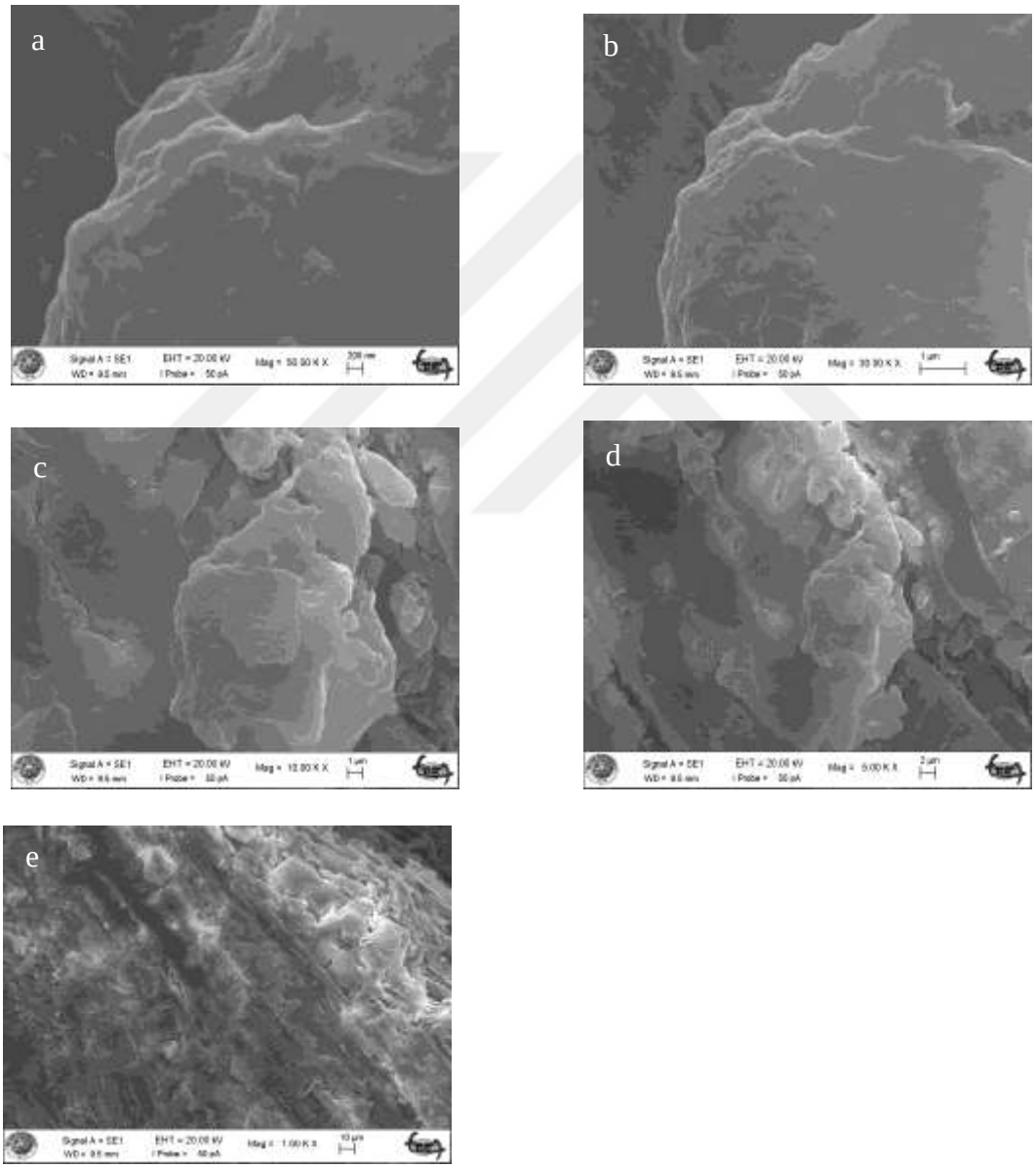

Figure 4.6.a-e SEM Micrographs of Raw sesame stem (50.00 KX, 30.00 KX, 10.00 KX, 5.00 KX, 1.00 KX) (200 nm, 1µm, 1µm, 2 µm, 10 µm)

After carbonization sesame stem at 500 °C without using the microwave, formation or waves and pits occurred on the surface of the material. As it is clearly seen from Figure 4.7, porosity formation was initiated and undulation surfaces appeared because of the removal of the tarry substance from the lignocellulose structure.

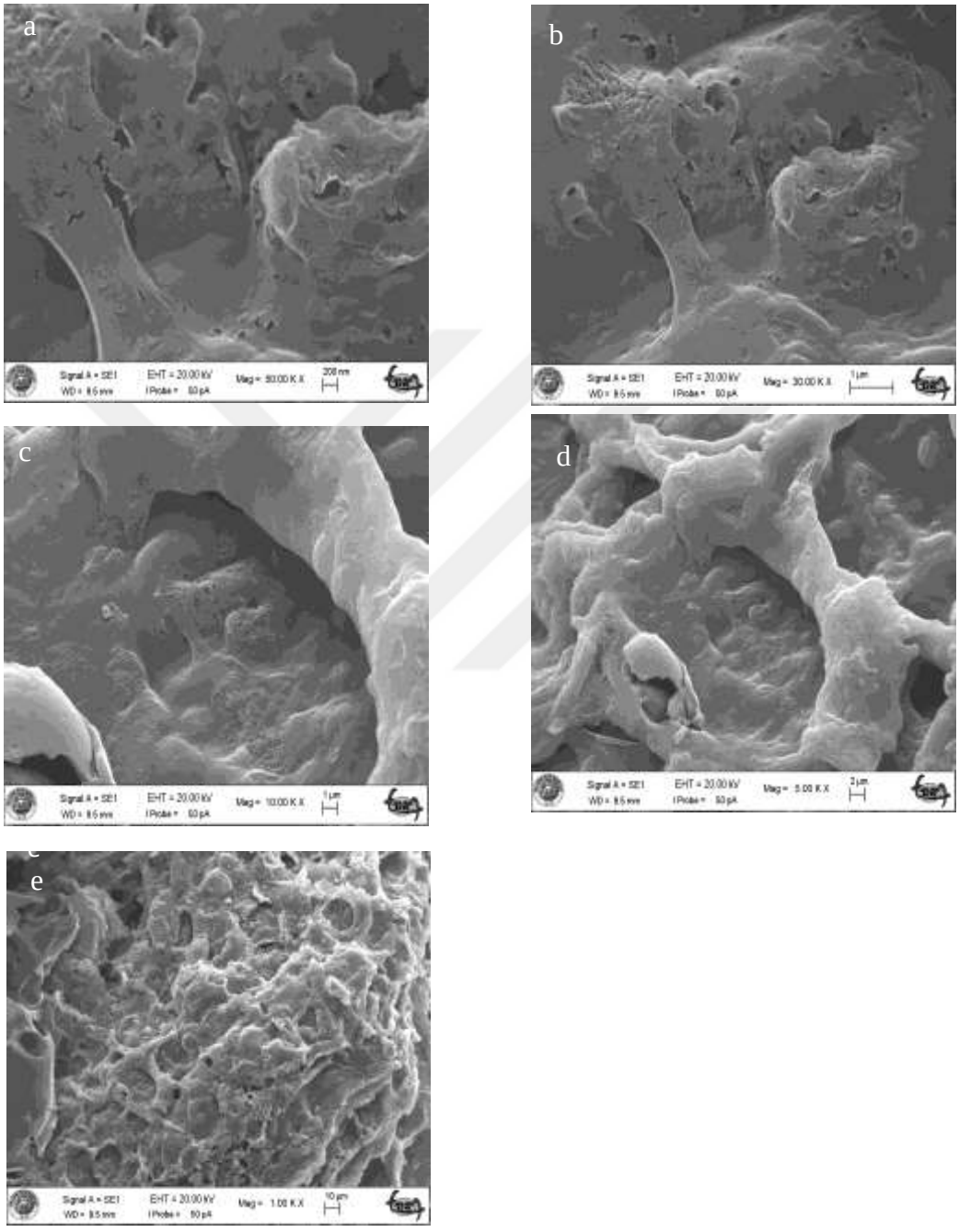

Figure 4.7.a-e SEM Micrographs of zinc chloride impregnated and carbonized raw sesame stem at 500 °C without a microwave. (50.00 KX, 30.00 KX, 10.00 KX, 5.00 KX, 1.00 KX) (200 nm, 1µm, 1µm, 2 µm, 10 µm)

SEM images are shown in Figure. 4.8. a-e. it can be seen from the micrographs that the external surface of the activated carbon has a crack, crevices, and some grains in various in large holes.it can be understood from SEM image thorough at the zinc chloride impregnated and carbonization temperature of 500 ºC after treatment into microwave using CO_2 gas that porous structure was formed because of most of the organic volatiles were release, leaving behind the ruptured surface of activated carbon with the most number of pores.

