REPUBLIC OF TURKEY
SİİRT UNIVERSITY
INSTITUTE OF SCIENCES

IMMOBILIZATION OF *Chaetomium erraticum* DEXTRANASE ON CARBON NANOTUBES

MASTER'S THESIS

Barzan Ismael GHAFOUR
(163108005)

Department of Food Engineering

Thesis Supervisor: Assist. Prof. Dr. Yakup ASLAN

May-2018
SİİRT

THESIS ACCEPTANCE AND APPROVAL

The thesis study titled **"Immobilization of *Chaetomium erraticum* dextranase on carbon nanotubes"** prepared by Barzan Ismael GHAFOUR, has been accepted a Master Degree thesis with unanimity / majority of votes by the following jury on the date of 14 / 05 / 2018 at Siirt University, Institute of Sciences, Department of Food Engineering.

Jury Members	**Signature**
President Prof. Dr. Ender Sinan POYRAZOĞLU	.....
Supervisor Assist. Prof. Dr. Yakup ASLAN	.....
Member Assist. Prof. Dr. Seda OĞUR	.....

I confirm the above results.

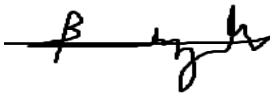
Assoc. Dr. Fevzi HANSU
Director of Institute of Science

This thesis study was supported by Scientific Research Projects Coordinatorship in Siirt University with the project numbered as 2018-SİÜFEB-003.

THESIS NOTIFICATION

This thesis, which is prepared in accordance with the thesis writing rules, complies with the scientific code of ethics, in case of exploitation of others' works it is referred to in accordance with the scientific norms, I declare that any part of the thesis, Every information bases, references and literature used it has not presented as another thesis work at this university or another universities.

Signature
Barzan Ismael GHAFOUR



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

PREFACE

I would like to extend my thanks to the republic of Turkey and University of Siirt to offer this chance to study in this country. I would like to express my sincere gratitude and appreciation to my supervisor Assist. Prof. Dr.Yakup ASLAN. He showed me different ways to approach a research problem and taught to be persistent to accomplish any goal and he was really great and kind for us.

There are too many people who have helped me during my study that I need to thank. However, there are some people that definitely deserve a place in my thesis.

I also record thank to Farhad X. Habib and thanks for all my friends who were sported me even by one word.

Many thanks to my special thanks to my wife, Zaytoon for supporting me at all time in my all stage in life and my sweet children Rewan, Ronya, Rouya and Rezhwan for their love, patience, sacrifice, and support, I owe all my achievements to them and am really thankful to know that they will support me in anything I do.

Finally, I would like to thank all those people who helped me at any single recommendation.

Barzan Ismael GHAFOUR
SİİRT-2018

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF ABBREVIATIONS AND SYMBOLS

Abbreviation	Description
BSA	: Bovine Serum Albumin
CED	: *Chaetomium erraticum* dextranase
c-MWCNT	: Carboxylated multi walled carbon nano tubes
DNS	: 3,5-dinitrosalcilic acid
FCED	: Free *Chaetomium erraticum* dextranase
f-CNT	: Functionalized carbon nano tubes
G	: Glucuronic acid
g	: Gram
ICED	: Immobilized *Chaetomium erraticum* dextranase
IU	: International unit of enzyme
L	: Liter
M	: Molarity
MA	: Mannuronic acid
mg	: Milligram
mL	: Milliliter
c-MWCNT	: carboxylated-multi walled carbon nanotubes
oC	: Degree of celsius
pH	: -log [H+]
PVDF	: Poly vinylidene difluoride filter
rpm	: Rolling per minute
UHT	: Ultra high temperature
UV	: Ultraviolet
V	: Volume
W	: Weight
µg	: Microgram
µL	: Microliter
µmol	: Micromole

ÖZET

YÜKSEK LİSANS TEZİ

Chaetomium erraticum DEXTRANAZIN KARBON NANOTÜPLERİNDE İMMOBİLİZASYONU

Barzan Ismael GHAFOUR

Siirt Üniversitesi Fen Bilimleri Enstitüsü
Gıda Mühendisliği Anabilim Dalı

Danışman: Dr. Öğr. Üyesi Yakup ASLAN
2018, 58 Sayfa

Bu çalışmada, *Chaetomium erraticum* dekstranaz (CED) enzimi adsorpsiyon yöntemi kullanılarak c-MWCNT üzerine immobilize edildi. İmmobilizasyon tamponunun pH ve molaritesi, c-MWCNT miktarı ve immobilizasyon süresi gibi immobilizasyon koşullarının optimize edilmesi %100 immobilizasyon verimi ve %114.15 aktivite verimi ile sonuçlandı. Serbest ve immobilize CED'in karakterizasyonu da incelendi. İmmobilizasyondan sonra CED'in optimum pH'sı 5.0'dan 6.0'a kayarken, optimum sıcaklığı (55 °C) değişmedi. Ayrıca, serbest ve immobilize CED için, Lineweaver-Burk grafiği kullanılarak kinetik sabitler de belirlendi. Serbest ve immobilize CED'in K_m değerleri sırasıyla 56.18 g/L ve 65.36 g/L ve V_{max} değerleri ise sırasıyla 523.56 µg indirgeyici şeker/L.dk.ve 595.24 µg indirgeyici şeker/L.dk. olarak hesaplandı. Diğer taraftan, tekrarlanan 20 kullanımdan sonra ve optimum depolama koşullarında 30 gün depolamadan sonra immobilize CED'in başlangıç aktivitesinde bir azalma olmamıştır. Son olarak, serbest ve immobilize CED kullanılarak, %10 dekstran çözeltisindeki (pH 6.0) dekstranın sırasıyla %17.15'i ve %17.53'ünün 12 saat içinde indirgenmiş şekerlere (IMOs ve Glukoz) dönüştürüldü. Sonuç olarak, bu çalışmada elde edilen immobilize CED, IMOs'un endüstriyel üretiminde ve dekstranın hidrolizinde kullanılabilir.

Anahtar Kelimeler: *Chaetomium erraticum*, dekstranaz, Dextran T70, immobilizasyon, izomaltooligosakkaritler, c-MWCNT,

ABSTRACT

MASTER'S THESIS
IMMOBILIZATION OF *Chaetomium erraticum* DEXTRANASE ON CARBON-NANOTUBES

Barzan Ismael GHAFOUR

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

Supervisior: Asst. Prof. Dr. Yakup ASLAN
2018, 58 Pages

In this study, *Chaetomium erraticum* dextranase (CED) enzyme was immobilized onto c-MWCNT using adsorption method. Optimization of the immobilization conditions such as the pH and molarity of the immobilization buffer, the amount of c-MWCNT and the time of immobilization resulted in 100% immobilization yield and 114.15% activity yield. Characterization of free and immobilized CED has also been studied. After immobilization, the optimum pH of the CED shifted from 5.0 to 6.0, while the optimum temperature (55 °C) did not change. Kinetic constants were also determined for free and immobilized CED using the Lineweaver-Burk plot. The K_m values of free and immobilized CED were 56.18 g/L and 65.36 g/L, respectively, and V_{max} values were 523.56 µg reductive sugar/ L.min. and 595.24 µg reductive sugar/L.min. On the other hand, there was no reduction in the initial activity of the immobilized CED after 20 consecutive uses and 30 days of storage at optimal storage conditions. Finally, 17.15% and 17.53% of the dextran, in 10% dextran solution (pH 6.0), were converted to reduced sugars (IMOs and Glucose) using free and immobilized CED respectively, in 12 hours. As a result, the immobilized CED obtained in this study can be used in the industrial production of IMOs and in the hydrolysis of dextran.

Keywords: c-MWCNT, *Chaetomium erraticum*, dextranase, Dextran T70, immobilization, isomaltooligosaccharides

1. INTRODUCTION

Dextranase (α-1, 6-D-glucan, 6-glucanohydrolase; E.C.3.2.1.11) is an enzyme which hydrolyzes the α-1, 6 glycosides linkages in dextran. Dextranase was found in several species of institute in higher plants mammalian tissues, bacteria and fungi, the fungal dextranase from *Chaetomium erraticum* have shown the best results on dextran's treatment both in juices and syrups, *Chaetomium erraticum* is one of the beast fungi among the other fungi to produce valuable enzymes biotechnologically because most of the fungi have *amylolytic* and *glucosidic* activities and the ability to degradation of carbohydrate the use of microorganisms, enzyme is ordinarily considered as environment friendly (Sidebotham, 1974).

The first appearance of the dextranase in the sugar industry was suggested by (Tilbury, 1971). More than 47 years ago, until today, when the enzyme was only discussed and studied for the preparations of medicinal dextranase, used as substitutes for blood plasma and more recently in the tooth to hydrolyze the dextran in the dental surface (Clarke et al., 1997b; Mendez, 2000) have debated several benefits with dextranase treatment.

The dextranase enzymes have many applications industrial such as medical and food, in sugar production dextranase to break down dextran to product IMOs, dextranase has extensive industrial application, however most of the time tough ecological conditions adversely affect the functionality and stability of the enzymes (Goulas et al., 2004b; Kubik et al., 2004; Thitaram et al., 2005).

There for tow enzymes must be used for the production of IMOs from sucrose, dextransucrase to convert sucrose to dextran (Goulas et al., 2004b) and dextranase to produce IMOs from dextran (Kubik et al., 2004). Dextran are undesirable compounds synthesized from sucrose by microbial contaminants during sugar production that increase viscosity of the flow and decrease industrial recovery, Dextran T2000 and Dextran T70 that was analogous to that of Dextran T90 and Dextran T2000 and Dextran T10, respectively a Dextran 500 or Dextran T500 is due to unforeseen circumstances powerful inside propagation limitation and to some extent due to aggregate size exclusion (Kim et al., 2011). Dextran was confirmed by the credibility European Commission for employ as a novel food constituent in bakery off spring's afflicted softness, crumb texture and loaf volume (Zannini et al., 2016).

This thesis study has been undertaken to forecast the immobilization CED on c-MWCNT. Immobilized enzymes have been widely used in food biotechnology, pharmaceutical, and chemical industries. The first immobilization enzymes it was published in 1953 and since then this field has gained increasing importance, more recently, enzymes have become attention for industrial application because of continue efforts to decrease energy consumption. More food manufacturing or processing applications would benefit from the availability of low-temperature enzymes. Enzymes have the ability to control the speed of chemical reactions in our body digestion, breathing, growth, reproduction, disease, healing, and blood coagulation (Corvis et al., 2005).

One of the different ways of obtaining IMOs is digestion of dextran polymer by immobilized dextranase enzyme.

Some methods are used for immobilization of enzymes such as covalent attachment, adsorption, entrapment, and cross linking. In the way the development of enzyme process, to employ the immobilized enzymes is best choise. Sundry procedure is ready to immobilize enzymes on upholding in classical reaction equipment and these one's techniques have also been applied to immobilize enzymes into a micro area in a technology known as micro response technology for the industrial implementation of dextranase (Tanaka and Kawamoto, 1999).

Several methods have been a suggestion to immobilize dextranase based on entrapment in polymers, cross-linking, adsorption and covalent binding (De Segura et al., 2004). According to Novick and Rozzell (2005), covalent immobilization is one of the best ways to beat this issue. The main benefits of covalently immobilized enzymes are those high operational and storage stability, but enzyme molecules linked to the bearer by powerful interactions cannot leak from the support through manufacturing industrial processes. The covalent immobilization of enzymes that is a well-known and easy process during stimulates and activates (Katchalski-Katzir and Kraemer, 2000; Miyazaki and Maeda 2006). It is renowned that different immobilization sum enzyme kinetic like enzyme concentration (Finocchiaro et al., 1980) yet the glutaraldehyde concentration leased midst enzyme immobilization impact the performance and the thermo stability of the enzyme in reaction (Betancor et al., 2008) description of the immobilized enzyme in order to compare together with free enzyme was done

procedures were also possessed for the expansion of a suitable batch hydrolysis of dextran in order that runs plentiful reactions equal, which would aid in acquisition more acquaintance during a moment or short time (Betancor et al., 2006).

Important note in sugar production is dextran pollution in the manufacturing process and bring about various problems like: reduce the capacity and production of crystallization and sucrose damages to the sugar molasses due to growing the viscosity of the juice that product blockage in the process line. The most harmful effects of elevated dextran concentrations in a technical sucrose solution are foreseen in the crystallization practicability because the influence of dextran increases strongly with the decrease of temperature (Clarke et al., 1997a).

The aim of this thesis study was the immobilization of CED on c-MWCNT for the production of IMOs due to c-MWCNT have the following advantages when used as support for immobilization of enzymes:

(1) They have a higher specific surface area to bind enzymes in larger amounts.

(2) Allows a variety of applications to modify the surface of carbon nanotubes by functionalizing the surface with OH, COOH, NH_2, F or other groups (Wei et al., 2007).

To this end, *Chaetomium erraticum* dextranase enzyme was first immobilized in optimum conditions at the first stage of this study by adsorption method. In the second step, the immobilized enzyme will be characterized by determining the kinetic constants, optimum pH and temperature, pH and thermal stability, operational and storage stability. In the third step, conditions of IMOs from Dextran T70 by using FCED and ICED were optimized to try to achieve the highest possible conversion rate.

2. LITERATURE REVIEW

2.1. Dextranases (DXs)

Dextranases are significant enzymes for their specific use in the industry. Dextranase that prepared from microbial used in chemistry, food technology, industry, producing sugarcane, pharmacy and textile or immobilization enzyme, dextranase enzyme that isolated from fungi, plant and bacteria are known for the production of isomaltooligosaccharides (IMOs), dextran-hydrolyzing enzymes like *Chaetomium erraticum, Acinetobacter* spp*, Enhydrobacter aerosaccus, Enterobacteriaceae* sp., *Porphyromonas sp., Leuconostoc pseudomesenteroides, Leuconostoc citreum, Weisella cibaria, Streptococcus equines, Streptococcus sp., Streptococcus parasanguinis sm21, Bacillus licheniformis, Micrococcus luteus, Propionibacterium acnes, mammalian tissues, Ascospheara apis, Gibberella fujikuroi, Beauveria bassiana, Penicillium sp., Fusarium sp., Fusarium acuminatum, Fusarium culmorum, Paecilomyces lilacinus, Funalia trogii, Phanerochaete chrysosporium, Trichoderma viride, Trichoderma viride* A*2, Trichoderma harzianum* D2*, H. insolens* and *Alternaria alternate* (Mouafi, 2016; Iqbal et al., 2015; Zohra et al., 2015).

