CHARACTERIZATION OF THE PHYSICOCHEMICAL PROPERTIES OF CROSS-LINKED LEVANASE AGGREGATES FOR LEVAN-TYPE FRUCTOOLIGOSACCHARIDES PRODUCTION

NOOR HIDAYAH BINTI ABD RAHMAN

UNIVERSITI TEKNOLOGI MALAYSIA

CHARACTERIZATION OF THE PHYSICOCHEMICAL PROPERTIES OF CROSS-LINKED LEVANASE AGGREGATES FOR LEVAN-TYPE FRUCTOOLIGOSACCHARIDES PRODUCTION

NOOR HIDAYAH BINTI ABD. RAHMAN

A thesis submitted in fulfilment of the
requirements for the award of the degree of
Doctor of Philosophy

School of Chemical and Energy Engineering
Faculty of Engineering
Universiti Teknologi Malaysia

MARCH 2021

DEDICATION

This thesis is dedicated to my beloved husband, who taught me that the best kind of knowledge to have is that which is learned for its own sake. It is also dedicated to my late mother (Norsiah binti Dali), *Mak* and *Abah* who taught me that even the largest task can be accomplished if it is done one step at a time. Also, to my little princess (Malisa, Maryam and Medina), who lend their playing time to me.

ACKNOWLEDGEMENT

Assalammualaikum w.b.t. and Alhamdulillah. First and foremost, I owe my deepest gratitude to my supportive supervisor, Prof. Dr. Rosli Md. Illias, whom give infinite encouragement, assistance, critics and guidance from the beginning to the final stage which assisted me to develop an understanding of the subject. I am also very thankful to my co-supervisor Prof. Dr. Hesham Ali El-Enshasy for his guidance, advices and motivation. In preparing this thesis, I was in contact with many people, researchers, academicians, and practitioners. They have contributed towards my understanding and thoughts. Without their continued support and interest, this thesis would not have been the same as presented here.

I would like to express my gratitude to those who have encouraged and guided me to complete this thesis. I am most indebted to my lovely hubby (Muhammad Faris Nasir), parents (Abd Rahman and Saipah), mother in-law (Siti Fadilah), my little princess (Fardanah Malisa, Faihanah Maryam, Nur Medina), my siblings and my family for their endless love, encouragement and prayer. A deep appreciation to my late mother, Allahyarham Norsiah binti Dali for her motivation and courage (20 years ago) which made me resilient in this life. May Allah bless her soul.

Special thanks go to the helpful laboratory staff and technician assistance throughout this work. Also, my sincere appreciation also extends to all my colleagues and others who helped me in many ways, particularly Dr. Nardiah, Dr. Namirah, Dr. Rabiatul, Dr. Hasmaliana, Dr. Izyan, Dr. Izawati, Dr Aishah, Dr. Intan, Dr. Joanne, Samson, Hakimi, Kak Dilin, Nashriq, Suhaili and Yip. Their views and tips are useful indeed. Unfortunately, it is possible to list all of them in this limited space.

I am also indebted to Universiti Teknologi Malaysia (UTM) for funding my Ph.D study. The research was supported through the Research University Grant (GUP) scheme (PY/2017/01569) (Q.J13000.2509.18H72).