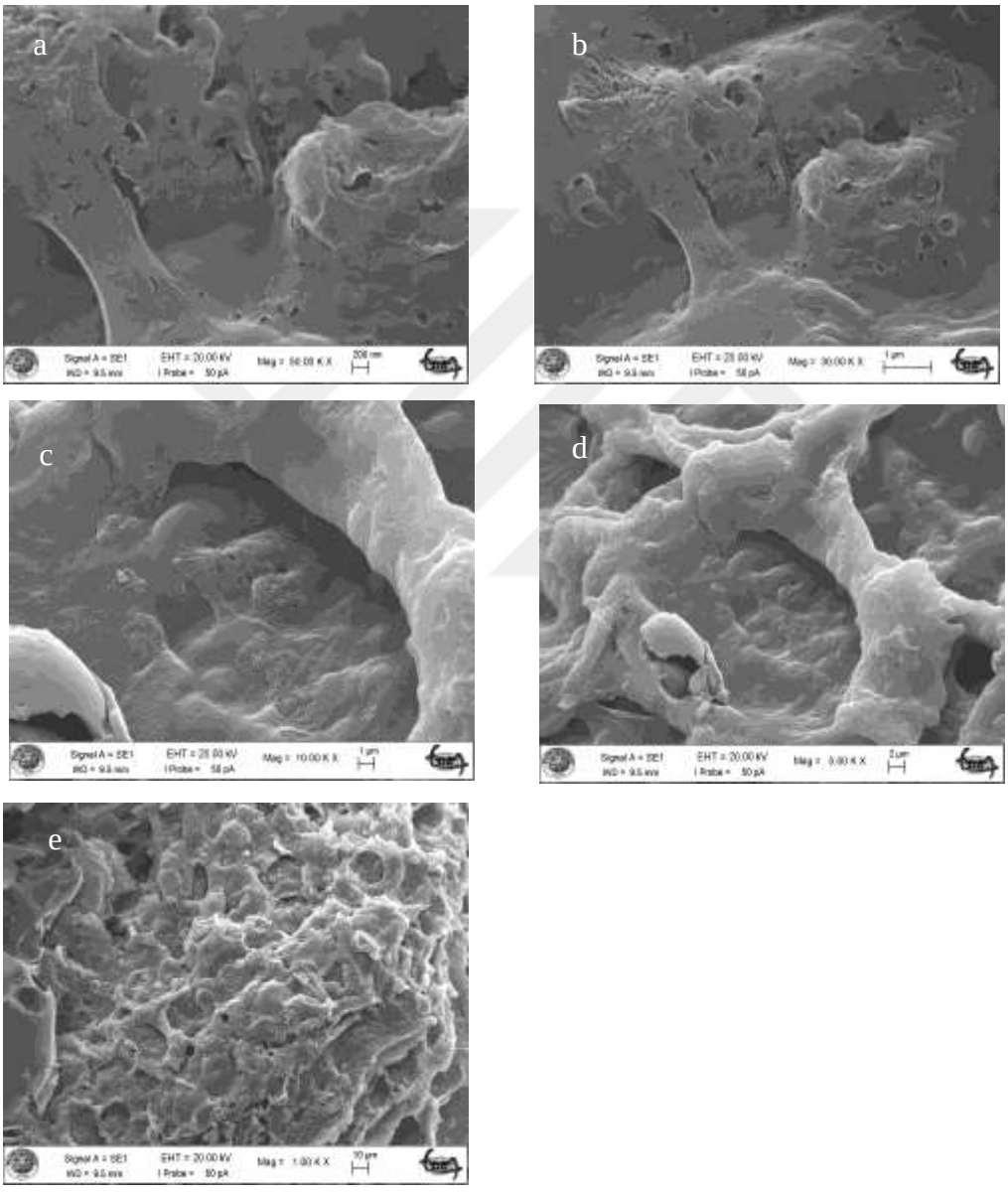

Figure 4.8.a-e SEM Micrographs of zinc chloride impregnated using the microwave then carbonized raw sesame stem at 500 °C. (50.00 KX, 30.00 KX, 10.00 KX, 5.00 KX, 1.00 KX) (200 nm, 1µm, 1µm, 2 µm, 10 µm)

4.1.9. Brunauer–Emmelt–Teller

Some of the higher iodine numbers from the synthesized activated carbons were sent to BET surface area analysis and the results obtained are given in the table below

Table 4.2. BET surface areas of synthesized activated carbons

using Gas	**Microwave power (watt)**	**microwave time (min)**	**microwave tempreature (C°)**	**activation temparature (C°)**	**activation time (min)**	**iodine number mg/g**	**BET Surface area(m^2/g)**
CO_2	750	20	80	500	45	1199	1254
non	non	non	non	500	45	852	408
CO_2	500	20	80	500	45	957	1078
CO_2	1000	20	80	500	45	831	628

As can be seen from the table.4.2. It is understood that the surface area of the active carbon obtained without microwave irradiation and the number of iodine are much lower than the activated carbon subjected to microwave irradiation. The probable cause is that as the activator enters the structure of the raw material, it enters into the inner zone in the microwave environment and consequently the activated carbon structure is considered to be more porous and causes a high BET surface area.

The change of the microwave power of the active carbon obtained by the microwave with the BET surface area is given in Figure 4.9.

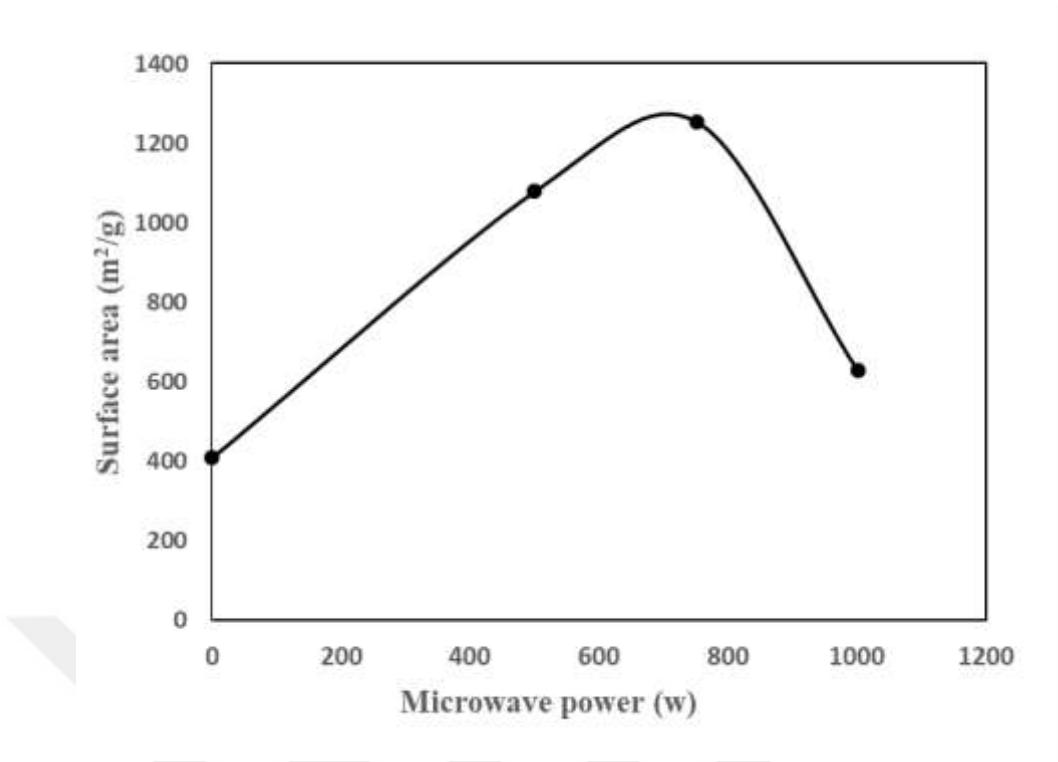

Figure.4.9. Effect of activation on BET surface area of activated carbon

As shown in Figure.4.9. , the microwave power increases from 0 to 750 watts, and the BET surface area increases and then decreases. The probable cause is that the increased microwave power opens up new pores in the matter structure and at 750 watts higher microwave power the micropores turn into macropores and the pore structure collapses.

4.1.10. FT-IR measurements

The FT-IR spectra obtained by scanning the sesame stem with the active carbon obtained by microwave treatment and the obtained active carbon obtained without microwave irradiation in the range of 4000-650 cm^{-1} wave are given in Figure 4.10.

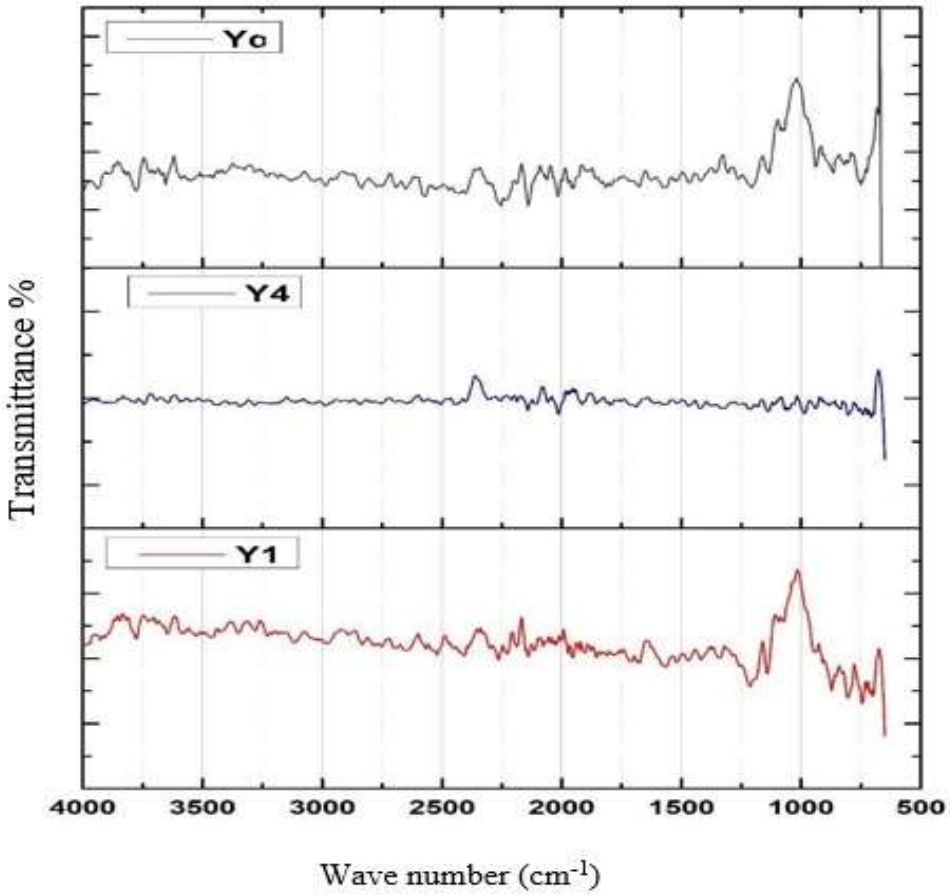

Figure 4.10. The FT-IR spectra of produced activated carbon for the sesame stalk sawdust (Y0), activated carbon with microwave (Y1) activated carbon without a microwave (Y4).