Fungi *Chaetomium erraticum* hydrolyze both dextran and sucrose to yield IMOs and dextran (Thitaram et al., 2005). CED is one of the industrial enzymes and uses the dextran polysaccharide as a substrate. CED is widely used in the food and sugar manufactory industries, especially in the food industry. By using CED the dextran is converted to IMOs (Goulas et al., 2004). CED is a member of endo-dextranases which hydrolyze the glycosidic bond of dextran hydrolyses dextranase which is produced by fungi and bacteria can catalyze the breakdown of organic molecules (like dextran) to IMOs with the presence of water, by dextranase enzyme.

Enzyme, dextranase has the systematic name (α-1,6-D-glucan, 6-glucanohydrolase; E.C.3.2.1.11) is an enzyme which hydrolyzes the α-1,6 glucosidic linkages in dextran, the dextranase enzyme It is the more efficient method for hydrolyzing the dextran at sugar mills especially in the made-up of Raw Sugar, the application of commercial dextranase to fracture down dextran (Abdel-Rahman et al., 2008; Aslan and Tanriseven, 2007b).

The high viscosity observed at the tip or high dextran focus also leads to the issue of sugar crystallization that's problem in sugar processing all togetherness of

dextran consumes almost four units of sucrose and hence it much affects the yield there is a considerable boost or increase of dextran and reducing sugars, with a concomitant lower in pH and sucrose proportion, over the trajectory of cane left to stale over sundry days, dextran, an extracellular glucose homo-polysaccharide, has been shown to interfere with downstream processing in sugar production and can result in significant losses in the recovery of sucrose (Hector et al., 2015).

2.1.1. Source of dextranase (DX) and microbial (DX) structure

The primary sources of dextranase (DX) are microorganism's fungi and bacteria (Table 2.1). Dextranase has been used for hydrolyzing dextran at sugar mills in order to improve efficiency of sugar production (Purushe et al., 2012; Eggleston et al., 2005). CED is an endo-dextranase which hydrolyzes 1,6 glucosidic linkages in dextran shows the best results on dextran hydrolysis in juices and syrups. Structure of CED is shown in Figure 2.1.

2.1.2. Mechanism of dextran hydrolysis using dextranase

Typically, enzymatic hydrolysis dextranase synthesis by more fungi is induced in the turnout of dextran. Dextran is a chemically and physically complex polymer, hydrolysis of which is carried out by a diversity of dextranases, about 90% of the substrate dextran was transformed to isomaltose or isomalto-oligosaccharides in a 4-h period at 40 °C (Madhu and Prabhu, 1984).

Table 2. The source of dextranases

Source	Name Microbial to produced Dextranase	References
Fungal	*Chaetomium erraticum* *Penicillium aculeatum* *Hypocrea lixii*	Frank and Hans-joachim, 2007; Gibriel et al., 2014
Bacteria	*Brevibacterium fuscum* *Aspergillus subolivaceus* *Aspergillus awamori* F-234 (*A. niger* F-93, *A. fumigatus* F-993, *P. funculosum* NRC289, T. *koningii* F-25 and *A. awamori* F-234), *Bacillus licheniformis*	Sugiura and Ito, 1974; El-Tanash et al., 2011; Foukia et al., 2016
Plants	mammalian tissues	Foukia et al., 2016
Yeast	*Lipomyces starkeyi*	Koenig and Day, 1989

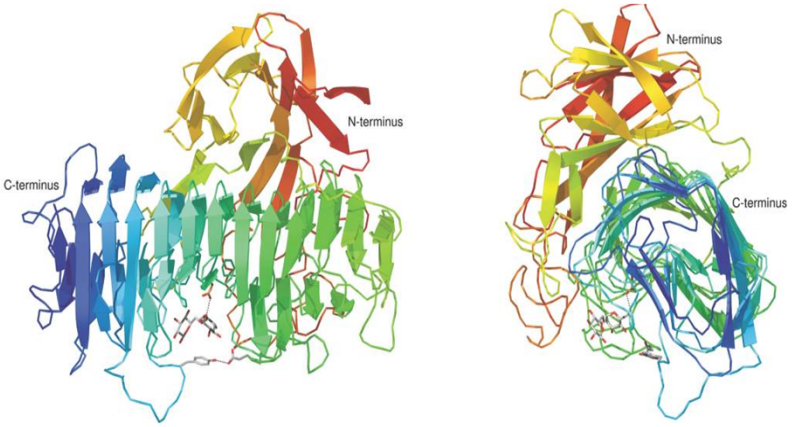


Fig.2.1. Crystal structure of Dextranase Dex49A from *Chaetomium erraticum* (Huang et al., 2015; Khalikova, et al., 2005).

Dextranase (1-6-α-D-glucan6-glucanohydrolases or glucanases, are the enzyme that specifically hydrolyze α-(1-6) linkages in dextran. Since many dextrans contain a relatively high concentration of secondary linkages, other than α-(1-6), and enzyme that can break (1-2), (1-3) and (1-4) linkages in dextran, is also included together with true dextranase. The hydrolysis products of dextran by dextranase are glucose with dextranase, isomaltose and isomalto-oligosaccharides' (Mao et al., 2012; Jaiswal and Kumar, 2011). The reaction mechanism is shown in Figure 2.2.

CED has been used in a study for immobilization of CED to consider the optimization of process provisions for enzymatic hydrolyzing dextran at sugar manufactory in order to progress or improve the efficiency of sugar production. The application of dextranase in the sugar manufactory industry to degrade dextran into smaller molecules was suggested by Tilbury (1971) and by Tilbury and French (1974).

In this way, the average molecular weight of dextran produced by CED can be modified by alteration of substrate concentration, pH and temperature. However, the optimal pH and temperature immobilization were pH 6.0 and 60 °C respectively. These results indicate is a novel GH31 dextranase with high trans glucosylation activity (Zhang et al., 2017; Gozu et al., 2016; Gutiérrez et al., 2016).

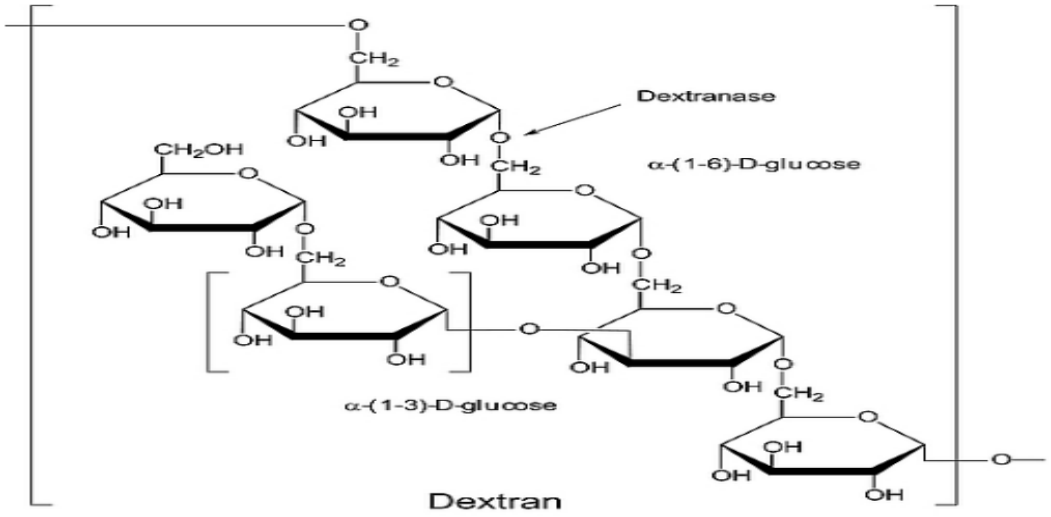


Figure 2.2. The reaction mechanism of dextranase of dextran hydrolysis (Leemhuis et al., 2013).

The α-glucans produced by dextransucrase and dextranase has the added characteristic of being indigestible by human stodgy enzymes are categorized classified as fiber (Leemhuis et al., 2013).

In a factory, dextran slows culmination the crystallization aggregate or even prevents crystallization. The decline of crystallization warmth is eligible for energy parts, however, is not viable on the occurrence of dextran but the weight of dextran boost strongly with the reduction of temperature (Anekella, 2012)

2.2. IMOs

The manufacturing IMOs crop of the enzymatic hydrolysis of dextran into great IMOs, followed by the progress of dextranase, which catalyzes the change of the non-reducing glucosyl moiety of dextran to the 6-OH group of glucose delivered by hydrolysis, the non-reducing glucosyl piece of dextran or any α-glucooligosaccharide instant in the solvent (Goffin et al., 2011). Dextran decrease depressed the crystallization rate or even inhibits crystallization due to enzyme dextranase to provide IMOs. The purpose of commercial dextranases to split dextran to result in IMOs. IMOs are good food additives according to (Gericke et al., 2016; Goffin et al., 2011) because of they have many biological functions like:

- Preferment of the majority of Bifidobacteria in the big intestine of humans and animals and modification of the cariogenic influence of sucrose, larger carbohydrates as starch, maltose, sucrose, and dextran, acceptor reactions of dextransucrase or processing of sucrose amidst dextransucrase and dextranase also

gives rise to IMOs (Kaneko et al., 1994).

- IMOs are used in biscuits, cakes, food bars, as sweeteners and as an additional prebiotic fiber in some commercial foods to impart the required flavor (Gericke et al., 2016).
- IMOs at a dose of 15-20 grams per day prevent abdominal bloating, nausea, excessive gas formation, constipation, diarrhea, bone erosion and colon cancer; especially tooth decay (Oku and Nakamura, 2003).
- IMOs can be added as dietary fibers in certain mercantile foods (Lattimer et al., 2010).
- IMOs are prebiotic and that is more advantageous to the increase of propitious bacteria in the colon (Hutkins, 2016).
- IMOs has approximately semi the calories of sucrose and it unites fiber to the meal,
- IMOs also killed off deleterious bad-bacteria, whose involve bacteria such as Shigella, Escherichia coli, and salmonella (Bouhnik et al., 2004).
- IMOs has also been shown to help the body's capacity to absorb metals from food and nutrition or IMOs are added to processed foods to improve the bulk and texture (Kaneko et al., 1992).
- IMOs have effect to reduce cholesterol and triglycerides in the body and blood, after then the organization. Metabolism especially human sex organization (Kohmoto et al., 1992).
- IMOs are not fermentable by flat mouth bacteria in order to it does not help surface tooth decay.

IMOs are generally synthesized by neopullulanase from starch and by dextranase from dextran (Sankpal et al., 2001; Mahmoud and Helmy, 2009) which will contribute to the technological development of our country, it's too important for food production industry, medicine and health sectors.

Aacceptor reactions have been widely studied for the production of IMOs from glucose, maltose, isomaltose, isomaltotriose, panose and kojibiose.

2.3. Properties of dextranase

2.3.1. Effect of temperature and pH on the free and immobilized dextranase activty

Many enzyme stability in a wider range of pHs between (1-12) some be stable in acidic conditions and other be stable in the basic condition and temperature and tolerance to a variety of organic solvents and more factors to affect growth microbial such as water activity, moisture content, temperature, pH, oxygen levels, and concentrations of nutrients to product enzymes (Tingirikari et al., 2017).

2.3.2. Dextranase kinetics

The topical of Michaelis–Menten kinetics with K_m and V_{max} values are to know the specificity of the enzyme towards several or different substrates which are used to differentiate the enzymes from several sources. The kinetic characteristics of one of the dextranase were studied. Two activity assays were compared to determine the kinetic parameters, this is about K_m, also for K_m determination advantages to recognized the standard condition of enzymatic effect, as well as it informs the relative suitability of other substrates against a particular enzyme being characteristic of enzyme affection towards the substrate (Wang et al., 2014; Jeanningros et al.,1976).

Virgen-Ortíz et al., (2015) reported K_m and V_{max} values estimated as 2.6 ± 0.1% and 2280 ± 9/mol min/mg protein, respectively from the, Dextranase catalyzed hydrolysis of dextran followed Michaelis-Menten kinetics., (Aslan and Tanrıseven (2007b) reported Kinetic parameters were also determined with

Table 2.2. The optimum temperature and pH stability of microbial dextranase from different sources

Sources	Optimum temperature (ºC)	Optimum pH	References
Chaetomium erraticum	50-55	5.0-6.0	Jiménez, 2009
Chaetomium erraticum	50-55	5.0–5.5	Naessens et al., 2005
Chaetomium erraticum	55-60	5.2-6.0	Virgen-Ortíz, 2015
Chaetomium gracile	60	5.0-6.0	Clarke et al., 1997b
Chaetomium erraticum	30	5.4-6.0	Erhardt et al., 2007

Lineweaver–Burk plot, The K_m values for dextran of soluble or immobilized enzyme are 13.1g and 15.7g dextran L−1, respectively. V_{max} for free and immobilized enzyme are 4.4g and 4.0 g IMO L−1 min−1, respectively. Kinetic parameters (K_m *and* V_{max}) were calculated fitting the initial rate values to the Michaelis–Menten equation:

$$V=V_{max}[S]\ (K_m+[S])-1$$

Kinetic constants, K_m *and* V_{max}, of the dextranase were determined by changing the dextran substrate concentration. Lineweaver –Burk double reciprocal (1/V versus 1/S) plot was constructed to calculate the K_m and V_{max}. With a slope of K_m/V_{max} and the intercept of $1/V_{max}$ estimated K_m and V_{max} values were calculated as given in double-reciprocal plot. (El-Tanash et al., 2011)

Enzymes are protein molecules which serve to accelerate the chemical reactions of living cells, 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. Immobilization of enzymes is of great importance in the processing and analysis of food (Tümtürk, et al. 2007; Khan and Alzohairy, 2010).

Immobilization enzyme means movement or inactivation, and this is a fully immobilized enzyme; an enzyme immobilized physically on a solid support through which a substrate is transferred and converted to product. Immobilized enzymes can operate at a multi-point pH and temperature range and exhibit higher thermal stability than native ones; multi-point, multi-subsided immobilization, or the creation of appropriate environments.