ABSTRACT

Levan-type fructooligosaccharide (L-FOS) are oligosaccharides that is in high demand in food-based and pharmaceutical industries and it can be produced from the levan hydrolysis. Recombinant levanase from *Bacillus lehensis* G1 (rlevblg1) is an enzyme that specifically converts levan to L-FOS. However, the use of free rlevblg1 presents a lack of stability and reusability, thus hinder the synthesis of L-FOS for continuous reactions. A carrier-free immobilization of cross-linked enzyme aggregates (CLEAs) were developed to overcome these drawbacks. However, low number of lysine residues of rlevblg1 may reduce cross-linking efficiency to form a stable and active biocatalyst. This issue can be solved by enzyme co-aggregation using additives. Moreover, the formation of CLEAs is also influenced by mass diffusion limitation as the degree of molecular cross-linking attained, significantly affects substrate accessibility especially at higher substrate concentrations. To address this problem, macromolecular cross-linker was used in the formation of CLEAs. In this study, formation of cross-linked levanase aggregates (CLLAs) was performed to improve stability and reusability of free rlevblg1. An active CLLAs using glutaraldehyde (CLLAs-GA), and with bovine serum albumin (CLLAs-GA-BSA) were obtained, and the factors affecting the formation of CLLAs were investigated. The highest activity recovery of CLLAs-GA (92.8 %; 169.5 U/mg) and CLLAs-GA-BSA (121.2 %; 221.3 U/mg) was achieved at optimized conditions. The optimum temperature of CLLAs-GA and CLLAs-GA-BSA increased to 35 °C and 40 °C, respectively, from 30 °C in its free rlevblg1. At high temperature (50 °C), the half-life of CLLAs-GA-BSA was higher than that of free rlevblg1 and CLLAs-GA. The reusability of CLLAs for 8 cycles was retained more than 50 % activity. The V_{max} value of CLLAs-GA-BSA (21.97 U/mg) was increased by 14.3 % from the free rlevblg1 (19.23 U/mg). Dialdehyde starch-tapioca (DAST) was successfully developed and used to cross-link levanase to form CLLAs-DAST and CLLAs-DAST-BSA which showed activity recovery of 65.6 % (119.8 U/mg) and 81.6 % (149.0 U/mg), respectively. After DAST cross-linking, the pH and thermal stability increased, and the tolerance in organic solvents improved which resulted in an activation of CLLAs. A kinetic study revealed that CLLAs-DAST (16.72 mg/mL) and CLLAs-DAST-BSA (16.58 mg/mL) had higher affinity (K_m) toward levan than that of CLLAs-GA (20.52 mg/mL) and CLLAs-GA-BSA (18.20 mg/mL). Thus, improving substrate accessibility with higher effectiveness factors especially at higher levan concentrations (10-12 mg/mL). The highest total L-FOS was achieved by CLLAs-DAST-BSA (78.9 % (w/v)), followed by CLLAs-DAST (62.4 %(w/v)), free rlevblg1 (51.2 % (w/v)), CLLAs-GA-BSA (50.1 % (w/v)) and CLLAs-GA (35.6 % (w/v)), after 3 h reaction. Although CLLAs formation using glutaraldehyde has produced an active and stable CLLAs, diffusion limitation at higher substrate concentrations reduced the L-FOS synthesis. In conclusion, DAST as a cross-linker may have application prospects as a promising and green biocatalyst for product formation such as L-FOS.