As seen in Figure 4.10.Y0. There is more than one functional group in the structure of sesame stems. Large peaks at maximum 3700 and 3600 cm^{-1} in the wave number range of 3700-3200 cm^{-1} indicate the presence of hydroxyl (-OH) groups bound by hydrogen bonds. The peak in the wave number of 2919 cm^{-1} shows the presence of C-H groups and the peak in the range of 2341-2151 cm^{-1} wave number shows the presence of C = C groups at the sesame stalk structure. The peak at the wave number of 2339 cm^{-1} shows the -COOH groups; the peak at a wave number of 1044 cm^{-1} shows C-OH groups. In addition, peak at 1627 cm^{-1} wave indicates that C-C peak originates from olefinic groups in sesame stalk structure.

It can be seen from Figure 4.10.Y4. There are no peaks which were found in the structure of raw sesame stalks in the active carbon obtained without a microwave.

As can be seen from Figure 4.10.Y1. Many functional groups found in the structure of sesame stalks are the same in the structure of active carbon obtained by subjecting to microwave.

4.2. Protein Assay

The protein (enzyme) concentrations in the immobilization solutions and in the filtrates were calculated Using Equation (4) obtained from BSA standard plot.

$$Y = 0.29958X \tag{4}$$

Accordingly, the enzyme concentration in a 5,2 mL immobilization solution containing 200 μL of free *ANGA* was calculated to be 6.842 mg / mL. The enzyme concentration in the powdered *ANGA* preparation was also calculated as 684.2 mg/g. This result is consistent with the declaration (GA content is between 62 – 82%) of the manufacturer company (Murphy, 2017).

4.3. Determination of *ANGA* activity

The amount of D-glucose formed at the end of the reaction of 200 μL of free *ANGA* at optimum activity assay conditions, was found to be 15655.0 μg by using Equation 4.1. Free *ANGA* activity was calculated as 40.74 IU / mg by using Equation 4.2. Furthermore, since 1 g of powder *ANGA* preparation contains 684.2 mg of free *ANGA*, the activity of powdered *ANGA* was also calculated to be 4074 IU/g for maltodextrin as substrate. The amount of free *ANGA* having 1 IU activity was also calculated as 0.168 mg.

$$Y = 0.00104X \tag{4.1}$$

$$IU / mg\ Enzyme = \frac{Released\ D\text{-}Glucose\ (\mu mol)}{Enzyme\ used\ (mg)\ x\ Duration\ of\ reaction\ (min)} \tag{4.2}$$

4.4. Optimization of immobilization procedure

After the optimization of the immobilization conditions, 100.00 % immobilization yield and 100.00 % activity yields were achieved.

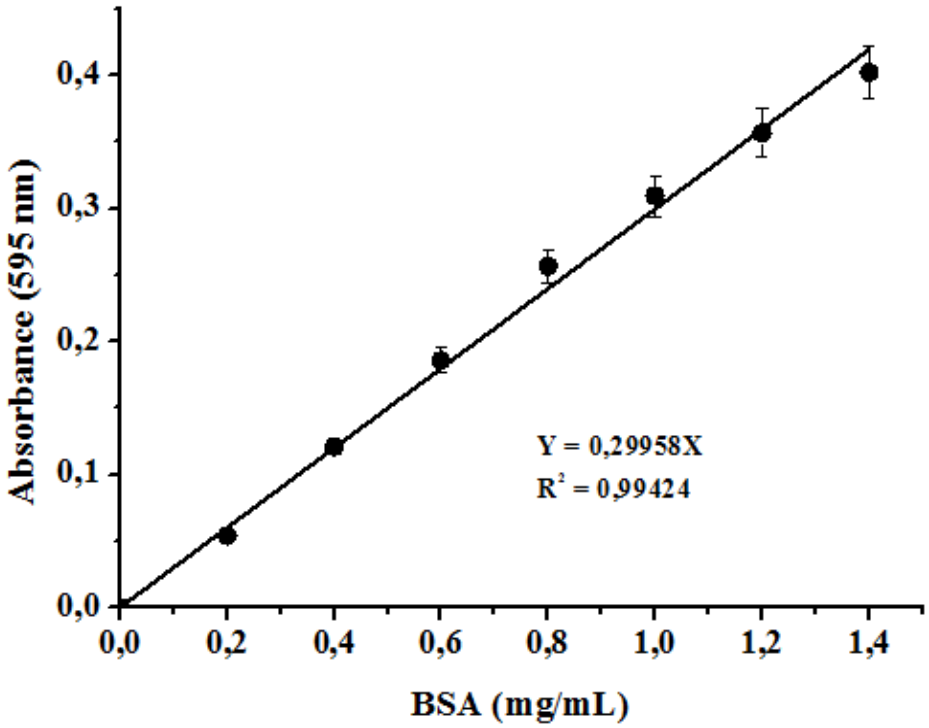

Figure 4.11. BSA standard graph. 100 µL standard BSA solutions at 0.2-1.4 mg/mL concentrations were mixed with 3 mL Bradford reagent solutions and incubated at room temperature for 45 minutes and the absorbance was measured at 595 nm by using UV spectrometer.

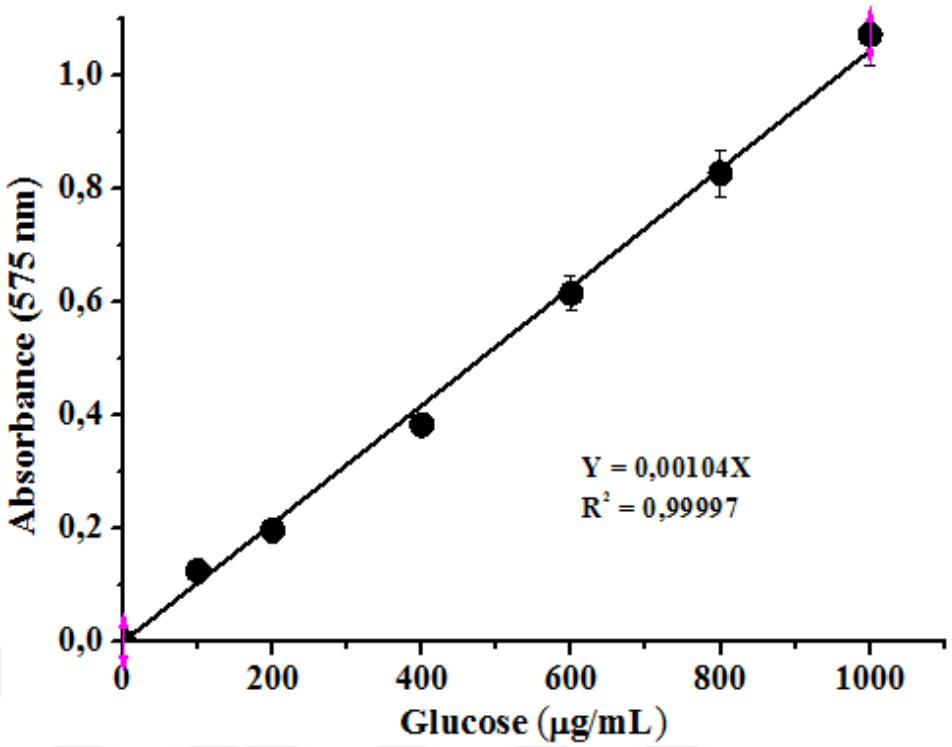

Figure 4.12. D-Glucose standard graph. 0.75 mL standard D-Glucose solutions at 100-1000 µg/mL concentrations were mixed with 0.75 ml DNS Reagent and incubated in an incubator at 90 °C for 15 minutes and then 0.25 mL sodium potassium tartrate solutions added and immediately cooled to room temperature and finally the absorbance were measured at 575 nm by using UV spectrometer.

4.4.1. Effect of immobilization buffer pH on immobilization efficiency

Table 4.3 shows the influence of pH on immobilization. 83.46 % immobilization yield and 76.56 % activity yield was obtained at optimum pH (5.5) of the enzyme. It is well known that highest activity yield is generally obtained at optimum pH of the enzyme.

Table 4.3. Effect of immobilization buffer pH on immobilization efficiency*

Immobilization Buffer pH	Immobilization Yield (%)**	Activity Yield (%)***
4,0	74.58 ± 0,04	70.17 ± 0,02
5,0	79.87 ± 0,02	72.44 ± 0,03
5,5	83.46 ± 0,03	76.56 ± 0,05
6,0	84.30 ± 0,05	69.28 ± 0,04
7,0	87.17 ± 0,02	66.15 ± 0,02

* Each value represents the mean for three independent experiments performed in triplicates. Data were analyzed by using Microsoft Windows Excell.
****** 200 µL *ANGA*s containing 40.74 IU activity were reacted with 100 mg of FAC in 5mL of sodium phosphate buffers (0.5 M) at different pHs and room temperature with shaking at 150 rpm in an incubator for 3 hours.
******* Free (200 µL) and 0.140 g immobilized *enzymes* containing 40.74 IU glucoamylases activity were reacted with 5 mL of 1 % (w/v) maltodextrin solutions (pH 5.5) at 150 rpm and at 55 ºC in an incubator for 60 minutes.