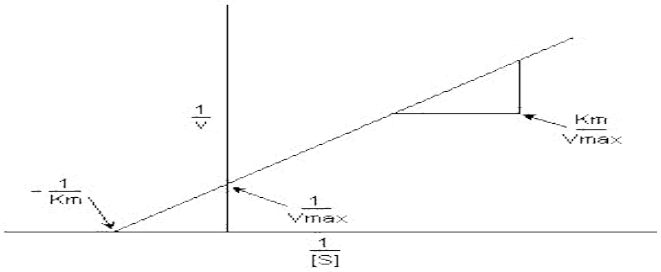


Figure 2.3. Double reciprocal plot of 1/v versus 1/[S]

2.4. Immobilization of enzymes

Reactions in which the composition can be accurately monitored, changing conditions and physiological (e.g. temperature, pH, coenzyme concentrations) throughout the reactions. Inactivity makes it easier to recapture and reuse costly enzymes in many applications in economic practice and allows for continuous, fixed bed operation. Another benefit is usually an increase in stability (Sheldon, 2007).

2.4.1. Advantages and disadvantages of enzyme immobilization

Immobilization enzymes are actually the case of considerable interest in order that their advantages or goods of using immobilized enzyme are shown below:

- ✓ The reaction can be separated from the environment by simple methods, or easy separation from reaction mixture,
- ✓ More resistant to high temperature, pH and thermal stability,
- ✓ Enables the implementation of continuous processes,
- ✓ Can be used repeatedly, or Re-use of enzymes for many reaction cycles,
- ✓ It can protect its activities for months in storage conditions,
- ✓ Reduces product cost,
- ✓ Enables pure product handling,
- ✓ It is possible to selectively synthesize some materials,
- ✓ Product inhibition can be avoided,
- ✓ Wider choice of reactor design.
- ✓ Control of biocatalytic microenvironment, protection from shear forces and air bubbles, limitation of side reactions multienzyme immobilization charge of matrix can enhance substrate affinity,
- ✓ The other major advantages include greater control over enzymatic reaction as well as high volumetric productivity with lower residence time, which are of great significance in the food industry, especially in the treatment of perishable commodities as well as in other applications involving labile substrates, intermediates or products

In spite of the advantages, the first approach to overcome these disadvantages has been to add stabilizers pumped into the water. Metals, surfactants, proteins, amino acids and some sugars are added to the enzymes. This inhibitory contribution is due to the fact that non-covalent, however, no additional compound is present in this assay

when the same enzyme is used 50 times more. The immobilization process has its shortcomings, for example; decline of the enzyme action subsequently immobilization or infiltration of the enzyme from the matrix, thus industrial applications are limited due to some disadvantages and these disadvantages are listed below:

- ✓ Wastage of enzyme action as a result of immobilization progression or wastage of biocatalyst in unsteadiness of material support mass transfer limitations (substrate) and restriction to smaller molecules Immobilization in particles may result in reduced access of macromolecules
- ✓ Capital recruitment required to present up-to-date equipment for implanting process.
- ✓ Immobilization costs (carriers may be not cheap immobilization techniques need fine tuning for each enzyme/cell type, The immobilization of biocatalysts consequences in non-incentive dry quantity of above 90% and hence decreases the volumetric and space-time yields (Tanaka and Kawamoto, 1999).

2.4.2. Methods for enzyme ımmobilization:

Many methods use for immobilization enzymes can also effect the kinetic parameters of the immobilized enzyme (Samoshina et al., 1987), all immobilization techniques are classified in to two as chemical and physical methods (Brahmachari, 2017). The methods used for immobilization of enzymes include adsorption, entrapment, cross-linking and covalent attachment are seen in Figure 2.4 (Tanaka and Kawamoto, 1999).

Enzyme immobilization methods are generally classified according to the type of binding reaction (Brena et al., 2013). Accordingly, enzyme immobilization methods

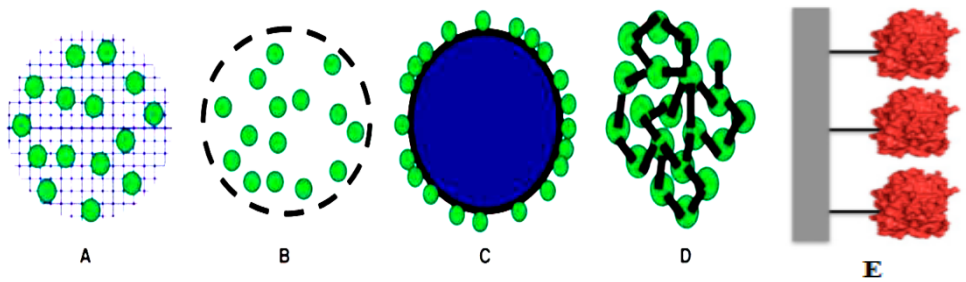


Figure 2.4. Methods of enzyme immobilization. **A.** Entrapment, **B.** Encapsulation. **C.** Adsorption, **D.** Cross-linking, **E.** Covalent Linking (binding).

are mainly divided into four main groups: Adsorption, entrapment, cross-linking covalent bonding with bi-functional reagents (Tanaka and Kawamoto, 1999) (Figure 2.6.).

2.4. 3. Physical adsorption and ionic binding

The adsorption method is to bind the enzyme to an inert matrix with the weakest attraction forces. 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 by the matrix. Ion exchangers easily adsorb proteins and are widely used for enzyme immobilization. But its simplest immobilization method is simple, simple and inexpensive, one of the important features of this technique, which is of great significance, is that, unlike ionic binding, hydrophobic interactions are usually stabilized by high ionic concentrations (Brena and Batsita-Viera, 2008).

Physical adsorption and ionic bonding are less conformational change of the enzyme and adsorption method, pore diffusion limitations will not be because the enzymes are externally immobilized on the support or carrier, but their disadvantages are the absorption of ineffective adsorption and adsorption such as physical adsorption and ionic binding and interactions, and enzyme leakage when the affinity is weak.

Physical adsorption and ionic bond immobilization of protein with epoxy support, metal-chelate formation, ion exchange and others have been investigated for this purpose in various commercially available ion exchange modifiers, In contrast to ionic bonding, one of the most important, hydrophobic interactions, stabilized with high ionic concentrations (Rivero et al., 2017; Guisan, 2013).

Ease of technique and obtaining great efficiency arrangements due to the surrounding circumstances can be the advantages of ionic binding method. Hence, the

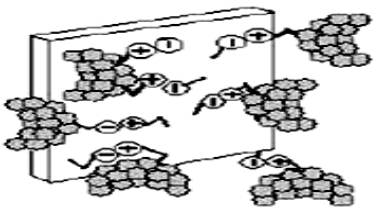


Figure 2.5. A carrier by adsorption and ionic binding (Costa et al., 2004)

ionic binding does little changes in the conformation and the active site of enzyme. The enzyme protein and carrier bind lower activity than in covalent binding, the leakage of enzyme may appear by converting the pH or the ionic strength (Costa et al., 2004).

2.4.4. Covalent binding

Enzymes can usually be immobilized with amino, hydroxyl, carboxyl, phenolic groups or imidazole groups, threonine group, indole group. Covalent bonding to a large number of natural and artificial rigid matrices, Covalent bonds among the enzyme remain and carrier (e.g. silica, wood chips, controlled pore glass) (Katchalski-Katzir and kraemer, 2000).

The matrices containing the active groups are immobilized by treatment with appropriate buffer solution enzymes. Urethane pre-polymers are commonly used in covalent immobilizations. Pre-polymers are obtained from the reaction of disocyanate derivatives with various compounds containing active hydrogen (glycol, polyglycol, polyol), (Brahmachari, 2017) and there are isocyanates when the pre-polymers are treated with water, the polymer in the sponge-like structure forms. When the enzyme is present in the medium, the isocyanate groups form covalent bonds with the active hydrogen-containing groups of the enzyme (Phathk et al., 2012).

Enzymes like dextranase, glucose oxidase, peroxidase, invertase, etc. have been immobilized using these technique different matrixes and techniques have been used for the covalent immobilization of dextranase.

The advantage of this method is most stable nature of bonds created amidst or between enzymes and beds (matrix) the enzyme is not released into the solution at use (Husain, 2010).

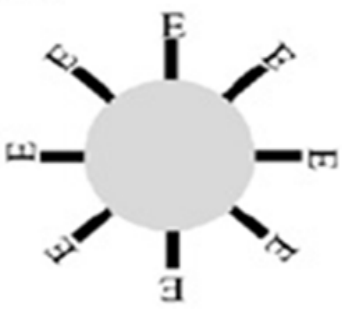


Figure 2.6. Covalent binding between the enzyme and carrier (Brahmachari et al., 2017)

The immobilization enzymes are typically very constant at conditions pH range and temperature and no enzyme permeation. The covalent binding should also be optimized so as not to convert its conformational plasticity. However, disadvantages covalent bindings are matrix and enzyme are not reconstitute major lack of activity can be denied by covalent bonding through the carbohydrate moiety when a glycoprotein is interested (Albayrak and Yang, 2002).

In her either natural polymers, such as modified cellulose, starch, dextran, agal polysaccharides, collagen, and gelatin; or they can be synthetic, such as polystyrene, polyacrylamide, polyacrylates, hydroxyalkyl methacrylates, and polyamides. Inorganic supports can also be used, such as porous glass, metal oxides, metals, sand, charcoal, Eupergit C, and Eupergit CM. Currently the immobilization processes that easy, in the Food Industry for e.g. application dextran hydrolysis production of sugar hydrolysates isomaltooligosaccharides (IMOs) by dextranase. Few other approaches including covalent binding, adsorption, entrapment, and cross-linking of the enzymes require to be tried out, some passed already to production scale but are not yet available and glucose isomerization granules of glutaraldehyde cross-linked microbial cells homogenates with glucose isomerase activity (current) (DiCosimo et al., 2013; Aslan et al., 2007b)

2.4.5. Entrapment

Entrapment's a standby type of immobilization method; however, the technique is more complex, the support itself may have several morphologies and one differentiates amidst surface engagement and trellis entrapment. Alginates are anionic, linear, branched, and cyclic, composed of α (1.4) -L-glucuronic acid (GA) and β (1,4) -D-mannuronic acid (MA) units gained from brown seaweed (Phaeophyceae, mainly Laminaria Hyperborea) polysaccharide (Blandino et al., 2001).

The chemical structure of the alginate molecule is shown in (Figure 2.7.) The ratio and sequence of the GA and MA monomers in the alginate molecules varies according to the source from which alginate is obtained. Alginates form gels in aqueous solutions by ionic bonding with divalent cat ions such as Ca^{2+}.Gel formation occurs as a result of the cooperative binding between the carboxyl groups of the GA's in the different alginate chains. Using alginate, enzymes can be immobilized by various methods. Enzyme, alginate calcium alginate beads are formed when they are added to the calcium chloride solution. When the enzyme is mixed with calcium chloride and

dropped into alginate solution, calcium alginate capsules are formed. When the enzyme-alginate-glycerol mixture is injected into the calcium chloride solution in the form of a syringe, calcium alginate fibers are formed. The pore size of the resulting bead, fiber or capsule depends on the concentration of alginate and calcium chloride solution used. (Rehman et al., 2016).

The polymers used in the Entrapment they belong to three major groups:

- ✓ Polysaccharides; dextran, starch, agar, agarose and gelatine carrageenans, cellulose, gellan, chitosan, alginates (not dextran is extremely hydrophilic and more stabilized over a spacious range of pH (i.e., alkaline conditions and mild acid) and its lazy degradation by human enzymes as compared to other polysaccharides and specific split by microbial dextranase in the relating to the stomach and the intestine or gastrointestinal tract (Riahi et al., 2017)**.**
- ✓ Proteins; collagen, albumin, and gelatine. The liposome type employs entrapment (Chen and Ou-Yang, 2004).
- ✓ Merging enzymes into the network of a semi- artificial polymers; epoxy resins, poly acryl amides, silicones, polyvinyl alcohol, polyurethanes and nylon permeable gel or enclosing the enzymes in a semi-permeable polymer membrane (Brahmachari et al., 2017).
- ✓ The entrapment strategies are simple to conduct due to Enzyme Immobilization by Entrapment during a gel network (Brady and Jordaan, 2009).

In this case, the enzyme is not binding to the matrix or film these methods different from the anther methods to immobilization enzyme itself does not bind to the membrane or gel matrix, so the advantage of entrapment form immobilization enzymes has a wide enforcement ability, gel matrix in entrapment methods fast process of

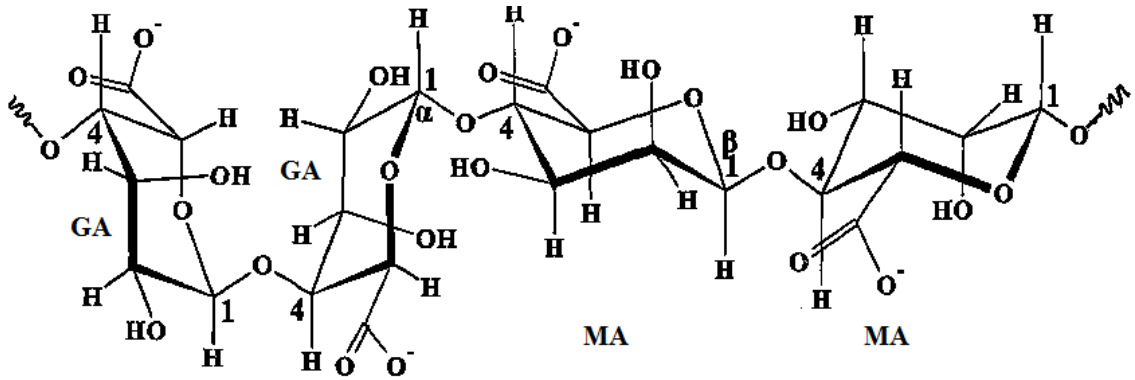


Figure 2.7. Molecular structure of alginate polymers. GA: Gluronic acid, MA: Mannuronic acid

immobilization, no expensive, easy to pursuit at little scale modest stipulations are required, lesser chance of conformational changes in enzyme, can be used for sensing implementation (Guisan, 2013; López-Gallego et al., 2013).

The disadvantage of entrapment form immobilization enzymes such as, poison publishing limitation problems between substrates and off spring's and infiltration of enzyme chance of microbial contamination not much success in industrial process (Guisan, 2013).