ABSTRAK

Fruktooligosakarida jenis levan (L-FOS) adalah oligosakarida yang mendapat permintaan tinggi dalam industri berasaskan makanan dan farmaseutikal, dan ia boleh dihasilkan melalui hidrolisis levan. Levanase rekombinan daripada *Bacillus lehensis* G1 (rlevblg1) adalah enzim yang menukar levan secara khusus kepada L-FOS. Walau bagaimanapun, terdapat kekurangan dari segi kestabilan dan kebolehgunaan rlevblg1 bebas yang menganggu sintesis L-FOS melalui tindak balas yang berterusan. Imobilisasi pembawa bebas jenis agregat enzim terpaut silang (CLEAs) telah dihasilkan untuk mengatasi kekurangan ini. Walau bagaimanapun, bilangan residu lisin rlevblg1 yang rendah boleh mengurangkan kecekapan paut silang untuk membentuk biomangkin yang stabil dan aktif. Isu ini boleh diselesaikan dengan pengagregatan enzim menggunakan bahan tambah. Selain itu, pembentukan CLEAs juga dipengaruhi oleh kekangan pemindahan jisim kerana tahap pautan silang molekul yang dicapai akan mempengaruhi akses substrat terutamanya pada kepekatan substrat yang tinggi. Bagi menangani masalah ini, pemaut silang makromolekul digunakan dalam pembentukan CLEAs. Dalam kajian ini, pembentukan agregat levanase terpaut silang (CLLAs) telah dilakukan untuk meningkatkan kestabilan dan kebolehgunaan rlevblg1 bebas. CLLAs aktif menggunakan glutaraldehida (CLLAs-GA) dan dengan albumin serum bovina (CLLAs-GA-BSA) telah dihasilkan, dan faktor-faktor yang mempengaruhi pembentukan CLLAs telah diuji. Perolehan aktiviti tertinggi oleh CLLAs-GA (92.8 %; 169.5 U/mg) dan CLLAs-GA-BSA (121.2 %; 221.3 U/mg) telah dicapai pada keadaan optimum. Suhu optimum CLLAs-GA dan CLLAs-GA-BSA masing-masing meningkat kepada 35 °C dan 40 °C, berbanding 30 °C pada rlevblg1 bebas. Pada suhu tinggi (50 °C), separuh hayat CLLAs-GA-BSA lebih tinggi berbanding separuh hayat rlevblg1 bebas dan CLLAs-GA. Kebolehgunaan semula CLLAs untuk 8 kitaran dapat mengekalkan aktiviti lebih daripada 50 %. Nilai V_{max} CLLAs-GA-BSA (21.97 U/mg) telah meningkat sebanyak 14.3 % daripada rlevblg1 bebas (19.23 U/mg). Kanji dialdehida ubi kayu (DAST) telah berjaya dihasilkan dan digunakan untuk pautan silang levanase bagi membentuk CLLAs-DAST dan CLLAs-DAST-BSA yang menunjukkan perolehan aktiviti masing-masing sebanyak 65.6 % (119.8 U/mg) dan 81.6 % (149.0 U/mg). Selepas pautan silang DAST, kestabilan pH dan haba meningkat, dan toleransi dalam larutan organik bertambahbaik yang menyebabkan pengaktifan CLLAs. Kajian kinetik mendedahkan bahawa CLLAs-DAST (16.72 mg/mL) dan CLLAs-DAST-BSA (16.58 mg/mL) mempunyai afiniti (K_m) yang lebih tinggi terhadap levan berbanding CLLAs-GA (20.52 mg/mL) dan CLLAs-GA-BSA (18.20 mg/mL). Oleh itu, meningkatkan kebolehcapaian substrat dengan faktor keberkesanan yang tinggi terutamanya pada kepekatan levan yang lebih tinggi (10-12 mg/mL). Jumlah L-FOS tertinggi telah dicapai menggunakan CLLAs-DAST-BSA (78.9 % (w/v)), diikuti oleh CLLAs-DAST (62.4 % (w/v)), rlevblg1 bebas (51.2 % (w/v)), CLLAs-GA-BSA (50.1 % (w/v)) dan CLLAs-GA (35.6 % (w/v)), selepas 3 jam tindak balas. Walaupun pembentukan CLLAs menggunakan glutaraldehida telah menghasilkan CLLAs yang aktif dan stabil, keterbatasan resapan pada kepekatan substrat yang lebih tinggi mengurangkan sintesis L-FOS. Kesimpulannya, DAST sebagai pemaut silang mungkin mempunyai prospek sebagai biomangkin hijau dan menjanjikan pembentukan produk seperti L-FOS.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