4.4.2. Effect of immobilization buffer molarity on immobilization efficiency

Table 4.4 shows that 83.46 % immobilization yield and 76.56 % activity yield were obtained with phosphate buffers of 0.5 M. According to Table 4.4, when the molarity of immobilization buffer increased, both of immobilization and activity yields have been decreased. Binding and activity yields in covalent enzyme

Table 4.4. Effect of immobilization buffer concentration on immobilization efficiency*

Buffer Concentration (M)	Immobilization Yield (%)**	Activity Yield (%)***
0.5	83.46 ± 0,03	76.56 ± 0,02
1.0	79.57 ± 0,03	73.91 ± 0,03
1.5	74.22 ± 0,04	72.50 ± 0,05
2.0	58.90 ± 0,05	71.13 ± 0,02

***** Each value represents the mean for three independent experiments performed in triplicates. Data were analyzed by using Microsoft Windows Excell.
** 200 uL *ANGA*s containing 40.74 IU activity were reacted with 100 mg of FAC in 5 mL of different concentrated sodium phosphate buffers (pH 5.5) at room temperature by shaking in an incubator at 150 rpm for 3 hours.
******* Free (200 µL) and 0.140 g immobilized *enzymes* containing 40.74 IU glucoamylases activity were reacted with 5 mL of 1 % (w/v) maltodextrin solutions (pH 5.5) at 150 rpm and at 55 ºC in an incubator for 60 minutes.

immobilization on synthetic carriers such as epoxy carriers was often affected by the properties of the salts and their concentration (Smalla K et al., 1988). It is well known that the higher salt concentrations can change the three-dimensional structures of enzyme molecules.

4.4.3. Effect of FAC amount on immobilization efficiency

According to Table 4.5, it can be seen that 83.46 % immobilization yield and 76.56 % activity yield were achieved for 100 mg of FAC. Furthermore, according to Table 4.5, the activity yield has decreased by increasing amounts of FAC while immobilization yield has increased. This is possibly the result of the deterioration of the three-dimensional structure of enzyme molecules due to multipoint attachment of enzyme molecules to the support via glutardialdehyde available on the support.

Table 4.5. Effect of FAC amounts on immobilization efficiency*

FAC Amounts (mg)	Immobilization Yield (%)**	Activity Yield (%)***
100	83.46 ± 0,03	76.56 ± 0,02
200	87.62 ± 0,04	71.93 ± 0,05
300	92.31 ± 0,05	67.68 ± 0,02
400	97.21 ± 0,02	64.01 ± 0,03
500	99.42 ± 0,02	63.21 ± 0,03

* Each value represents the mean for three independent experiments performed in triplicates. Data were analyzed by using Microsoft Windows Excell.
** 200 µL *ANGA*s containing 40.74 IU activity were reacted with different amounts of FAC in 5mL of sodium phosphate buffers (0.5 M, pH 5.5) and room temperature with shaking at150 rpm in an incubator for 3 hours.
*** Free (200 µL) and 0.140 g immobilized *enzymes* containing 40.74 IU glucoamylases activity were reacted with 5 mL of 1 % (w/v) maltodextrin solutions (pH 5.5) at 150 rpm and at 55 ºC in an incubator for 60 minutes.

4.4.4. Effect of immobilization time on immobilization efficiency

The effects of the immobilization time on the immobilization efficiency are shown in Table 4.6. The maximum immobilization and activity yields (100.00 %) were achieved for 12 hours and then is gradually decreased to 90.87 after 24 hours. This probably is due to unfavorable changing the three-dimensional structure of enzyme molecules due to multipoint attachment of the enzyme to the support via excess glutardialdehyde molecules available on the support.

Table 4.6. Effect of immobilization time on immobilization efficiency*

Immobilization Time (hours)	Immobilization Yield (%)**	Activity Yield (%)***
3	83.46 ± 0,03	76.56 ± 0,02
6	91.63 ± 0,03	89.94 ± 0,02
9	97.38 ± 0,04	94.35 ± 0,03
12	100.00 ± 0,05	100.00 ± 0,05
15	100.00 ± 0,02	97.32 ± 0,03
18	100.00 ± 0,03	96.97 ± 0,04
21	100.00 ± 0,05	94.35 ± 0,02
24	100.00 ± 0,02	90.87 ± 0,02

* Each value represents the mean for three independent experiments performed in triplicates. Data were analyzed by using Microsoft Windows Excell.
** 200 uL *ANGA*s were reacted with 100 mg of FAC in 5 mL of sodium phosphate buffers (0.5 M, pH 5.5) at room temperature with shaking in an incubator at 150 rpm for different durations.
******* Free (200 µL) and 0.140 g immobilized *enzymes* containing 40.74 IU glucoamylases activity were reacted with 5 mL of 1 % (w/v) maltodextrin solutions (pH 5.5) at 150 rpm and at 55 ºC in an incubator for 60 minutes.

4.5. Characterization of immobilized enzyme

4.5.1. Optimum pH

According to Figure 4.13, the optimum pH (5.5) of *ANGA* was not affected by immobilization. On the other hand, as seen in the Figure, the immobilized *ANGA* is more active than a free counterpart at all pH range tested. There are some similar results in the related literature (Arıca et al., 1998; Wang et al., 2007; Milosavic et al., 2005; Tanrıseven and Ölçer, 2008; Milosavic et al., 2012; Uygun et al., 2015).

4.5.2. Optimum temperature

Figure 4.14 shows that the optimum temperature range (55-60 °C) was not affected by immobilization and the immobilized enzyme shows higher activity than free enzyme at the higher temperatures. This result agrees with two of previous studies (Oh JT and Kim, 2000; Tanrıseven and Ölçer, 2008).

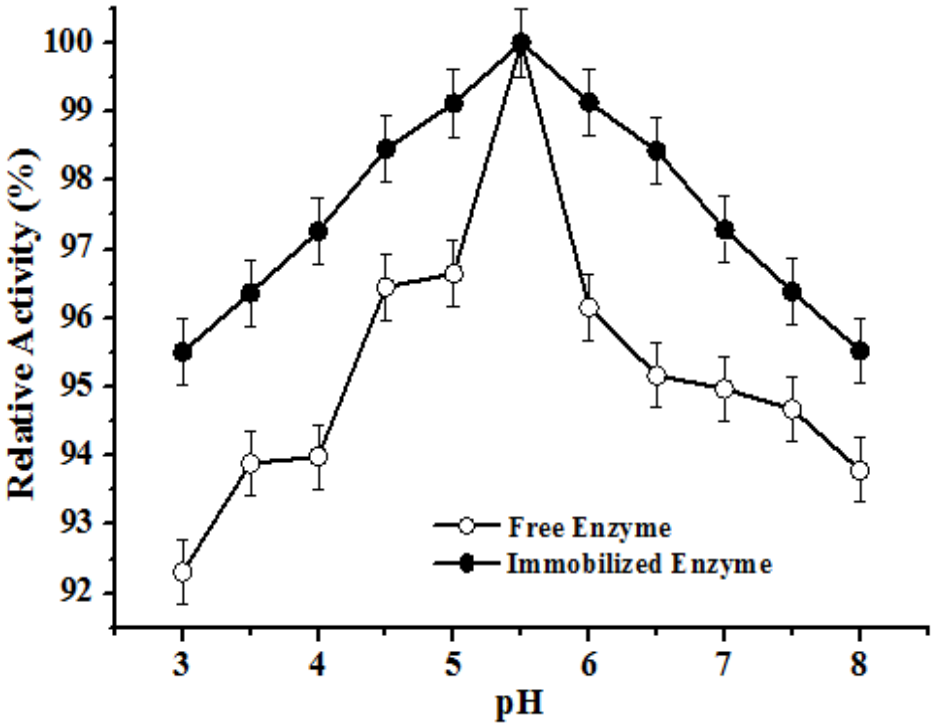

Figure 4.13. Optimum pH of free and immobilized *ANGA.* The effect of pH on enzyme activity was investigated by performing the activity assay for 200 µL free or 0.312 g immobilized *ANGA*s containing 4.07 IU activity with 5 mL of 1 % (w/v) maltodextrin solutions, at different pHs and at 55 ºC. 100% relative activity refers to 4.07 IU activity used for activity assay.

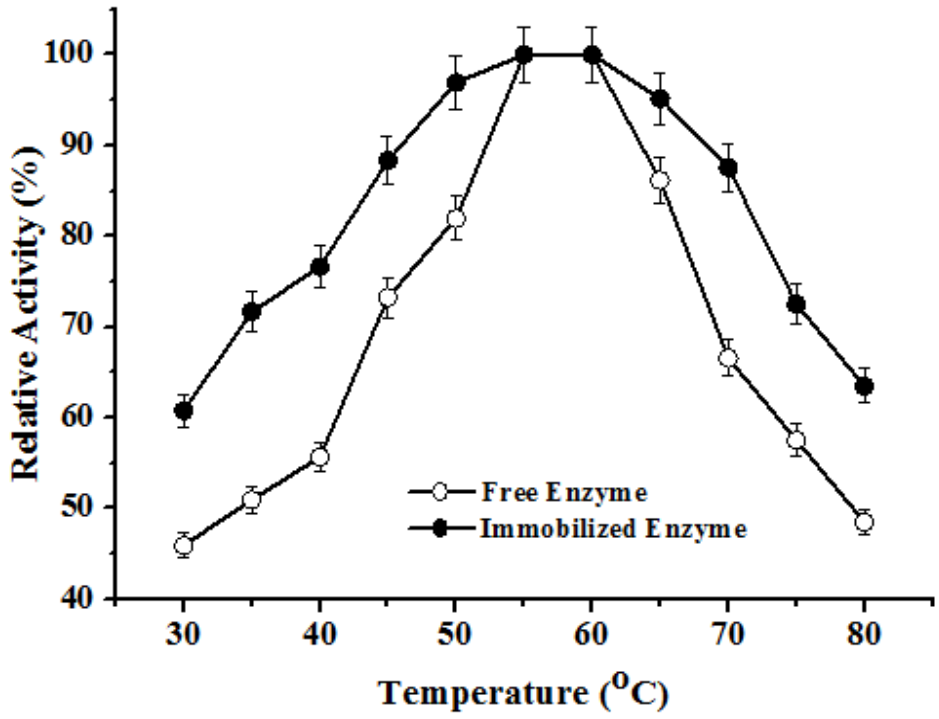

Figure 4.14. The optimum temperature of free and immobilized *ANGA.* The effect of temperature on the enzyme activity was determined by performing the activity assay for 200 µL free or 0.312 g immobilized *ANGA*s containing 4.07 IU at pH 5.5 and at different temperatures. 100% relative activity refers to 4.07 IU activity used for activity assay.