An alginate beads was used to immobilize dextranase through the entrapment technique as described by Frank and Hans-Joachim (2007) and dextranase from *P. aculeatum* was immobilized by entrapment in polyacrylamide gel, hydroxyapatite and alginate beads (Gibriel, 2014) and results of *Penicillium aculeatum* NRRL-896 dextranase immobilization by entrapment in polyacrylamide were higher than that reported for other immobilized *P. funiculosum* 258 dextranase by entrapment in polyacrylamide (Mohamed et al., 1999).

2.4.6. Cross-linking

In this style of enzymes immobilization is too named as co-polymerization, Cross-linking enzyme immobilization (Figure 2.9) is related by covalent bonds in the midst different groups of enzymes via many-functional reagents in these styles different to other methods due to no use matrixes or support implicated in the way of immobilization enzymes (Dong and Zhang, 2017).

Commonly used multifunctional reagents are heterogeneous catalysts which are amino acid NH2 groups or are typically glutaraldehyde, which reacts with amino groups of enzymes such as glutaraldehyde, aldehyde, dextran, and which reacts with aggregate cross-linked enzymes (which form CLEs) which are insoluble in aggregates or a lot of

(a) Entrapped in a matrix (b) microencapsulation

Figure 2.8. Entrapped in a matrix and microencapsulation between matrix (carrier) and enzymes (Hill, 1991)

cross-linking reagents such as glutardialdehyde, aldehyde, carbodiimide, and sulfate-de (Brahmachari et al., 2017 and Kim, 2007). Glutaraldehyde is the cross-linking reagent used for this purpose (Klibanov, 1983).

In the cross-linking method turnout of an indolent protein or an inert protein such us bovine serum albumin (BSA), collagen and gelatin in this way cross-linked protein competence be easily separated by centrifugation the technique established by (Babu and Panda, 1991).

Aldehyde groups in the bi-functional glutaraldehyde molecule react with the amino groups of the enzymes to cross-link the enzymes. Cross-linking can occur intermolecular or and protein precipitates. The cross-linked protein can be easily separated by centrifugation (Klibanov, 1983). However, starting conditions of the biocatalyst (concentration of reaction components, temperature, reaction time and organic solvents) are given more stable between of carrier-free immobilized enzymes and lipase and penicillin amidase are used as cross-linked enzyme crystals as cross-linked enzymes trypsin, and papain, the penicillin acylase and cross-linked spray-dried enzymes as the cross-linked enzyme from the aggregates are the best known enzyme cross-linked products This procedure allows enzymes to be immobilized without the use of a carrier, which not only decreases the cost, but also avoids “dilution” of the enzymes' activity (Panesar et al., 2010; Šulek et al., 2011; Cao et al., 2003).

Usefulness in this Cross-linking is biocatalyst settlement, plain and inexpensive or cheap but not predominating used with neat enzymes and high qualification; this process is widely utilized in industrial implementations but disadvantage or demerit of this whey is that the bi-functional reagents used for cross linking the enzyme may denature or structurally modify the enzyme leading to the loss of activity due to the participation of the active site in bound figuration (Guisan, 2013; Cowan and Ferendez-lafuente, 2011).

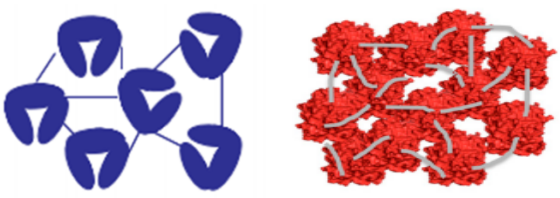


Figure 2.9. Cross-linking immobilization enzymes between bi-functional reagents and covalent bonds in the midst different groups of enzymes (Xiuyun and Shunichi, 2013)

Immobilized dextranase are also used in processing sugar industry by cross-linking dextran used to products isomalto-oligosaccharides (Gibriel, 2014).

2.5. Enzyme immobilization with c-MWNT

Nanoparticles are excellent matrices for enzyme immobilization (Prlainovic et al., 2013) because they have high surface area and mechanical stability, which are interesting and important features for the immobilization process. There are numerous studies in the literature about the use of single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs) in adsorption and covalent binding methods for enzyme immobilization. Immobilization by adsorption is carried out by incubating the enzyme solution with the matrix-containing buffer solution at the appropriate time (Garlet et al., 2014). In covalent immobilization (Figure 2.10), the carboxyl (-COOH) groups on c-CNTs are first activated by N-ethyl-N'-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC). The enzyme molecules are then matrically linked through these groups (Yin et al., 2009). The enzyme immobilization studies with c-MWNTs are summarized in Table 2.3.

2.6. Previous studies related to immobilization of CED

Immobilization of CED by covalent attachment, physical adsorption on various carriers including Eupergits, silica gel and bentonite reported by Erhardt and Jördening (2007) and on Ca-Alginate gels by Bashari et al., (2014).

In some study with c-MWCNT, enzyme immobilization resulted in 5 times (Jamie et. al., 2016) and 12 times (Zniszczoł et al., 2016) increases of the activity. Therefore, in this thesis study, immobilization of CED on c-MWCNT by adsorption method was investigated.

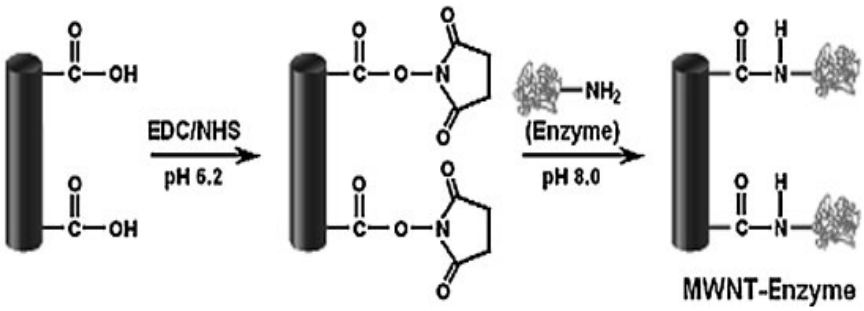


Figure 2.10. Enzyme immobilization with c-MWNT

Table 2.3. Immobilization studies using c-MWCNT

Enzyme	Immobilization yield (%)	Activity yield (%)	Operational Stability (%)	Storage stability (%)	References
Aspergillus niger glucose oxidase	Not determined	Not determined	Not determined	Not determined	Soo-Keun et al., 2005
Candida antarctica lipase B	Not determined	Not determined	Not determined	% 55 after 6 months	Pavlidis et al., 2010
Papain from Genview, USA	18.8	78.9	61 after 7 using	Not determined	Wang et al., 2011
Candida rugosa lipase	52	48	Not determined	Not determined	Rastian et al., 2013
Aspergillus niger inulinase	90	Not determined	Not determined	100 after 5 weeks	Garlet et al., 2014
Candida rugosa lipase	86.7	492.5	123,7 after 7 using	Not determined	Rastian et al., 2014
fungal peroxidase	100	Not determined	0 after 9 using	34 after 40	Azevedo, 2014
α-Amylase ve glucoamylase	Not determined	95.1	95.1 after 8 using	Not determined	Feng et al., 2015
Candida rugosa lipase	85.6	500	Not determined	Not determined	Jamie et al., 2016
Pseudomonas fluorescens lipase	59	1200	60 after 8 using	Not determined	Zniszczoł et al., 2016

3. MATERIALS AND METHODS

3.1 Materials

Chaetomium erraticum Dextranase (E.C.3.2.1.11) which have standardized to 30,000 units/ml activity was provided as a gift by Bio-Cat (Troy, USA). Dextran T70, purchased from Carl Roth GmbH-Co. KG. Polyvinylidene di fluoride (PVDF) membrane (48mm and 0.1 μm) were purchased from Co. (Cork, Ireland). Carbon nanotubes were purchased from Grafen Co. (Ankara, Turkey). 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). The 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). The 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, sodium sulfite, phenol, 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 (Rochelle salt) was purchased from VWR Prolabo Chemicals (Leuven Belgium). Sodium azide was purchased from Merck Millipore (Darmstadt, Germany).

3.2. Methods

3.2.1. Determination of protein

The quantities of proteins, amounts of an enzyme in the immobilization solutions before and after immobilization will be determined according to Bradford Protein Assay Method (1976) method using UV-VIS Spectrometer.

Accordingly, the 100 μL samples were added to 3 mL Bradford reagents at 45 minutes at room temperature after than for measuring the protein amount by using Bradford reagents to protein assay protocol read at 595 nm by using UV spectrophotometer. The amount of immobilized enzyme protein was checked from the

difference between the amount of protein used for immobilization minus that recovered into the supernatant plus washings (Manjon et al., 1995).

Immobilization the binding and activity yields using the amounts of enzyme in the immobilization solutions before and after will be calculated using the following Eequations:

$$Immobilization\ Yield\ (\%) = \frac{Enzyme\ used\ -\ enzyme\ in\ filtrate}{Enzyme\ used} x100 \quad (3.1)$$

$$Activity\ Yield\ (\%) = \frac{Activity\ of\ immobilized\ enzyme}{Activity\ of\ soluble\ enzyme} x100 \quad (3.2)$$

3.2.2. Determination of dextranase activity

In this case, 1% (w/v) of dextran solutions prepared 5 mL 25 mM sodium dihydrogen phosphate buffer (pH 5 for free enzyme and pH 6 for immobilized enzyme) was reacted with 0.2 ml/L of free or 0.785 g the immobilized enzymes having 19.41 IU and 22.15 IU dextranase activities, respectively at 55 ^{o}C for 60 min in an incubator shaking rate 150 rpm. 200 µL of samples from reaction mixtures were added to 1.8 ml of distilled waters and boiled for 10 min in bath boiling to inactivate the dextranase after than add DNS and R.S. The amount of D-glucose created was determined by measuring its absorbance employing a UV spectrophotometer at 575 nm, released reducing sugars were measured by the 3.5-dinitrosalicylic acid according to method of Miller (1959). One IU CED activity was determined as the amount of enzyme forming 1 µg reducing sugar equivalent to 1 µg D-glucose from dextran per minute, under the optimum activity assay conditions.

3.2.3. Immobilization procedure

Adsorption tests were carried out to examine the impact of some variables on the dextranase immobilization from watery solution. The adsorption of dextranase was perfect by using an Immobilization Technique. Typically, 100 mg carbon nanotubes and 200 µL CED were reacted in buffer solutions (0.5 M, pH 6) at room temperature for 5 hours in an orbital shaking incubator with gentle shook at 150 rpm and then the 100 µL aliquots from the immobilization buffer were taken after 5 hours. After immobilization, the beads were filtered through a PVDF membrane on a sintered glass filter by suction

under vacuum pump, and washed with 10 mL of distilled water as two aliquots respectively. The dextranase activity in the aqueous solution was calculated by determining the reducing sugars according to method of Miller (1959).

After then, the beads have been stored in 5 mL of disodium phosphate buffer (0.5 M, pH 6) in a refrigerator at +4 ^{o}C until the next use.

3.2.4. Optimization of immobilization conditions

The amount of bead used, the pH and solution molarity immobilization with immobilization period affects the efficiency of the activity and immobilization (Katchalski-Katzir and Kraemer, 2000). The effect of each of these factors, in turn, can be determined by examining the immobilization conditions have been optimized. Optimum conditions for immobilization were determined by changing individually the conditions, (pHs from 3.0 to 7.0; buffer concentration from 0.025 mM to 0.500 mM; amount of c-MWCNT from 25 mg to 100 mg; and duration of immobilization from 1 h to 5 h).

3.2.5. Characterization of FCED and ICED

3.2.5.1. Optimum pH

Optimum pH for the free and immobilized CED were investigated by determining the activity after the reaction between FCED or ICED with 1% (w/v) dextran solutions, at several pH (3.0-7.0) and 55 ^{o}C for 60 minutes (Aslan and Tanriseven, 2007a).

3.2.5.2. Optimum temperature

The maximum temperature of free and immobilized enzyme was found by conducting the activity assay method with 1% (w/v) of dextran solutions (pH is 5.0 for the FCED and 6.0 for ICED at several temperatures from (30 ^{o}C to 70 ^{o}C) (Aslan and Tanriseven, 2007a).

3.2.5.3. pH stability

In this case, firstly, 200 μL free or 0.785 g immobilized CED having 19.41 IU and 22.15 IU dextranases activities, were incubated with 2.5 ml of 25 mM sodium phosphate buffers with several pH (3.0-3.5-4.0-4.5-5.0-5.5-6.0-6.5-7.0) in an incubator at 55 ^{o}C for 60 minutes. Lastly, the retained activities were determined according to standard activity assay method by adding 2.5 mL of 2% (w/v) buffered dextran solutions (pH is 5.0 for FCED and pH is 6.0 for ICED), (Aslan and Tanriseven, 2007a).

3.2.5.4. Thermal stability

Firstly, 200 μL FCED solution and 0.785 g wet ICED were incubated with 2.5 ml of 0.25 mM sodium phosphate buffers at another pH in an incubator at different temperatures (30 to 90 ^{o}C) for 60 minutes, individually. Secondly, both of FCED and ICED were cooled in a deep-freezer for ten minutes for stopping the temperature effect. Lastly, the retained activities were determined according to standard activity assay method by adding 2.5 mL of 2% (w/v) buffered dextran solutions (pH is 5.0 for FCED and pH is 6.0 for ICED) (Aslan and Tanriseven 2007a).

3.2.5.5. Kinetic constants

Initial velocities for kinetic parameters were determined by performing the reactions between 200 μL of free or 0.785 g of the wet ICED having 19.41IU and 22.15 IU dextranase activities and dextran solutions (pH is 5.0 FCED and 6.0 for ICED) at several concentrations (0.5 g/L to 20 g/L) for 10 min. K_m and V_{max} were determined from Line-weaver–Burk plots (Aslan and Tanriseven 2007a).

3.2.5.6. Operational and storage stabilities

The operational and the storage stabilities of ICED were determined by performing the standard activity assay method after 20 repeated batch experiments and every two days when storing in sodium phosphate buffer (0.5 M, pH 6.0) in the refrigerator at +4 ^{o}C, respectively. The immobilized enzymes used for the determination of storage stability were stored in 0.5 M sodium phosphate buffers (pH 6.0) in a refrigerator at +4 ^{o}C continuously before next use. Before each use, the immobilized enzymes were filtered and washed with 10 mL of distilled water using PVD membrane filter on a sintered glass under vacuum (Aslan and Tanriseven, 2007a).