A600	-	Absorbance at optical density of 600 nm
AS	-	Ammonium sulfate
B. lehensis	-	*Bacillus lehensis*
B. subtilis	-	*Bacillus subtilis*
BSA	-	Bovine serum albumin
BSA	-	Bovine serum albumin
C	-	Celsius
Ca	-	Calcium
$CaCl_2$	-	Calcium chloride
CLEAs	-	Cross-linked enzyme aggregates
CLLAs	-	Cross-linked levanase aggregates
Co	-	Cobalt
Cu	-	Copper
DAST	-	Dialdehyde starch-tapioca
dH2O	-	Distilled water
DMA	-	Dimethylacetamide
DMSO	-	Dimethyl sulphoxide
DNA	-	Deoxyribonucleic acid
DNS	-	3, 5-Dinitrosalicylic acid
E. coli	-	*Escherichia coli*
E	-	Activation energy
Fe	-	Iron
$FeCl_3$	-	Iron chloride
FTIR	-	Fourier transform infrared
ga	-	Glutaraldehyde
H_3BO_3	-	Boric acid
HCI	-	Hydrochloric acid
HPLC	-	High-performance liquid chromatography
kDa	-	Kilo Dalton
KH_2PO_4	-	Potassium phosphate

LB	-	Luria bertani
L-FOS	-	Levan-type fructooligosaccharide
$MgCl_2$	-	Magnesium chloride
Mn	-	Manganese
Mo	-	Molybdenum
MW	-	Molecular weight
Na_2HPO_4	-	Sodium phosphate
$(NH_4)_2SO_4$	-	Ammonium sulfate
NaCl	-	Sodium chloride
NaOH	-	Sodium hydroxide
Ni	-	Nickel
NPS	-	Sodium phosphate solution
O.F.A.T	-	One factor at a time
PEI	-	Polyethylenimine
pH	-	Potential of hydrogen
PMSF	-	Phenylmethylsulfonyl fluoride
R	-	Universal gas constant
rlevblg1	-	Recombinant levanase from *Bacillus lehensis* G1
Rpm	-	Rotation per minute
SDS-PAGE	-	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
Se	-	Selenium
SEM	-	Scanning electron microscopy
V	-	Velocity
Zn	-	Zinc

LIST OF SYMBOLS

%	-	Percentage
~	-	Approximately
°C	-	Degree celcius
°F	-	Degree Fahrenheit
d. µm	-	Diameter in micrometer
g	-	Gram
h	-	Hour
J	-	Joule
K	-	Kelvin
M	-	Molar
min	-	Minute
mL	-	mililiter
mM	-	Millimolar
TM	-	Trademark
V	-	Velocity

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Introduction

In recent years, consumers pay considerable attention to their lifestyle. It generates increased demand for functional food promoting and improving wellness and health. In traditional food practice, a combination with herbal medicines is widely used in dietary supplements and as functional foods for health improvement purposes. The principal concept is related to the improvement of the circulation system, control of ageing and disease prevention (Shi *et al*., 2010). Prebiotics are short-chain carbohydrates that are non-digestible oligosaccharides by the digestive enzyme in humans (Quigley *et al*., 1999). The International Scientific Association for Probiotic and Prebiotics (ISAPP) suggested the new prebiotics definition as, 'a substrate that selectively consumed by host microorganisms conferring a health benefit' (Tomasik and Tomasik, 2020).

Presently, only di-, oligo-, polysaccharides of non-digestible carbohydrates, resistant starches, and sugar polyols have prebiotic properties. They found in many different sources such as chicory, asparagus, artichoke, bananas, tomatoes, milk, starch, lactose and many more (Al-Sheraji *et al*., 2013). Prebiotics used in the human diet, including lactulose, galactooligosaccharides (GOSs), maltooligosaccharides (MOSs), xylooligosaccharides (XOSs) and fructooligosaccharide (FOSs). FOSs are usually used in food industries due to their nutrition and health-relevant properties. FOSs plays a crucial role in the improvement of gut microbiota balance and individual health. FOSs have been produced by the hydrolysis of inulin, sucrose, and levan using inulinases, sucrase, and levansucrase or levanase, respectively (Roberfroid, 2007; Porras-domínguez *et al.*, 2014). Generally, all types of FOSs production can mainly be performed by two methods: chemical hydrolysis or enzymatic synthesis. Chemical hydrolysis of inulin exhibited high toxicity and lacks specificity, which produced