4.5.3. pH stability

In Figure 4.15, the stability of immobilized enzyme appears that is higher than free enzyme and it is also seen that the immobilized enzyme is more stable than the free enzyme at all tested pH values. It is well known that immobilization improves the pH stability.

4.5.4. Thermal stability

Figure 4.16 shows the effect of temperature on the stability of the free and immobilized enzyme. It is seen that immobilized enzyme more stable than the free enzyme at the higher temperatures than 55 ^{o}C. It is generally known that immobilization improves the thermal stability of enzymes (Torres-Bacete et al., 2000).

4.5.5. Michaelis-Menten constants

The Michaelis-Menten constants of free and immobilized enzyme were calculated using the Lineweaver-Burk plot (Figure 4.17). After immobilization, V_{max} value has been decreased from 1464.1 to 1342.3 μmol D- Glucose / L.min, while K_m value decreased from 116.3 to 109.9 g maltodextrin / L. while K_m value decreased from 116.3 to 106.3 g maltodextrin / L. K_m value indicates the affinity of an enzyme to a substrate (Torres-Bacete et al., 2000). It is well known that when the K_m value decreased, the affinity of the enzyme to substrate increases. The lower K_m value of immobilized *ANGA* shows that its affinity to the substrate has increased. But, the lower V_{max} value of immobilized *ANGA* shows that substrate molecules could not reach easily as a free enzyme to the active site due to diffusional limitations for the substrate into the active site of enzyme molecules. Therefore, it can be said that the optimization of the immobilization conditions results in 100 % activity yield, probably due to the increased affinity despite the decreased maximum velocity.

4.5.6. Operational and storage stabilities of the ımmobilized *ANGA*

The activity of immobilized enzyme decreased to 99.3 % of initial value after the repeated twenty uses under optimum conditions (Figure 4.18). The activity of immobilized enzyme decreased to 98.3 % of initial value after thirty days under optimum storage conditions (Figure 4.19). These results show that the immobilized *ANGA* can be used in the industrial production of glucose syrup in the continuous processes

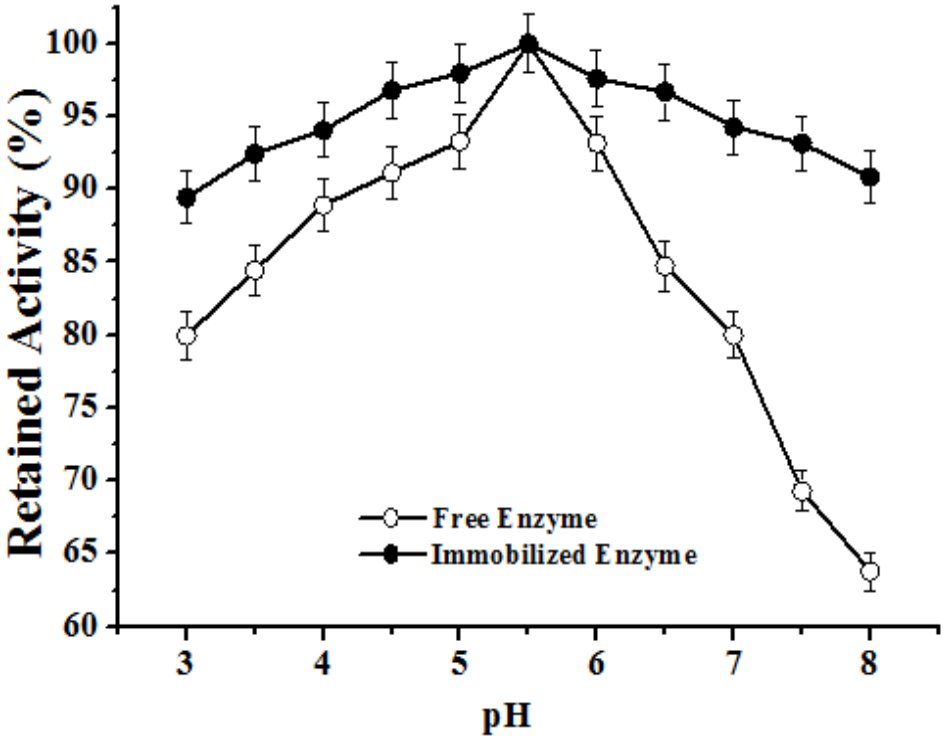

Figure 4.15. pH stability of free and immobilized *ANGA*. 200 µL free or 0.312 g immobilized *ANGA*s containing 4.07 IU activity were incubated in buffer solutions at various pHs (3.0–8.0) and at room temperature for 1 h and the retained activities were determined under the standard activity assay conditions.

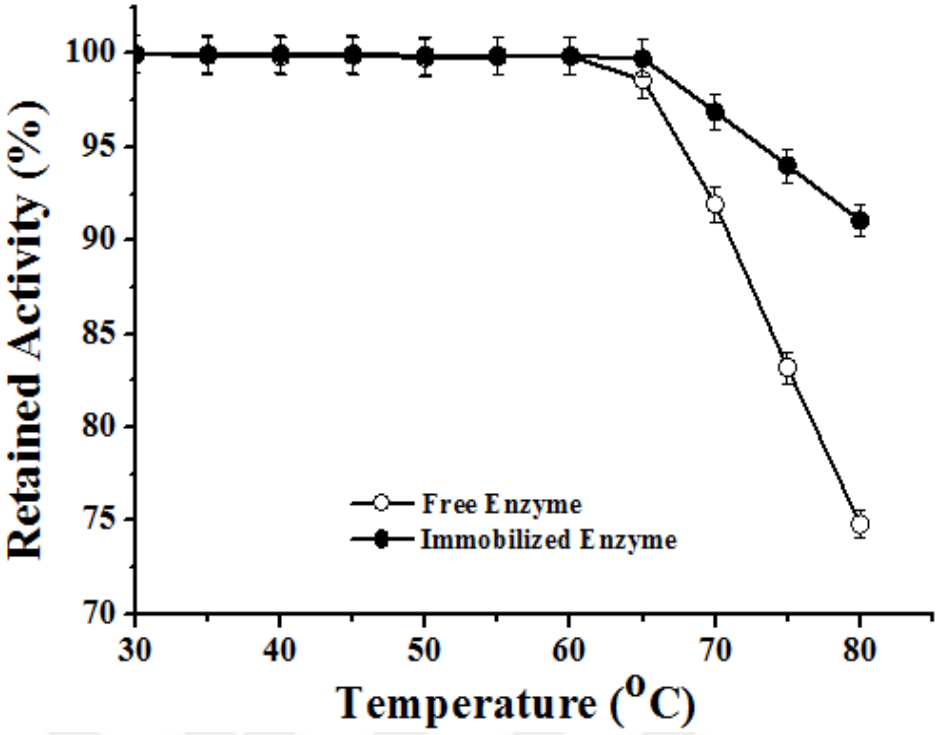

Figure 4.16. Thermal stability of free and immobilized *ANGA*. 200 µL free or 0.312 g immobilized *ANGA*s containing 4.07 IU activity were incubated in buffer solutions (25 mM, pH 5.5) at temperatures from 30 to 80 ◦C for 1 h and the retained activities were determined under the standard activity assay conditions.

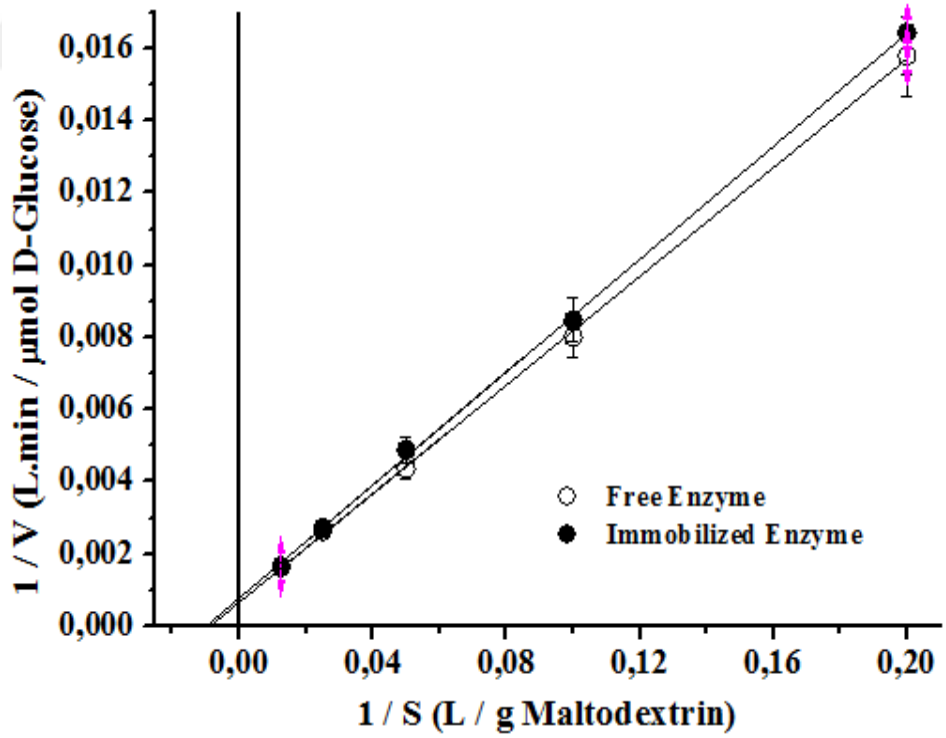

Figure 4.17. Lineweaver–Burk plots of free and immobilized *ANGA*. Initial velocities were determined by performing the reactions between 200 µL free or 0.312 g immobilized *ANGA*s containing 4.07 IU activity with maltodextrin solutions (pH 5.5) at different concentrations (10 to 80 g/L) for 15 min.