3.2.6 Hydrolysis of dextran using FCED and ICED

For the effect of substrate concentration on dextran hydrolysis, 200 μL FCED solutions or 0.785 mg wet ICEDs reacted with dextran solutions at different concentrations (from 5 g/L to 20 g/L) at optimum pH and temperatures for 10 minutes.

For the effect of duration of hydrolysis time on the dextran hydrolysis, 400 μL FCED solutions or 1.57 g ICED was reacted with 10 mL of 10% dextran solution (pH 6.0) for 12 hours.

During or after the reactions, 200 μL aliquots withdrawn from reaction mixtures and added to 1800 μL of distilled water. After inactivating the enzymes in boiling water bath for 10 minutes, the released reducing sugars were determined by DNS method of Miller (1959).

3.2.7. Statistical analysis

Each value represents the mean of values obtained in triplicates. Each graphics were drafted by using Origin Pro 8.0 software. Data were investigated using Microsoft Windows Excel each data were given as the mean of three values.

4. RESULTS AND DISCUSSION

4.1. Determination of protein

The BSA standard plot obtained according to Bradford (1976) method is shown in Figure 4.1. The coefficient of determination (R^2) for the correct equation (Equation 4.1) obtained from the plot is 0.99424.

$$Y = 0.29958X \tag{4.1}$$

The enzyme concentrations in the solutions are calculated by using Equation 4.1. Accordingly, CED concentration in the commercial liquid enzyme preparation was calculated as 6.686 mg/mL.

4.2. Determination of CED activity

The amount of reducing sugar released at the end of the reaction of 200 µL of FCED with 1% (w/v) dextran solution (pH 5.0) at optimum activity assay conditions, was found to be 8885.0 µg by using Equation 4.2 obtained from Figure 4.2. Specific FCED activity was calculated as 19.41 IU/mg by using Equation 4.3. Furthermore, the activity of commercial liquid CED preparation was also calculated to be 740.4 IU/mL for DextranT70 as substrate. The amount of FCED having 1 IU activity was also calculated as 0.302 mg.

$$Y = 0.00104X \tag{4.2}$$

$$IU / mg\ Enzyme = \frac{Released\ Reducing\ sugar\ (\mu g)}{Enzyme\ used\ (mg)\ x\ Duration\ of\ reaction\ (min)} \tag{4.3}$$

4.2. Optimization of CED amount

As seen in the Figure 4.3, the released reducing sugar increased by increasing amount of CED. When the higher amounts of CED than 200 µL were used, the released reducing sugar was not increased significantly. Therefore 200 µL CED solutions were used in the next steps.

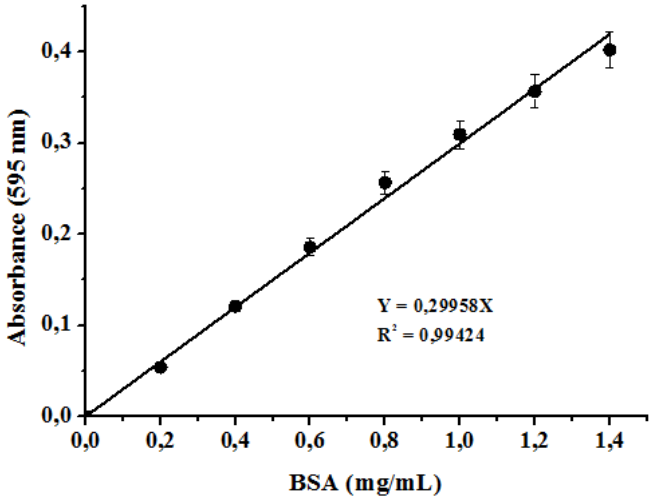


Figure 4.1. BSA standard plot

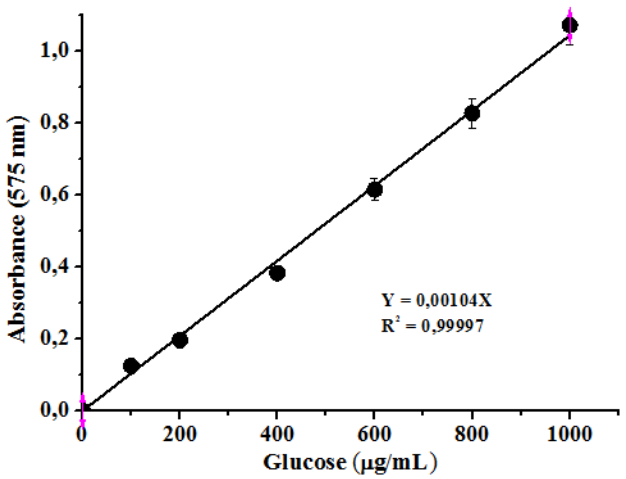


Figure 4.2. Glucose standard plot

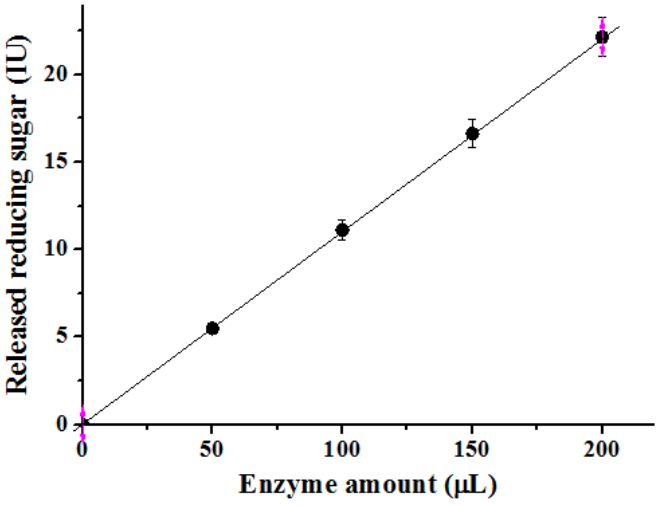


Figure 4.3. Optimization of CED amount

4.3. Optimization of immobilization conditions

The effects of factors affect the immobilization efficiency (amount of matrices versus to fixed amount of enzyme, duration of immobilization, pH and molarity of immobilization buffer) were optimized by changing individualy.

4.3.1. Effect of c-MWCNT amount on immobilization efficiency

The effect of c-MWCNT amount on immobilization efficiency is shown in Table 4.1. The immobilization yield was not changed by increased amount of c-MWCNT. But, the activity yield was increased by increasing amount of c-MWCNT. The highest activity yield (75.59%) was achieved for 100 mg c-MWCNT.

4.3.2. Effect of immobilization duration on immobilization efficiency

As is shown in Table 4.2, Duration of immobilization did not effect the immobilization yield but the activity yield increased by the duration of immobilization. Activity yield was reached to 114.13% after 5 hours.

Table 4.1. Effect of c-MWCNT amount on immobilization efficiency

c-MWCNT (mg)	Immobilization Yield* (%)	Activity Yield** (%)
25	100.00 ± 0.03	32.57± 0.05
50	100.00 ± 0.02	47.41 ± 0.05
75	100.00 ± 0.04	60.37 ± 0.02
100	100.00 ± 0.03	75.59 ± 0.05

*200 µL CED solutions having 19.41 IU dextranase activity were incubated with different amounts of c-MWCNT in 5mL of sodium phosphate buffers (0.5 M, pH 5.5) and room temperature with shaking at150 rpm in an incubator for 1 hour.

**200 µL free or 0.785 g immobilized CED having 19.41 IU and 22.15 IU dextranase activities, respectively were reacted with 5 mL of 1 % (w/v) buffered dextran solutions (pH 5.0 for the FCED and 6.0 for the ICED) at 150 rpm and at 55 ^{o}C in an incubator for 60 minutes.

Table 4.2. Effect of immobilization duration on immobilization efficiency

Duration of Immobilization (hours)	Immobilization Yield* (%)	Activity Yield** (%)
1	100.00 ± 0.04	75.59 ± 0.05
2	100.00 ± 0.03	85.55 ± 0.03
3	100.00 ± 0.02	89.53 ± 0.04
4	100.00 ± 0.03	101.48 ± 0.02
5	100.00 ± 0.05	114.13 ± 0.03

*200 µL of CED solutions having 19.41 IU dextranase activity were incubated with 100 mg of c-MWCNT in 5 mL of sodium phosphate buffers (0.5 M, pH 6.0) at room temperature with shaking in an incubator at 150 rpm for different durations.

**200 µL of free or 0.785 g of immobilized CED having 19.41 IU and 22.15 IU dextranase activities, respectively were reacted with 5 mL of 1% (w/v) buffered dextran solutions (pH 5.0 for the FCED and 6.0 for the ICED) at 150 rpm and at 55 ^{o}C in an incubator for 60 minutes.

4.3.3. Effect of buffer's pH on immobilization efficiency

Table 4.3 shows the influence of pH on immobilization. According to the table, there are no correlation between the buffer's pH and the immobilization yield. At all pH values tested, immobilization yield was 100%. But, the highest activity yield (114.13%) was achieved at pH 6. It is well known that maximum activity yield is achieved at optimum pH of the enzyme, but sometimes this can be changed (Katchalski-Katzir and Kraemer, 2000).

4.3.4. Effect of buffer molarity on immobilization efficiency

According to the Table 4.4, there was also no correlation between the buffer's molarities and the immobilization yield, but the activity yield is increasing when molarities increased. Immobilization yield is 100% for all the buffer's molarities. The highest activity yield (114.13%) was achieved for 0.5 M buffer. Immobilization and activity yields in enzyme immobilization were often affected by the properties of the salts and their concentration, because of salts can change the three-dimensional structures of enzyme molecules (Smalla et al., 1988).

After optimizing the immobilization conditions 100% immobilization yield (0.348 g/g c-MWCNT) and 114.13% activity yield (22150 U/g) was achieved. The immobilization of CED was studied by another researchers. Despite the loaded CED on c-MWCNT is lower than the loaded CED on bentonite (0.8 g/g), the achieved activity in

Table 4.3. Effect of immobilization buffer's pH on immobilization efficiency

Immobilization Buffer's pH	Immobilization Yield* (%)	Activity Yield ** (%)
3.0	100.00 ± 0.05	87.48 ± 0.03
4.0	100.00 ± 0.03	86.77 ± 0.02
5.0	100.00 ± 0.02	96.98 ± 0.04
6.0	100.00 ± 0.04	114.13 ± 0.05
7.0	100.00 ± 0.03	90.04 ± 0.04

*200 µL of CED solutions having 19.41 IU dextranase activity were incubated with 100 mg of CNT in 5mL of sodium phosphate buffers (0.5 M) at different pHs and room temperature with shaking at 150 rpm in an incubator for 5 hours.

**200 µL of free or 0.785 g of immobilized CED having 19.41 IU and 22.15 IU dextranase activities, respectively were reacted with 5 mL of 1% (w/v) buffered dextran solutions (pH 5.0 for the FCED and 6.0 for the ICED) at 150 rpm and at 55 ^{o}C in an incubator for 60 minutes.

Table 4.4. Effect of immobilization buffer's molarity on ımmobilization efficiency

Buffer's Molarity (M)	Immobilization Yield* (%)	Activity Yield** (%)
0.025	100.00 ± 0.03	66.54 ± 0.04
0.050	100.00 ± 0.02	71.42 ± 0.05
0.100	100.00 ± 0.04	91.07 ± 0.02
0.250	100.00 ± 0.05	94.41 ± 0.03
0.500	100.00 ± 0.03	114.13 ± 0.05

*200 µL of CED solutions having 19.41 IU dextranase activity were incubated with 100 mg of CNT in 5 mL of sodium phosphate buffers (pH 6.0) at different concentraions at room temperature by shaking in an incubator at 150 rpm for 5 hours.

**200 µL of free or 0.785 g of immobilized CED having 19.41 IU and 22.15 IU dextranase activities, respectively were reacted with 5 mL of 1% (w/v) buffered dextran solutions (pH 5.0 for the FCED and 6.0 for the ICED) at 150 rpm and at 55 ^{o}C in an incubator for 60 minutes.

our study is higher than achieved activity (12000 U/g) in another study (Erhardt and Jördening, 2007). On the other hand, both of the loaded CED on c-MWCNT (100%), immobilization yield (100%) and activty yield (114.13%) is higher than loaded CED, immobilization yield (93%) and activity yield (90%) in the immobilization of CED on Ca-alginate gels in the study of Bashari et al.(2014). Therefore, the immobilization efficiency achieved in our study is the better than previous two studies.

4.4. Characterization of free and immobilized CED

4.4.1. Optimum pH

According to Figure 4.4, optimum pH of CED was shifted from 5.0 to 6.0 after immobilization. This result is agree with the study of Bashari et al. (2014). Similar result can be see in the related literature. On the other hand, ICED is more active than FCED at the pH tested except 5.0.

4.4.2. Optimum temperature

As shown in Figure 4.5, optimum tepmperature of CED was not changed after immobilization. But in the study of Bashari et al. (2014), optimum tamperature of CED was shipted from 50 ^{o}C to 60 ^{o}C after immobilization on Ca-Alginate gels. This difference may resulted from the differencies of the structure of two support. On the other hand, it is also seen in the Figure 4.5 that ICED is more active than FCED at all temperatures tested.