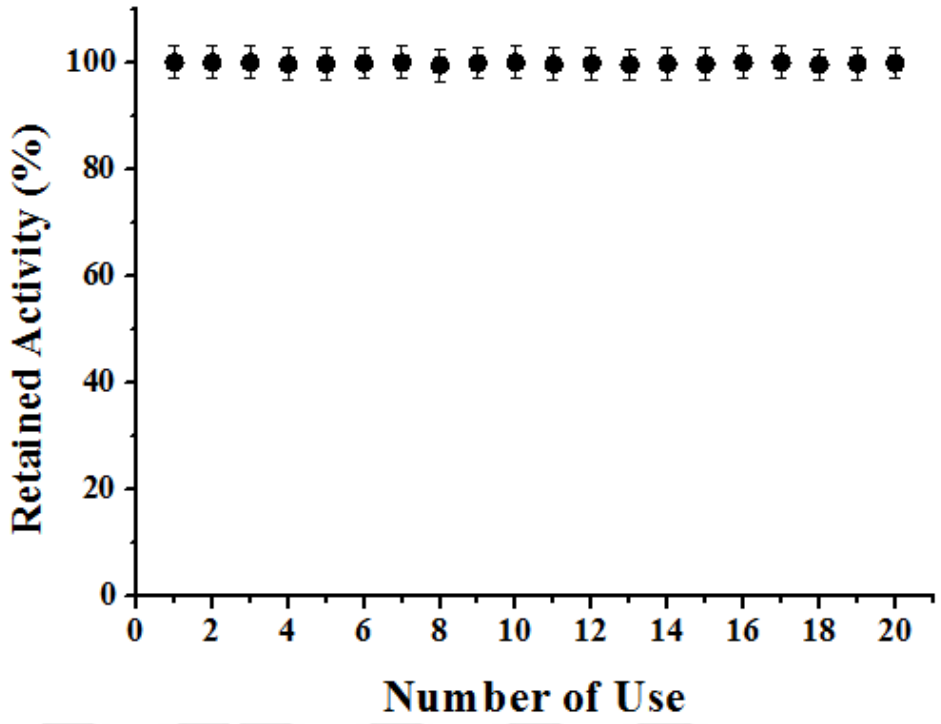

Figure 4.18. Operational stability of immobilized *ANGA*. Operational stability of the immobilized enzyme was determined by using the standard activity assay method, in repeated batch experiments.

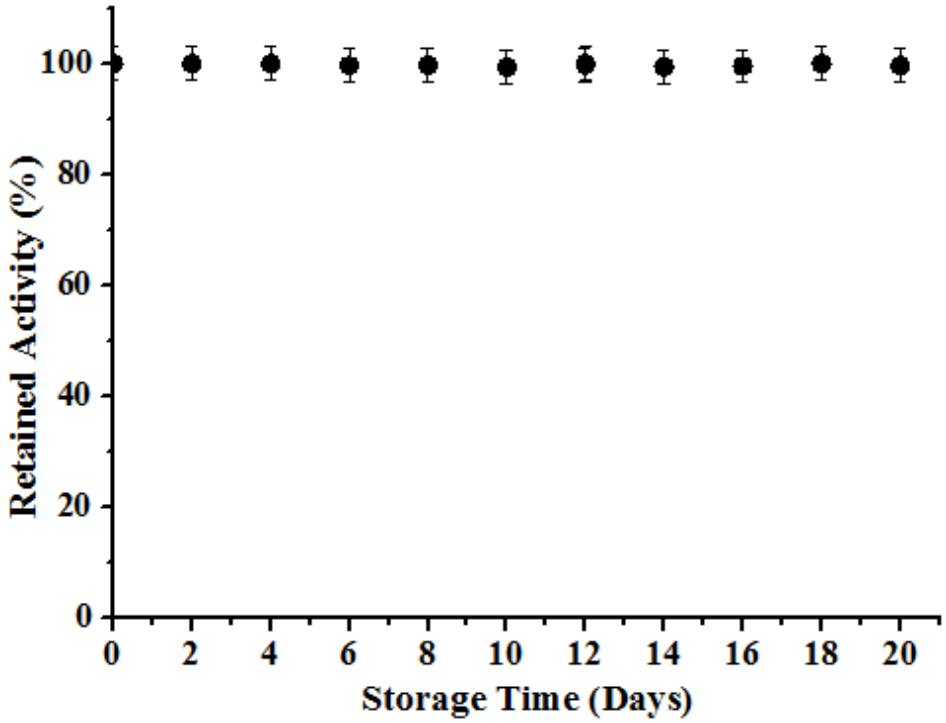

Figure 4.19. Storage stability of immobilized *ANGA*. Storage stability of the immobilized enzyme was determined by performing the standard activity assay method two days intervals for thirty days.

4.5.7. Production of glucose syrup from maltodextrin by using immobilized *ANGA*

The changes in the maltodextrin and D-glucose concentration during the production of glucose syrup were evaluated by investigating Figure 4.20. As seen in the figure, all of the maltodextrins have converted to D-glucose in four hours.

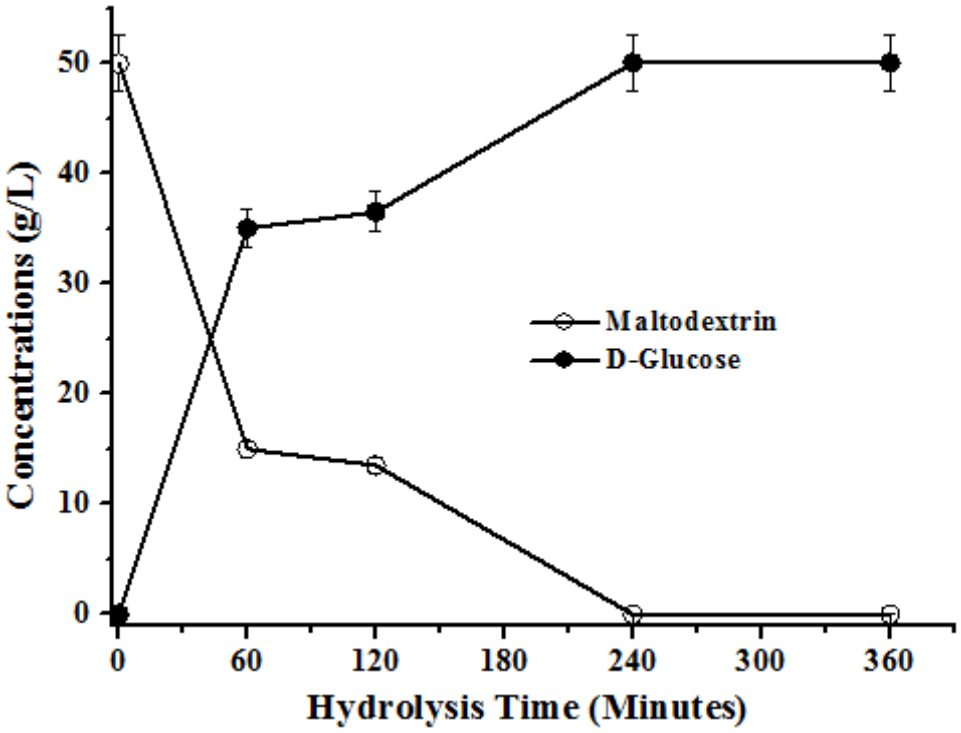

Figure 4.20. Production of glucose syrup from maltodextrin by using immobilized *ANGA*. 0.312 g immobilized *ANGA* containing 4.07 was reacted with 5 mL of 5 % (w/v) maltodextrin solution (pH 5.5) at 55 ◦C for 6 h and released D-glucose content was determined at 1 h intervals by using UV spectrophotometer.

5. CONCLUSION AND RECOMMENDATIONS

5.1. Conclusions

In the present study, there are several topics that can be addressed by activated carbon which I've produced it in my work in the laboratory because of its Characterization of BET surface area, SEM, FT-IR analysis of the obtained activated carbons with high iodine numbers will be performed. For that suitable for immobilization *ANGA*. That's why I've used it in this thesis for immobilization of *ANGA* after functionalization activated carbon activated by glutaraldehyde.

the present study, firstly *ANGA* has been covalently immobilized onto FAC with 100.00 % immobilization and 100.00 % activity yields by optimizing the immobilization conditions. This result is the best between the immobilization studies of *ANGA* in the literature. Secondly, the immobilized *ANGA* obtained in the present study exhibit high operational and storage stabilities. Lastly, maltodextrin has completely converted to glucose by using immobilized *ANGA*. Consequently, it can be said that immobilized *ANGA* obtained in the present study can be used in the industrial production of glucose syrup and in the other industrial applications.

5.2. Recommendations

Firstly, the results achieved in this thesis can be published in any SCI-indexed journal.

Secondly, the prepared activated carbon can be used for the removal of toxic metals, dyes from industrial wastewater, air pollution control, food processing, chemical, pharmaceutical industries, and other purposes.

Thirdly, this method for the preparation of activated carbon can be used for other low-cost biomass materials and the by-products of agriculture.

Fourthly, a patent application can be made for the production of glucose syrup using the immobilization method developed in this thesis for *ANGA*

Finally, we can work with the large starch industry companies in our country to make the industrial production of glucose syrup.