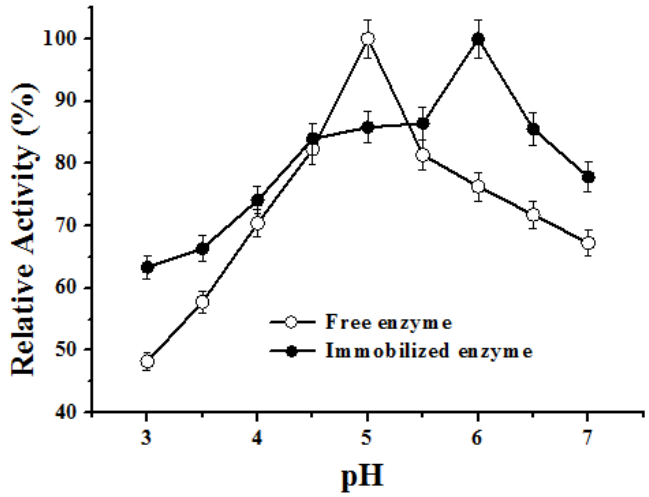


Figure 4.4. Optimum pH of the free and the immobilized CED

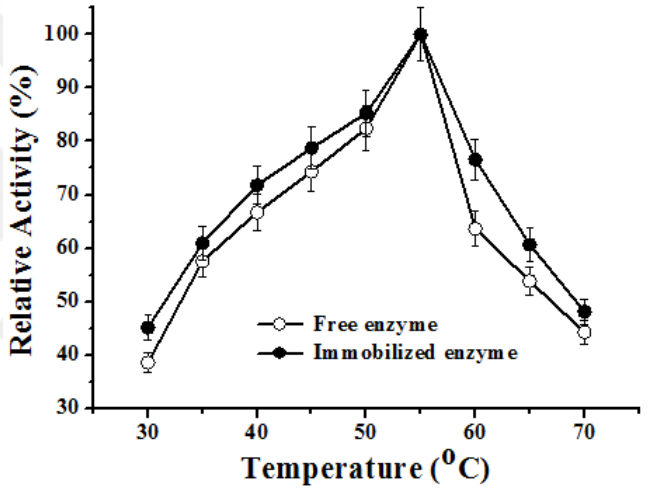


Figure 4.5. Optimum temperature of free and immobilized CED

4.4.3. pH stability

Figure 4.6 shows the effect of pH on the stability of CED. Accordingly, ICED is more stable than FCED at the all pH values tested. This result can be understand, because of it is well known that immobilization increase the pH stability of enzyme.

4.4.4. Thermal stability

According to Figure 4.7, ICED is more stable than FCED at higher temperatures. at 65 ^{o}C ICED didn't lose its activity, while FCED lost ~ 4 of its activity. At the upper temperatures than 65 ^{o}C FCED lost rapidly its activity. When FCED was completely inactivated at 80 ^{o}C, ICED retained 50% of its activity. Furtermore, ICED retained 20% of its activity at 90 ^{o}C. It is also well known that immobilization incresae the thermal stability of enzyme.

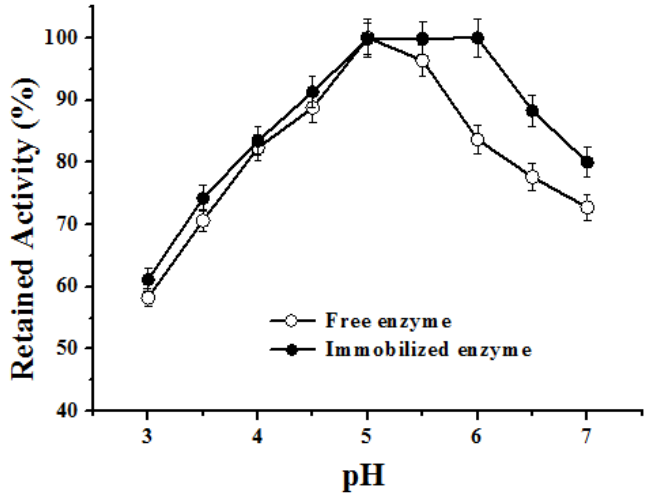


Figure 4.6. pH stability of free and immobilized CED

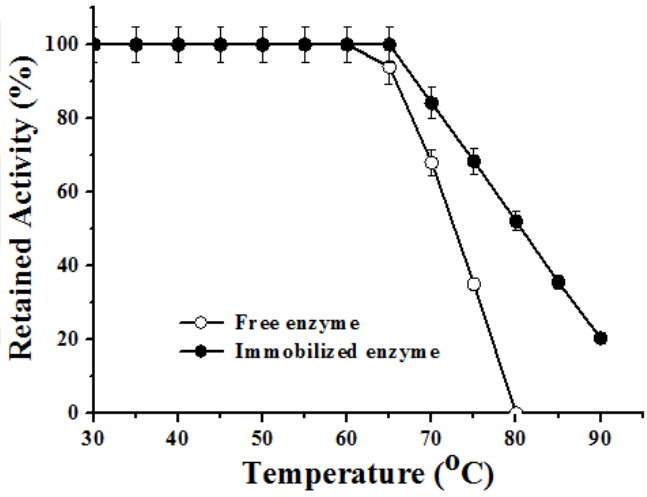


Figure 4.7. Thermal stability of free and immobilized CED

4.4.5. Kinetic constants

The kinetic constants for the free and for the immobilized CED were also determined by using the Lineweaver-Burk plot (Figure 4.8). The K_m values of the free and immobilized enzymes were determined to be 56.18 g/L and 65.36 g/L, respectively, while the V_{max} values were determined to be 523.56 µg reducing sugar/L.min and 595.24 µg reducing sugar/L.min respectively. The K_m represents the affinity of an anzyme to its substrate. There are negative correlation between the affinity and K_m. When K_m decreases the affinity increases.

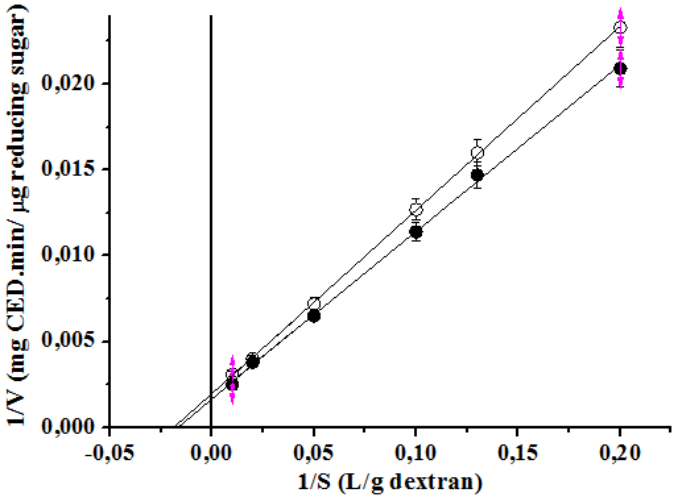


Figure 4.8. Kinetic constant of free and immobilized CED

4.4.6. Operational and storage stabilities of immobilized enzyme

As shown in Figure 4.9 and Figure 4.10, ICED has perfect operational and storage stabilities. This result better than achieved operational and storage stabilities in previous two studies (Erhardt and Jördening, 2007; Bashari et al., 2014). CED immobilized on all supports lost more than 80% of their activities after six recycle (Bashari et al., 2014). Erhardt and Jördening (2007), did not determine the operational and storage stabilities of CED immobilized on Ca-Alginate gels.

4.5. Hydrolysis of dextran using FCED and ICED

The optimum condition of dextran hydrolysis was determined by changing the factors such as substrate (dextran) concentration and duration of hydrolysis that affect the hydrolysis yield, individually. According to Figure 4.11, the released reducing

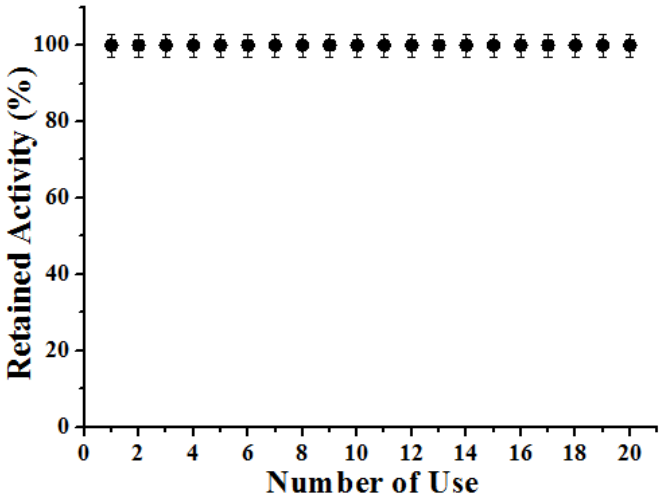


Figure 4.9. Operational stability of immobilized CED

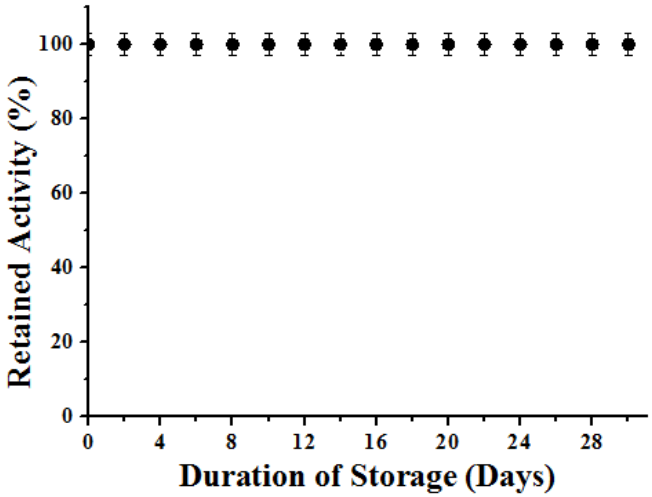


Figure 4.10. Storage stability of immobilized CED

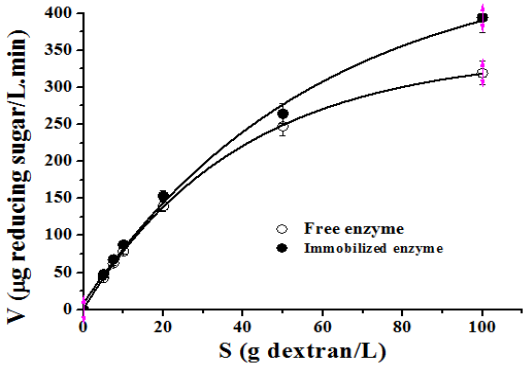


Figure 4.11. The effect of substrate concentration on the released reducing sugars

sugars (IMOs and glucose) concentration were increased and reached the maximum level at 100 g/L. As shown in Figure 4.12, the released reducing sugar concentration was increased by the incresing hydrolysis time until 10 hours. According to Table 4.5, 17.15% and 17.52% of dextran in the 10 mL of dextran solutions (pH is 5.0 and 6.0 for FCED and ICED respectively) was converted to reducing sugars at the end of 10 hours, using FCED and ICED respectively. Dextran hydrolysis ratio in our study lower than achieved dextran hydrolysis ratio (~ 60%) in the study of Erhardt and Jördening (2007). This difference may resulted from the difference between the moleculer weights of dextrans. Because, we used dextran T70, but they have used dextan T40.

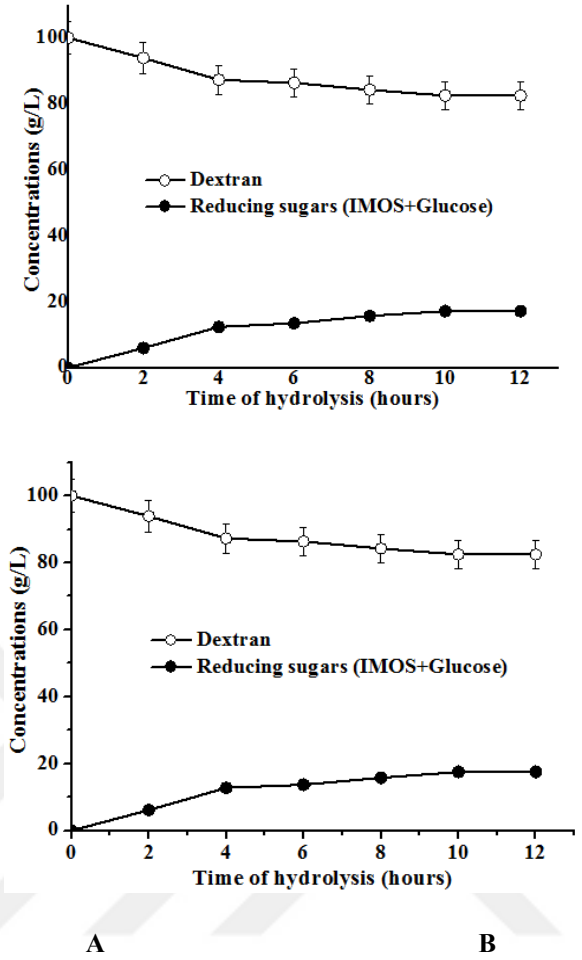


Figure 4.12. Time course of the production of IMOs from dextran by FCED (A) and ICED (B)

Table 4.5. Production of IMOs from dextran by FCED and ICED*

Enzyme	Hiydrolysis Time (hours)	Dextran (g/L)	Reducing sugars (IMO+Glucose) (g/L)
FCED	0	100.00	0.00
	2	94.01	5.99
	4	87.63	12.37
	6	86.51	13.49
	8	84.28	15.72
	10	82.86	17.14
	12	82.85	17.15
ICED	0	100.00	0.00
	2	93.88	6.12
	4	87.26	12.74
	6	86.31	13.69
	8	84.23	15.78
	10	82.48	17.52
	12	82.47	17.53

*1.57 g ICED was reacted with 10 mL of 10% dextran solution (pH 6.0 for 12 hours. 200 µL aliquats were taken and added to 1800 µL of distilled water. After inactivating the enzymes in boiling water bath for 10 minutes, released reducing sugars were determined two hours intervals by DNS method during hydrolysis.

5. CONCLUSION AND RECOMMENDATIONS

5.1. Conclusion

Firstly, CED was successfully immobilized c-MWCNT with a high immobilization yield (100%) and (114.13%) activity yield by optimizing the immobilization conditions.

Secondly, there was no decrease in the initial activity of the ICED, after repeated 20 batch experiments and after storage for 30 days under optimum storage conditions.

Lastly, 17.53% of dextran in the dextran solution (10%) was converted to reducing sugars (IMOs and D-glucose) after 12 hours by using ICED.

As a result, we can say that the CED obtained in this study can be used in the industrial production of IMOs from dextran.

5.2. Recommendations

Firstly, the results achieved in this thesis can be published in SCI indexed journals.

Secondly, it can be apply to the national or international patent institute to get patent for the immobilization of CED on c-MWCNT that developed in this thesis study.

Finally, in the future, a new research project could be made to increase the conversion rate of dextran to IMOs using immobilized ICED.

6. REFERENCES

Abdel-Rahman, E. A., Smejkal, Q., Schick, R., El-Syiad, S., and Kurz, T., 2008. Influence of dextran concentrations and molecular fractions on the rate of sucrose crystallization in pure sucrose solutions. *Journal of food engineering*, 84(4), 501-508.

Albayrak, N. and Yang S.T., 2002. Production of galacto-oligosaccharides from lactose by *Chaetomium erraticum*beta-galactosidase immobilized on cotton cloth. *Biotechnology and Bioenginnering* 7(1):8-19.

Anekella, K., 2012. Microencapsulation of probiotics (Lactobacillus acidophilus and Lactobacillus rhamnosus) in raspberry powder by spray drying: optimization and storage stability studies. McGill University (Canada).

Aslan, Y., and Tanrıseven, A., 2007a. Immobilization of Pectinex Ultra SP-L to produce galactooligosaccharides. *Journal of Molecular Catalysis B: Enzymatic*, 45(3), 73-77.

Aslan, Y., and Tanriseven, A., 2007b. Immobilization of *Penicillium lilacinum* dextranase to produce isomaltooligosaccharides from dextran. *Biochemical Engineering Journal,* 34(1), 8-12.

Azevedo, R.A.M. 2014. Immobilization of peroxidase on functionalized carbon nanotubes for synthesis of biocatalysts with high performance. *Master's Thesis. Porto University*, 1-123.

Babu, P. S. R., and Panda, T. 1991. Studies on improved techniques for immobilizing and stabilizing penicillin amidase associated with E. coli cells. *Enzyme and Microbial Technology,* 13(8), 676-682.

Bashari M., Abbas S., Xueming Xu X., and Jin Z. 2014. Combined of ultrasound irradiation with high hydrostatic pressure (US/HHP) as a new method to improve immobilization of dextranase onto alginate gel. *Ultrasonics Sonochemistry*, 21, 1325–1334.

Betancor, L., López-Gallego, F., Hidalgo, A., Alonso-Morales, N., Mateo, G. D. O. C., Fernández-Lafuente, R., and Guisán, J. M., 2006. Different mechanisms of protein immobilization on glutaraldehyde activated supports: effect of support activation and immobilization conditions. *Enzyme and Microbial Technology,* 39(4), 877-882.

Betancor, L., Luckarift, H.R., Seo, J.H., Brand, O. and Spain, J.C., 2008. Three-Dimensional immobilization of Dextranase on a silicon surface. *Biotechnology and Bioengineering* 99(2) 261-267.

Blandino, A., Macias M., Cantero, D., 2001, Immobilization of glocose oxidase within calcium alginate gel capsules. Process Biochemistry, 36, 601-606.

Bouhnik, Y., Raskine, L., Simoneau, G., Vicaut, E., Neut, C., Flourié, B. and Bornet, FR., 2004. A dose-response relation study was performed with a double-blind, randomized, placebo-controlled, parallel-group study of fecal bifidobacteria in healthy humans. *The American Journal of Clinical Nutritio,*80 (6), 1658-1664.

Bradford, M. M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry,* 72(1-2), 248-254.

Brady, D., and Jordaan, J. 2009. Advances in enzyme immobilisation. Biotechnology Letters, 31(11), 1639.

Brahmachari, G., Adrio, J.L., Demain, A.L., 2017 Biotechnology of microbial enzymes: production, biocatalysis and industrial application. *Elsevier Academic press, Amsterdam*, 1–11.

Brena, B.M. and Batista-Viera, F., 2008. Immobilization of Enzymes. In: Methods in Biotechnology: Immobilization of Enzymes Cells, edited by Gordon Bickerstaff. Totowa, New Jersey: Humana Press.

Brena, B.M., González-Pombo, P., and Batista-Viera, F., 2013. Immobilization of enzymes. In: Methods in biotechnology: Immobilization of Enzymes and Cells, edited by Guisan, J.M., *Humana Press,* New Jersey, 15-32.

Cao, L.Q., Van-Langen, L. and Sheldon, R.A., 2003. Immobilized enzymes carrier-bound or carrier free. *Current Opinion Biotechnology*, 14, 387-394.

Chen, W.C. and Ou-Yang, C.C., 2004. Bounded water kinetic model of Dextranase in reverse micelles, *Bioprocess and Biosystems Engineering*, 26, 307-313.

Clarke, M. A., Edye, L. A., and Eggleston, G., 1997a. Sucrose decomposition in aqueous solution, and losses in sugar manufacture and refining. *Advances in Carbohydrate Chemistry and Biochemistry, Elsevier,* 52, 441-470.

Clarke, M. A., Edye, L. A., Cole, F., and Kitchar, J. L., 1997b. Sugarcane factory trials with dextranase enzyme. *Sugar Journal*, 60, 20-22.

Corvis, Y., Walcarius, A., Rink, R., Mrabet, N. T., and Rogalska, E., 2005. Preparing catalytic surfaces for sensing applications by immobilizing enzymes via hydrophobin layers. *Analytical Chemistry*, 77(6), 1622-1630.

Costa, S.A, Azevedo, Helena S., and Reis, R.L., 2004. Enzyme immobilization in biodegradable polymers for biomedical applications, *Biodegradable Systems in Tissue Engineering and Regenerative Medicine,* 1(4), 301- 324

Cowan, D. A., and Fernandez-Lafuente, R., 2011. Enhancing the functional properties of thermophilic enzymes by chemical modification and immobilization. *Enzyme and Microbial Technology*, 49(4), 326-346.

De Segura, A. G., Alcalde, M., Yates, M., Rojas-Cervantes, M. L., López-Cortés, N., Ballesteros, A., and Plou, F. J., 2004.Immobilization of Dextransucrase from Leuconosto cmesenteroides NRRL B-512F on Eupergit C Supports. *Biotechnology progress*, 20(5), 1414-1420.

DiCosimo, R., McAuliffe, J., Poulose, A. J., and Bohlmann, G., 2013. Industrial use of immobilized enzymes. *Chemical Society Reviews*, 42(15), 6437-6474.

Dong, Li, Y., and Zhang, W. B., 2017. Polyhedral oligomeric silsesquioxane meets “click” chemistry: Rational design and facile preparation of functional hybrid materials. Polymer, 125, 303-329.

Eggleston, G., Monge, A., Montes, B., and Stewart, D., 2005. Factory trials to optimize the application of dextranase in raw sugar manufacture: Part II. *International sugar journal*, 1308, 757.

El-Tanash ,A. B. El-Baz, E., and Sherief, A.A., 2011. Properties of Aspergillus subolivaceus free and immobilized dextranase*, journal. IN Springer-Verlag, European Food Technology* 233:735–742

Erhardt, F.A., Jördening, and H.J., 2007. Immobilization of dextranase from *Chaetomium erraticum. Journal of biotechnology*, 131(4), 440-447.

Erişim tarihi: 07.05.2018

Feng, W., and Ji, P. 2015. Enzymes immobilized on carbon nanotubes. *Biotechnology* advances, 29, 889–895.

Finocchiaro, T., Richardson, T., and Olson, N. F., 1980. Lactase immobilized on alumina. *Journal of Dairy Science,* 63(2), 215-222.

Foukia E, M, Karam E, A and Hassan, H.M., 2016, dexranase *Journal of Pharmaceutical, Biological and Chemical Sciences in Egypt* ID 60014618.

Frank A. E. and Hans-Joachim J., 2007. Immobilization of dextranase from *Chaetomium erraticum*. *Journal of Biotechnology*, 131: 440 - 447.

Garlet, T. B., Weber, C.T., Klaic, R., Foletto, E.L., Jahn, S.L., Mazutti, M.A., and Kuhn, R.C. 2014. Carbon Nanotubes as Supports for Inulinase *Immobilization. Molecules,* 19, 14615-14624.

Gericke, B., Amiri, M., and Naim, H. Y., 2016. The multiple roles of sucrase-isomaltase in the intestinal physiology. Molecular and cellular pediatrics, 3(1), 2.

Gibriel, A. Y., Amin, A. A., Yassien, N. M., El Banna, H. A., and Khaled, F. M., 2014. Immobilization techniques for *P.aculeatum* dextranase. Int. *Journal of Microbiology.* Application. Scientific in Dokki, Giza, Egypt, 3(9), 1114-1134.

Goffin, D., Delzenne, N., Blecker, C., Hanon, E., Deroanne, C., and Paquot, M., 2011. Will isomalto-oligosaccharides, a well-established functional food in Asia, break through the European and American market? The status of knowledge on these prebiotics. Critical Reviews in Food Science and Nutrition, 51(5), 394-409.

Goulas, A. K., Cooper, J. M., Grandison, A. S., and Rastall, R. A., 2004a. Synthesis of isomaltooligosaccharides and oligodextrans in a recycle membrane bioreactor by the combined use of dextransucrase and dextranase. *Biotechnology and bioengineering,* 88(6), 778-787.

Goulas, A. K., Fisher, D. A., Grimble, G. K., Grandison, A. S., and Rastall, R. A., 2004b. Synthesis of isomaltooligosaccharides and oligodextrans by the combined use of dextransucrase and dextranase. *Enzyme and Microbial Technology*, 35(4), 327-338.

Gozu, Y., Ishizaki, Y., Hosoyama, Y., Miyazaki, T., Nishikawa, A., and Tonozuka, T., 2016. A glycoside hydrolase family 31 dextranase with high transglucosylation activity from Flavobacterium johnsoniae *Bioscience, Biotechnology, and Biochemistry*, 80(8), 1562-1567.

Guisan, J. M., 2013. New opportunities for immobilization of enzymes. In: Guisan J.M. (eds) Immobilization of enzymes and cells, *Humana Press*, New Jersey, 1-13.

Gutiérrez-A, P, Gimeno-Pérez M, Ramírez-Escudero M., 2016 Molecular characterization and heterologous expression of a Xanthophyllomyces dendrorhous α-glucosidase with potential for prebiotics production. Application. *Microbiol in Biotechnology* 100: 3125_3135.

Hector, S., Willard, K., Bauer, R., Mulako, I., Slabbert, E., Kossmann, J., and George, G. M., 2015. Diverse exopolysaccharide producing bacteria isolated from milled sugarcane: implications for cane spoilage and sucrose yield. PloS one, 10(12), e0145487.

Hill, F., 1991. U.S. Patent No. 5,070,019. Washington, DC: U.S. Patent and Trademark Office. United States Patent. http://www.scribd.com/doc/12707839/Enzyme-Immobilisation.

https://tr.scribd.com/document/83042104/Enzyme-Immobilization

Huang, C., Miao, M., Janaswamy, S., Hamaker, B. R., Li, X., and Jiang, B., 2015. Polysaccharide modification through green technology: role of endodextranase in improving the physicochemical properties of (1→ 3) (1→ 6)-α-D-glucan. *Journal of Agricultural and Food Chemistry*, 63(28), 6450-6456.

Husain, Q., 2010. β Galactosidases and their potential applications: a Review. *Critical Reviews in Biotechnology,* 30(1), 41-62.

Hutkins, R. W., Krumbeck, J. A., Bindels, L. B., Cani, P. D., Fahey, G., Goh, Y. J., ... and Vaughan, E., 2016. Prebiotics: why definitions matter. Current Opinion in Biotechnology, 37, 1-7.

Iqbal, S., Aman, A., Bano, S., Sidduiqui, N. N., Ansari, A., and Ali-Ul-Qader, S., 2015. Role of nutrients and environmental conditions for the production of dextransucrase from L. mesenteroides KIBGE-IB26. *Pakistan Journal of Pharmaceutical Sciences,* 28(6).

Jaiswal, P., and Kumar, S., 2011. Impact of media on isolation of dextranase producing fungal strains. *Journal of Scientific Research*, in India *55*, 71-76.

Jamie, A., Alshami, A.S., Maliabari, Z.O., Ateih, M.A., and Al Hamouz, O.C.S. 2016. Immobilization and enhanced catalytic activity of lipase on modified MWCNT for Oily Wastewater treatment. *Environmental Progress and Sustainable Energy*, 35(5), 1-9.

Jeanningros, R., Creuzet-Sigal, N., Frixon, C., and Cattaneo, J. 1976. Purification and properties of a debranching enzyme from Escherichia coli. *Biochimica et Biophysica Acta (BBA)-Enzymology,* 438(1), 186-199.

Jiménez, E. R. 2009. Dextranase in sugar industry: a review. *Sugar technology*, 11(2), 124-134.

Kaneko, T., Kohmoto, T., Kikuchi, H., Fukui, F., Shiota, M., Yatake, T. and Iino, H.,1992. Digestibility of isomaltooligosaccharides by rats and effects on serum lipids. *Journal of the Agricultural Chemical Society of Japan.*

Kaneko, T., Kohmoto, T., Kikuchi, H., Shiota, M., Iino, H., and Mitsuoka, T., 1994. Effects of Isomaltooligosaccharides with different degrees of polymerization on human fecal bifidobactcria. *Bioscience, Biotechnology, and Biochemistry*, 58(12), 2288-2290.

Katchalski-Katzir, E., and Kraemer, D.M., 2000. Eupergit C, a carrier forimmobilization of enzymes of industrial potential. *Journal of Molecular Catalysis B: Enzymatic*, 10, 157–176.

Khalikova, E., Susi, P., and Korpela, T., 2005. Microbial dextran-hydrolyzing enzymes: fundamentals and applications. *Microbiology and Molecular Biology Reviews*, 69(2), 306-325.

Khan, A.A., and Alzohairy, M.A., 2010. Recent advances and applications of immobilized enzyme technologies: A Review. Research *Journal of Biological Sciences,* 5(8), 565-575.

Kim, S.W., 2007. Development of enzyme immobilization technique, Lecture Notes, Department of Chemical and Biological Engineering, Korea University, http://www.cheric.org/ippage/e/ipdata/2004/02/file/e200402-1001.pdf (accessed December 13, 2017)

Kim, Y. M., Shimizu, R., Nakai, H., Mori, H., Okuyama, M., Kang, M. S. and Kimura, A., 2011. Truncation of N-and C-terminal regions of Streptococcus mutans dextranase enhances catalytic activity. *Applied Microbiology and Biotechnology*, 91(2), 329-339.

Klibanov A.M., 1983, Immobilized enzymes and cells as practical catalysts., Science, 219, 722-727.