6. REFERENCES

Aktar T (2011) Preparation and characterization of low-cost high porous activated carbon from agricultural by-products and their adsorption capacities. Master Thesis, Ankara University Institute of Science, 20-44.

Aygün, A., 2002. Investigation of active carbon production and adsorption properties of indigenous natural raw materials. Master Thesis., Istanbul Technical University, Institute of Science, Istanbul.

Abdel-Nasser A, El-Hendawy A, Andrew J, Alexander b, Robert J, Andrews b, Gavin b (2008) Effects of activation schemes on porous, surface and thermal properties of activated carbons prepared from cotton stalks. *J. Anal. Appl. Pyrolysis* 82: 272–278

Aiyer PV (2005) Amylases and their applications. *African Journal of Biotechnology* 13:1525-1529

Arıca MY, Alaeddinoğlu NG, Hasırcı V (1998) Immobilization of glucoamylase onto activated pHEMA/EGDMA microspheres: properties and application to a packed-bed reactor. *Enzyme Microb Technol* 22:152-157

Arıca MY, Yavuz H, Patir S, Denizli A (2000) Immobilization of glucoamylase onto spacer-arm attached magnetic poly_methylmethacrylate/ microspheres: characterization and application to a continuous flow reactor. *J Mol Catal B-Enzym* 11:127-138

Aslan Y, Tanrıseven A (2007) Immobilization of Pectinex Ultra SP-L to produce galactooligosaccharides. J Mol Catal B-Enzym 45:73-77

Aslan Y, Handayani N, Stavila E, Loos K (2013) Improved performance of Pseudomonas fluorescens lipase by covalent immobilization onto Amberzyme. Turk J Biochem 38(3):313-318

Aslan Y, Handayani N, Stavila E, Loos K (2014) Covalent immobilization of Pseudomonas fluorescens lipase onto Eupergit CM. Int J Curr Res 6(2):5225-5228

Alexander M (1983) Stabilization of Enzymes against Thermal Inactivation. *Advances in Applied Microbiology* 29:1-28

Arulkumar M, Thirumalai K., Sathishkumar P, Palvannan T (2012). Rapid removal of chromium from aqueous solution using novel prawn shell activated carbon. *Chemical Engineering Journal* 185– 186:178– 186.

Aehle W, 2004.enzymes in industry, WILEY-VCH Verlag GmbH & Co.KgaA, Weinheim.

Bhatnagar A, Hogland W, Marques M, Sillanpaaää M (2013) An overview of the modification methods of activated carbon for its water treatment applications. *Chemical Engineering Journal* 219: 499–511

Bailey E., Cho Y.K. (1981) High Loading of Immobilized Enzymes on Activated Carbon Supports. *United States Patent* 289,853.

Bouhamed F, Elouear Z, Bouzid J (2012) Adsorptive removal of copper (II) from aqueous solutions on activated carbon prepared from Tunisian date stones: Equilibrium, kinetics, and thermodynamics. *Journal of the Taiwan Institute of Chemical Engineers* 43: 741–749

Bradford MM, (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72:248-254

Chui WK, Wan LS (1997) Prolonged retention of cross-linked trypsin in calcium alginate microspheres. *J. Microencapsulation* 14:51961

Caturla F, Molina-Sabio M, (1991) Rodriguez-Reinoso., Preparation of activated carbon by chemical activation with ZnCl, P ergamonP rcwp lc, 999-1007

Drevon G,2002. Enzyme immobilization into polymers and coatings, BS, University of Pittsburgh, School of Engineering, Chimie Physique Electronique Lyon

Diao Y, Walawender W, Fan L, (2001) Activated carbons prepared from phosphoric acid activation of grain sorghum. *Elsevier Science Ltd*, Bioresource Technology 81: 45-52

Emneus J, Nilsson G, Gorton L (1993) Flow injection system for the determination of starch in starch from different origins with immobilized o-amylase and glucoamylase reactors. *Starch-Starke* 45:267-270

El-Hendawy A, Abdel-Nasser A. Alexander, Andrew J.Andrews, Robert J, 2008. Effects of activation schemes on porous, surface and thermal properties of activated carbons prepared from cotton stalks. Journal of Analytical and Applied Pyrolysis, 82: 272–278 Contents

Girgis B, Ishak M, (1999) Activated carbon from cotton stalks by impregnation with phosphoric acid. Elsevier Science.Materials Letters 39: 107–114

Güngör C., 2010. Production of activated carbon from grape seed and its use in copper removal. Master Thesis, Eskisehir Osmangazi University Institute of Science, Eskişehir.

George R, Sugunan S (2014) Kinetic and thermodynamic parameters of immobilized glucoamylaseon different mesoporous silica for starch hydrolysis: a comparative study. *J Mol Catal B-Enzym* 106:81-89

Gupta K, Jana AK, Kumar S, Jana MM (2015) Solid state fermentation with recovery of Glucoamylase from extract by direct immobilization in cross linked enzyme aggregate for starch hydrolysis. *Biocatal Agric Biotechnol* 4:486-492

Gupta K, Jana AK, Kumar S, Maiti, M (2013) Immobilization of glucoamylase from SSF of Aspergillus niger bycrosslinked enzyme aggregate onto magnetic nanoparticles usingminimum amount of carrier and characterizations. J *Mol Catal B-Enzym* 98:30-36

Haycarb (Pvt) Ltd., 2017. Activated Carbon Suppliers . Haycarb PLC Sri Lanka, Available from: http://www.haycarb.com/activated-carbon

Jain A, Ong V,Jayaraman S,Balasubramanian R, Srinivasan M.P (2016) Supercritical fluid immobilization of horseradish peroxidase on high surface area mesoporous activated carbon. *The Journal of Supercritical Fluids* 107: 513–518

Jiang Li, Gao Y, Liang G, 2015. Optimization of Activated Carbons Production from Sesame Stalks Using Response Surface Methodology. International Conference on Chemical, Material and Food Engineering. Kunming, Yunnan, China 25 – 26

Kong H, He J, Wu H, Wu H, Gao Y (2012) Phenanthrene Removal from Aqueous Solution on Sesame Stalk-based Carbon. *Clean – Soil, Air, Water* 00 (0), 1–8

Kılıç M., 2009. Investigation of adsorption properties and kinetics of active carbon obtained from Euphorbia rigida in aqueous solutions. Graduate Thesis, Anadolu University Institute of Science, Department of Chemistry Engineering, Eskişehir.

Köseoğlu, E., 2005. Chemical activation of agricultural byproducts and characterization of active carbon: characterization and examination of aqueous solution cation adsorption. İnönü University Institute of Science, Department of Chemistry Engineering, Malatya.

Küçükgül, E. Y., 2004. Determination of commercial activated carbon production and properties. Dokuz Eylül University, Faculty of Engineering, Science and Engineering Journal, 6 (3): 41-56.

Kirk O, Borchert V, Fuglsang C (2002) Industrial enzyme applications. *Current Opinion in Biotechnology* 13: 345-351

Lemus-Yegres L.J., Such-Basáñez I., Román-Martínez M.C., and Salinas-Martínez de Lecea C., 2007. Catalytic properties of an Rh-diamine complex anchored on activated carbon: Effect of different surface oxygen groups. Applied Catalysis A: General. 331: 26-33.

Mines P.D., Thirion D., Uthuppu B., Hwang Y., Jakobsen M.H., R. Andersen H., and Yavuz C.T., 2017. Covalent organic polymer functionalization of activated carbon surfaces through acyl chloride for environmental clean-up. Chemical Engineering Journal 309: 766–771.

Mani Arulkumara, 1, Kasinathan Thirumalaia, 1, Palanivel Sathishkumarb, Thayumanavan Palvannana, 2012. Rapid removal of chromium from aqueous solution using novel prawn shell activated carbon. Chemical Engineering Journal 185– 186 (2012) 178– 186

Manjon A, Iborra JL, Ozano PL, Canovas M (1995) A practical experiment on enzyme immobilization and characterization of the immobilized derivatives. *Biochem Educ* 23 (4):213-216

Miller GL (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal Chem* 31:426-428

Milosavic N, Prodanovic R, Jovanovic S, Novakovic I, Vujcic Z (2005) Preparation and characterization of two types of covalently immobilized glucoamylase. J Serb Shem Soc 70(5):713-719

Milosavic N, Prodanovic R, Jovanovic S, Novakovic I, Vujcic Z (2007) Immobilization of glucoamylase via its carbohydrate moiety on macroporous poly(GMA-co-EGDMA). *Enzyme Microb Technol* 40:1422-1426

Milosavic N, Pristov JB, Velickovic DV, et al (2012) Study of the covalently immobilized glucoamylase on macroporous polymer by mathematical modeling of the pH optima. *J Chem Technol Biotechnol* 87(10):1450-1457

Murphy M (2017) Product information. Bio-Cat, Troy, p 1

Mattiasson B, Kaul, R. (1991) Determination of coupling yields and handling of labile proteins in immobilization technology. In:Protein immobilization. Fundamentals and Applications (Taylor, R. F., ed.), Marcel Dekker, New York, NY, pp. 161–179.

Messing, R. A. (1976) Adsorption and inorganic bridge formations. In: Methods in Enzymology, volume XLIV, (Mosbach, K., ed.), Academic Press, New York, NY, pp. 148–169.

Namvar-Asl M, Soltanieh M, Rashidi A (2008) Modeling and Preparation of Activated Carbon for Methane Storage. *Energy Conversion and mANGAement*, 49: 2478-2482.

Özaşık, A., 2002. Adsorption of heavy metal and heavy metal complexes on activated charcoal and the effect of tannic acid on adsorption. Master Thesis, Ondokuz Mayıs University, *Institute of Science and Technology*, Samsun.