Koenig, D., and DAY, D., 1989. The purification and characterization of a dextranase from *Lipomyces starkeyi*. *The FEBS Journal,* 183(1), 161-167.

Kohmoto, T., Tsuji, K., Kaneko, T., Shiota, M., Fukui, F., Takaku, H. and KOBAYASHI, S., 1992. Metabolism of 13C-isomaltooligosaccharides in healthy men. Bioscience, Biotechnology, and Biochemistry, 56(6), 937-940.

Kubik, C., Sikora, B., and Bielecki, S., 2004. Immobilization of dextransucrase and its use with soluble dextranase for glucooligosaccharides synthesis. *Enzyme and microbial technology,* 34(6), 555-560.

Lattimer, J. M., and Haub, M. D., 2010. Effects of dietary fiber and its components on metabolic health. Nutrients, 2(12), 1266-1289.

Leemhuis, H., Pijning, T., Dobruchowska, J. M., van Leeuwen, S. S., Kralj, S., Dijkstra, B. W., and Dijkhuizen, L., 2013. Glucansucrases: three-dimensional structures, reactions, mechanism, α-glucan analysis and their implications in biotechnology and food applications. *Journal of Biotechnology*, *163*(2), 250-272.

López-Gallego, F., Fernandez-Lorente, G., Rocha-Martin, J., Bolivar, J. M., Mateo, C., and Guisan, J. M., 2013. Stabilization of enzymes by multipoint covalent immobilization on supports activated with glyoxyl groups. In Immobilization of Enzymes and Cells (pp. 59-71). Humana Press, Totowa, NJ.

Madhu, and K. A. Prabhu., 1984. Studies on dextranase from *Penicillium aculeatum.* Enzyme Microbiology *Technology Journal* in India 6:217-220

Mahmoud, A. D., and Helmy, A. W., 2009 Application of cold-active dextranase in dextran degradation and isomaltotriose synthesis by micro-reaction technology. *Australian Journal of Basic and Applied Sciences*, 3(4), 3808-3817.

Manjon, A., Iborra, J.L., Lozano, P.L., and Canovas, M., 1995. A practical experiment on enzyme immobilization and characterization of the immobilized derivatives, Biochemical Educcation, 23(4), 213-216

Mao, X., Wang, S., Kan, F., Wei, D., and Li, F., 2012. A novel dextran dextrinase from Gluconobacter oxydans DSM-2003: purification and properties. *Applied Biochemistry and Biotechnology*, *168*(5), 1256-1264.

Mendez, F., 2000. Dextranase in sugar production: factory experience. In American Society of Sugar Cane Technologists (Vol. 20, p. 95).

Miller, G.L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analitical Chemistry*, 31, 426-428.

Miyazaki, M., and Maeda, H. 2006. Microchannel enzyme reactors and their applications for processing. *TRENDS in Biotechnology*, 24(10), 463-470.

Mohamed A. A., S. I. Abdel-Mohsen, M.A. Azza and F. A. Ahmed, 1999. Preparation and some properties of immobilized Penicillium funiculosum 258 dextranase. Process Biochemistry, 34: 391 398.

Mouafi, F. E., Karam, E. A., and Hassan, H. M., 2016. Production of Dextranase from Agro-industrial Wastes by Aspergillus awamori F-234 under Solid State Fermentation. *Research journal of Pharmaceutical Biological and Chemical Sciences,* 7(6), 1451-1459.

Naessens, M., Cerdobbel, A., Soetaert, W., and Vandamme, E. J., 2005. Dextran dextrinase and dextran of Gluconobacter oxydans. *Journal of Industrial Microbiology and Biotechnology*, 32(8), 323.

Novick, S. J., and Rozzell, J. D., 2005. Immobilization of enzymes by covalent attachment. *Microbial Enzymes and Biotransformations*, 247-271.

Oku, T., and Nakamura, S., 2003. Comparison of digestibility and breath hydrogen gas excretion of fructo-oligosaccharide, galactosyl-sucrose, and isomalto-oligosaccharide in healthy human subjects. *European Journal of Clinical nutrition*, 57(9), 1150-1156.

Panesar, P. S., Kumari, S., and Panesar, R. 2010. Potential applications of immobilized β-galactosidase in food processing industries. *Enzyme Research*, 2010.

Pavlidis, I., V., Tsoufis, T., Enotiadis, A., Gournis, D., and Stamatis, H., 2010. Functionalized multi-wall carbon nanotubes for lipase immobilization. *Advanced Biomaterials,* 12(5), 179-183.

Phathk, A.K., Sahu, N., and Dubey P., 2012. Report about Enzyme immobilization, in Barkatullah university.

Prlainović, N. Ž., Bezbradica, D. I., Knežević-Jugović, Z. D., Stevanović, S. I., Ivić, M. L. A., Uskoković, P. S., and Mijin, D. Ž., 2013. Adsorption of lipase from Candida rugosa on multi walled carbon nanotubes. *Journal of Industrial and Engineering Chemistry*, 19(1), 279-285.

Purushe, S., Prakash, D., Nawani, N. N., Dhakephalkar, P., and Kapadnis, B., 2012. Biocatalytic potential of an alkalophilic and thermophilic dextranase as a remedial measure for dextran removal during sugar manufacture. *Bioresource technology,* 115, 2-7.

Rastian, Z., Khodadadadi, A.A., Vahabzade, F., and Mortazavi, Y. 2013. Functionalization of multi -walled carbon nanotubes for lipase immobilization. *The Journal of Macro Trends in Technology and Innovation*, 1(1), 65-71.

Rastian, Z., Khodadadadi, A.A., Vahabzade, F., Bortolini, C., Dong, M., Mortazavi, Y., Mogharei, A., Naseh, M.V., and Guo, Z. 2014. Facile surface functionalization of multiwalled carbon nanotubes bysoft dielectric barrier discharge plasma: Generate compatibleinterface for lipase immobilization. *Biochemical Engineering Journal,* 90, 16–26.

Rehman, H. U., Aman, A., Nawaz, M. A., Karim, A., Ghani, M., Baloch, A. H., & Qader, S. A. U. (2016). Immobilization of pectin depolymerising polygalacturonase using different polymers. *International journal of biological macromolecules,* 82, 127-133.

Riahi, N., Liberelle, B., Henry, O., and De Crescenzo, G., 2017. Impact of RGD amount in dextran-based hydrogels for cell delivery. *Journal Elsevier Carbohydrate Polymers*, 161, 219-227.

Rivero, C. W., De Benedetti, E. C., Gallego, F. L., Pessela, B. C., Guisán, J. M., and Trelles, J. A., 2017. Biosynthesis of an antiviral compound using a stabilized phosphopentomutase by multipoint covalent immobilization. *Journal of Biotechnology*, 249, 34-41.

Samoshina, N. M., Lotmentzena, E. Y., Borisona, V. N., and Nakhepetyan, L. A., 1987. Studies on the effective kinetic parameters of Dextranase immobilized on modified carriers. Biotekhnologiya, 3, 478-480.

Sankpal NV, Joshi AP, Sainkar AP, Kulkarni BD., 2001 Production of dextranz by Rhizopus sp. Immobilized on porous cellulose support. Processing Biochemistry 37,395_403.

Sheldon, R.A., 2007. Enzyme Immobilization: The Quest for Optimum Performance. Advanced Synthesis and Catalysis, 349, 1347-1389.

Sidebotham, R. L.,1974. Dextrans. Advances in carbohydrate *chemistry and biochemistry,* 30, 371-444.

Smalla, K., Turkova, J., Coupek, J., and Hermann, P., 1988. Influence of salts on the covalent immobilisation of proteins to modified copolymers of 2-hydroxyethyl methacrylate with ethylene dimethacrylate. Biotechnology and Applied Biochemistry,10, 21–31.

Soo-Keun, J., Chae, Y.R., Yoon, J.M., Cho, B.W., and Ryu, K.G. 2005. Immobilization of Glucose Oxidase on Multi-Wall Carbon Nanotubes for Biofuel Cell Applications. *Journal of Microbiology and Biotechnology*, 15(2), 234–238.

Sugiura, M., and Ito, A., 1975. Studies on dextranase. III. Action patterns of dextranase from Penicillium funiculosum on substrate and inhibition on hydrolysis reaction by substrate analogues. *Chemical and Pharmaceutical Bulletin*, 22(7), 1593-1599.

Šulek, F., Fernández, D. P., Knez, Ž., Habulin, M., and Sheldon, R. A., 2011. Immobilization of horseradish peroxidase as crosslinked enzyme aggregates (CLEAs). Process biochemistry, 46(3), 765-769.

Tanaka A., and Kawamoto T., 1999. Cell and Enzyme Immobilization. Manual of industrial microbiology and Biotechnology, *American Society for Microbiology*, Washington; pp: 504-513.

Thitaram, S. N., Chung, C. H., Day, D. F., Hinton Jr, A., Bailey, J. S., and Siragusa, G. R., 2005. Isomaltooligosaccharide increases cecal Bifidobacterium population in young broiler chickens. *Poultry Science*, 84(7), 998-1003.

Tilbury, R. H., 1971. Dextrans and dextranase. Proccessing Manufacturing *Research Centre Sugar Cane Technology, England,* 14, 1444-1458.

Tilbury, R. H., and French, S. M., 1974. Further studies on enzymic hydrolysis of dextran mill juices by dextranase and fungal-amylase. In Processing. *International Society Sugar Cane Technology* (Vol. 15, pp. 1277-1287).

Tingirikari, J.M.R., Gomes, W.F., Rodrigues, S., 2017. Efficient production of prebiotic gluco-oligosaccharides in orange juice using ımmobilized and co-immobilized dextransucrase. *Applied Biochemistry And Biotechnology*, 183 (4), 1265-1281.

Tümtürk, Hayrettin, Şahin, F. and Demirel, G., 2007. A new method for immobilization of acetylcholinesterase. *Bioprocess Biosystem Engineering* 30,141–145.

Virgen-Ortíz, J. J., Ibarra-Junquera, V., Escalante-Minakata, P., Ornelas-Paz, J. D. J., Osuna-Castro, J. A., and González-Potes, A., 2015. Kinetics and thermodynamic of the purified dextranase from *Chaetomium erraticum*. *Journal of Molecular Catalysis B: Enzymatic,* 122, 80-86.

Wang, Q., Zhou, L., Jiang, Y., Gao, J., 2011. İmproved stability of the carbon nanotubes-enzyme bioconjugates by biomimetic silicification. *Enzyme and Microbial Technology,* 49, 11-16.

Wang, X., Lu, M., Wang, S., Fang, Y., Wang, D., Ren, W., and Zhao, G. 2014. The atmospheric and room-temperature plasma (ARTP) method on the dextranase activity and structure. *International journal of biological macromolecules*, 70, 284-291.

Wei, W., A. Sethuraman, C. Jin, N. A. Monteiro-Riviere and R. J. Narayan., 2007. Biological Properties of Carbon Nanotubes. *Journal of Nanoscience and Nanotechnology* 7(4-1): 1284-1297.

Xiuyun .W., and Shunichi U., 2013. Polymers for Biosensors Construction, Science, book of Technology and Medicine, page 70 in http://dx.doi.org/10.5772/54428

Yin, S., Shen, P. K., Song, S., and Jiang, S. P. 2009. Functionalization of carbon nanotubes by an effective intermittent microwave heating-assisted HF/H2O2 treatment for electrocatalyst support of fuel cells. *Electrochimica Acta,* 54(27), 6954-6958.

Zannini, E., Waters, D. M., Coffey, A., and Arendt, E. K., 2015. Production, properties, and industrial food application of lactic acid bacteria-derived exopolysaccharides. *Applied Microbiology and Biotechnology*, 100(3), 1121-1135.

Zhang, Y. Q., Li, R. H., Zhang, H. B., Wu, M., and Hu, X. Q., 2017. Purification, characterization, and application of a thermostable dextranase from Talaromyces pinophilus. *Journal of Industrial Microbiology and Biotechnology,* 44(2), 317-327.

Zniszczoł, A., Herman, A.P., Szymanska, K., Mrowiec-Białon, J., and Krzysztof, Z. Walczak, Andrzej Jarzebski, Sławomir Boncel, 2016. Covalently immobilized lipase on aminoalkyl-, carboxy- andhydroxy-multi-wall carbon nanotubes in the enantioselectivesynthesis of Solketal. *Enzyme and Microbial Technology*, 87-88, 61–69.

Zohra, R.R., Aman, A., Ansari, A., Haider, M. S., and Qader, S.A.U., 2015. Purification, characterization and end product analysis of dextran degrading endodextranase from *Bacillus licheniformis* KIBGE-IB25. *International Journal of Biological Macromolecules,* 78, 243-248.

CURRICULUM VITAE

PERSONAL INFORMATION		
Name and Surname	Barzan Ismael GHAFOUR	
Date of birth	**23 -02-1984**	
Address	**zylan, Erbil, Iraq**	
Phone (Iraq)Phone(Turkey)	**+9647504887015** **+9647508332737** **+905522275588**	
E-mail	**barzanismael.1984@gmail.com** **b.mstafa@yahoo.com**	

EDUCATION AND TRAINING		
Degree	**|Institution**	**Time Interval**
Bachelor of Science	-Salahaddin University, College of Agriculture, Erbil (IRAQ) Food Science and Technology.	2003 – 2007 Bachelor of Food Technology Department.
Master of science Master of science Food	Republic of turkey Siirt university Institute of sciences	2016 – 2018 Master of science Food Department Of Food Engineering

WORK EXPERIENCE		
Organizntion	**Position and Responsibilities**	**Time Interval**
-Employer -Sept. 2008 Pre-seller	-Employer government., Erbil (IRAQ -PIPSI Company. -Golden Great Wall Company	September 2008 – July 2017… Administrative

And During placement with Dream City company for house building I was supervisor for the green zone in the city, I contributed to projects such as the preparation of soil and choosing the proper plant for the city and managed my research for the best of the new built city, liaised with various divisions, formulated reports and participated in group project meetings. Utilizing excellent communication skills, I developed and maintained successful working relationships with both internal and external staff

PEROSONEL SKILS	
Languages	Kurdish mother language
	Arabic
	English and Turkish
Computer skills	MS word, MSexcel, MSPublisher, MS PowerPoint and MS access Database, MS NOTE One
Driving licence	Yes