Oh JT, Kim JH (2000). Preparation and properties of immobilized glucoamylase on nonporous PS/PNaSS microspheres. *Enzyme Microb Technol* 27:356-361

O'Driscoll K.F., 1976. Techniques of enzyme entrapment in gels. In: Methods in Enzymology In: Immobilized Cells and Enzymes: A Practical Approach, *IRL, Oxford, UK*, pp. 3–17.

Ole Kirk*, Torben Vedel Borchert and Claus Crone Fuglsang (2002). Industrial enzyme applications. Elsevier Science Ltd. 13:345–351

Pragya1 P, Sripal1 S and Maheshkumar Y (2013). Preparation and Study of Properties of Activated Carbon Produced from Agricultural and Industrial Waste Shells. Research Journal of Chemical Sciences 3(12), 12-15

Puziy A. M., poddubnaya O. I., Matinez-Alonso A., Suarez-Garcia F. and Tascon J. M. D., Characterization of synthetic carbon activated with phosphoric acid, applied surface science 200,196-202 (2002).

Ramani K, Karthikeyan S, Boopathy R, John Kennedy L, Mandal A.B, and Sekaran G (2012) Surface functionalized mesoporous activated carbon for the immobilization of acidic lipase and their application to hydrolysis of waste cooked oil: Isotherm and kinetic studies. *Process Biochemistry* 47: 435–445.

Rani AS, Das M, Satyanarayana S (1999) Characterization of amyloglucosydase bound on chitin. *J Sci Ind Res* 58:31-33

Rani AS, Das MLM, Satyanarayana S (2000) Preparation and characterization of glucoamylase adsorbed on activated charcoal. *J Mol Catal B-Enzym* 10:471-476

Roy I, Gupta MN (2004) Hydrolysis of starch by a mixture of glucoamylase and pullulanase entrapped individually in calcium alginate beads. *Enzyme Microb Technol* 34:26-32.

Ramani K, Karthikeyan S, Boopathy R, John Kennedy L, Mandal A.B, Sekaran G, (2012) Surface functionalized mesoporous activated carbon for the immobilization of acidic lipase and their application to hydrolysis of waste cooked oil: Isotherm and kinetic studies. *Process Biochemistry* 47: 435–445.

Ramani K, Boopathy R, Vidyac C, Kennedy L, Velan M., Sekaran G (2010) Immobilisation of Pseudomonas gessardii acidic lipase derived from beef tallow onto mesoporous activated carbon and its application on hydrolysis of olive oil. *Process Biochemistry* 45: 986–992.

Švorčík V., Makajová Z., Kasálková N.S., Kolská Z., Žáková P., Karpíšková J., Stibor I., and Slepička P.,, 2014. Cytocompatibility of polymers grafted by activated carbon nano-particles. Materials Science and Engineering. 69: 361–371.

Sanjay G, Sugunan S (2005) Glucoamylase immobilized on montmorillonite: synthesis, characterization and starch hydrolysis activity in a fixed bed reactor. *Catalysis Communications* 6:525-530

Smalla K, Turkova J, Coupek J, Hermann P (1988) Influence of salts on the covalent immobilisation of proteins to modified copolymers of 2-hydroxyethyl methacrylate with ethylene dimethacrylate. *Biotechnol Appl Biochem* 10:21-31

Švec F, Kálal J, Menyailova II, Nakhapetyan LA (1978) Immobilization of glucoamylase on poly [(glycidyl methacrylate) Co(ethylene dimethacrylate)] carrier and its derivatives. *Biotechnol Bioeng* 9:1319-1328

Suma Y, Lim H, Kwean O. S, Cho S, Yang J, Kim Y, Kang C. S, and Kim H. S (2016) Enzymatic degradation of aromatic hydrocarbon intermediates using a recombinant dioxygenase immobilized onto surfactant-activated carbon nanotube. *Bioresource Technology* 210: 117–122

Scouten W.H. (1987) A Survey of Enzyme Coupling Techniques.In: Methods in Enzymology, *Academic Press*135:30–65

Tan I.A.W., Hameed B.H, Ahmad A.L (2007) Equilibrium and kinetic studies on basic dye adsorption by oil palm fibre activated carbon. Chemical Engineering Journal, 127:111-119.

Tengyan Zhanga, Walter P. Walawendera, ∗, L.T. Fana, Maohong Fanb, Daren Daugaardb, R.C. Brownb., 2004. Preparation of activated carbon from forest and agricultural residues through CO2 activation. Chemical Engineering Journal 105: 53–59

Tanrıseven A, Ölçer Z (2008) A novel method for the immobilization of glucoamylase onto polyglutaraldehyde-activated gelatin. *Biochem Eng J* 39:430-434

Tardioli PW, Vieira MF, Vieira AMS et al (2011) Immobilization–stabilization of glucoamylase: chemical modification of the enzyme surface followed by covalent attachment on highly activated glyoxyl-agarose supports. *Process Biochem* 46:409-412

Torres-Bacete J, Arroyo M, Torres-Guzman R et al (2000) Covalent immobilization of penicillin V acylase from Streptomyces lavendulea. *Biotechnol Let* 22:1011-1014

Tosa T, Mori T. Fuse N, and Chibata I. (1966) Studies on continuous enzyme reactions. I. Screening of carriers for preparation of water-insoluble aminoacylase. *Enzymologia.* **31,** 214–224.

Uludag O, 2016. Preparation of activated carbon from chestnut shell by microwave assistance and analysis on its surface**.** Proceedings of Academics World 29th International Conference, San Francisco, USA, ISBN: 978-93-85973-73-4

Uygun M, Akduman B, Ergönül B et al (2015) Immobilization of glucoamylase onto macroporous cryogels for continuous glucose production from starch. *J Biomater Sci Polym Ed* 26(16):1112-1125

Vernersson T, Bonelli P.R., Cerrella E.G, Cukierman A.L (2002). Arundo donax cane as a precursor for activated carbons preparation by phosphoric acid activation. *Bioresource Technology*, 95-104.

Virgen M, Gómez R, Castillo D, Petriciolet A (2012). Applications of Activated Carbons Obtained from Lignocellulosic Materials for thenWastewater Treatment, Lignocellulosic Precursors. *InTech* 10: 5772-39367

Wang F, Chen G, Liu HZ, Liu CZ (2007) Reversible immobilization of glucoamylase by metal affinity adsorption on magnetic chelator particles. *J Mol Catal B-Enzym* 48:1-7

Woodward, J. (1985) Immobilized enzymes: adsorption and covalent coupling. In: Immobilized Cells and Enzymes: A Practical Approach, (Woodward, J., ed.), IRL, Oxford, UK, pp. 3–17.

Žáková P., Slepičková Kasálková N., Kolská Z, Leitner J, Karpíšková J., Stibor I., Slepička P., and Švorčík V., 2016. Cytocompatibility of amine functionalized carbon nanoparticles grafted on polyethylene. Materials Science and Engineering C 60: 394–401

CURRICULUM VITAE

PERSONAL INFORMATION

Name and surname: Yousif Mohammed Sharif Ali
Birthplace and Date: Duhok, Iraq, 1971
Telephone: 05393994971
Email: yosif@uod.ac

EDUCATION

Started in Turkey to study M.Sc. at Siirt University of science Chemical Engineering Department in 2016©. I can speak Kurdish, English, and Arabic.

Degree	Specialization and Title	Awarding Institution or Body	Academic Year
Diploma	Chemical Industry	Institute of Technology in Mosul	1991-1992
B. Sc.	Chemical Engineering	College of: Chemical Engineering University of Technology-Baghdad / Country Iraq	2008-2009

Academic Project:

Guide: Dr. Balasim Ahmed

-Low-Density polyethylene production (LDPE)

Practical Experience:

Plants: Charcoal briquette

detailed description: Studied the HAZARDS OF Medical Waste Incinerator Project

COMPUTER SKILLS:

International Computer Driving Licence (ICDL)/2013

Computer course (Maintenance of Computers) Joint Humanitarian Information Center .31st 2004 in University of Duhok.

Work Experience:

Faculty of Medical Sciences, University of Duhok, Kurdistan Region-Iraq (August 2009- till now) start working as a demonstrator.

December 2014- May 2015: I was employed as a demonstrator in Biochemistry Department at Faculty of Medical Sciences, University of Duhok.

- Duties entailed giving practical lectures of biochemistry for first stage health sciences students (anesthesia and biomedical laboratory schools), and also the job involved working in the lab and assisting undergraduate 1st stage medical and dentistry students to know the techniques of general chemistry.

September 2012- November 2014: I was employed as a demonstrator in Biochemistry Department at Faculty of Medical Sciences, University of Duhok.

- The job involved working in the lab and assisting undergraduate 3rd and 4th stages pharmacy students to know the techniques of medicinal chemistry and pharmaceutical analysis.

Faculty of Medical Sciences - University of Duhok/Kurdistan Region- Iraq (Nov 2009 – August 2012).

September 2010- August 2012: I was employed as a demonstrator in Biochemistry Department at Faculty of Medical Sciences, University of Duhok.

- The job involved working in the lab and assisting undergraduate 1st stage pharmacy students to know the techniques of the general chemical test. It also involved observer junior students for their project work.

August 2009 – August 2010: I was employed as a demonstrator in biochemistry Department at Faculty of Medical Sciences, School of Medicine, and the University of Duhok.

- Duties entailed assisting researchers in the lab by giving practical lectures for 1st stage medical students in Analytical Chemistry, plus dealing with any of their inquiries.

Areas of Interest:

- Drying principles and technologies
- industrial Crystallization
- Thermodynamics
- Equipment Design for Chemical Industries plants
- Chemical Safety and Security

Workshops Attendances:

1) Workshop on (Chemical Safety and Security Officer Training) / University of Sulaimani, Iraq June 2012.

2) Workshop on (Chemical Safety and Security Officer Training) / University of Salahaddin, Iraq June 2013.

3) Workshop on (Atomic absorption) / University of Duhok, Iraq May 2017