synthetic sugars that may be rejected by the consumer. Thus, in industrial production, the β-2,1-linked FOSs has been produced commercially from the enzymatic hydrolysis of inulin and transfructosylation of sucrose. Besides that, although β-2,6-linked levan-type FOSs (L-FOS) are not commercially manufactured, L-FOS have potential applications in the food and feed additive, agriculture and pharmaceutical industries (Kumar and Dubey, 2019; Martins *et al.*, 2019; Sánchez-Martínez *et al.*, 2020). Levan does not exist in plants abundantly. However, it can be produced by enzymatic synthesis and microbial fermentation. Microbial levan can biologically produce using enzymatic reaction from sucrose by levansucrase (Srikanth *et al*., 2015). Moreover, microbial levan has been produced from microorganisms such as *Bacillus atrophaeus, Acinetobacter nectaris* (González-Garcinuño *et al*., 2017), *Halomonas* sp. (Poli *et al*., 2009), *Zymomonas mobilis* (Silbir *et al*., 2014) and *Pseudomonas fluorescens* (Jathore *et al*., 2012). L-FOS are new potential prebiotic products with improved functional properties and had a higher ability to modulate microbiota for health purposes (Meyer *et al*., 2016).

Production of β-2,6-linked L-FOS from microbial levan is possible using levanase, due to the less availability of levan from plant sources. (Porras-Domínguez *et al*., 2014). Levanases [2,6-β-D fructan fructohydrolase, EC 3.2.1. 65] are enzymes that specifically catalyze the random hydrolysis of (2,6)-β-D fructofuranosidic linkages in levan, a high molecular weight fructose polymer. Levanase catalyzes the enzymatic hydrolysis of levan to produce L-FOS with a variable degree of polymerization (DP 1-10) (Dahech *et al*., 2013). The specificity of levanase toward levan has been explored by Porras-Domínguez *et al*. (2014), and all types of levan produce a low molecular weight of L-FOS and fructose. The production of levanase from various microorganisms was studied. The optimum pH and temperature of levanase were in the range of pH 6-8 and 30-40 °C, respectively(Srikanth *et al.*, 2015; González-Garcinuño *et al.*, 2017). Thus, L-FOS production using levanase requires rigorous control of fermentation conditions so that they can withstand in a broad pH and temperature range, especially for industrial-scale processes.

In the present study, recombinant levblg1 (rlevblg1) was used to synthesize levan polysaccharide for L-FOS production. Recombinant rlevblg1 was originated from mesophilic bacteria. Thus rlevblg1 showed low optimum temperature and a lack of thermostability (Fattah, 2018).

Moreover, the use of soluble enzymes as biocatalyst is not practical for extensive scale processes. Immobilization of enzyme is a preferred approach to increase enzyme activity, stability and thermostability. Immobilized enzymes have several benefits compared to free enzymes, including high enzyme stability, volumetric and specific productivities, and improved reusability and selectivity (Cao, 2005). Several techniques of enzyme immobilization which commonly used are adsorption, covalent binding, affinity binding, entrapment, and cross-linking (Datta *et al*., 2013). Enzyme immobilization typically involves the binding of the enzyme to support or encapsulates in inert support, offering high operational stability.

Nevertheless, some weaknesses of carrier-bound enzymes are low product formation due to a high amount of carrier and lead to substrate diffusion limitation, loss of enzyme activity caused by high enzyme loading on carrier and, expensive support materials (Sheldon, 2007; Pervez *et al.*, 2019). On the other hand, carrier-free immobilized enzymes offer high productivities and low cost because the support material is not required (Sheldon, 2011a). The carrier-free immobilized enzyme, such as CLEAs is a versatile and straightforward approach in enzyme immobilization. CLEAs were prepared by physically precipitating the enzyme molecules using either non-ionic polymer, organic solvent or salts. Then, the enzymes were cross-linked using bifunctional cross-linker such as glutaraldehyde (GA) (Zerva *et al*., 2020). CLEAs have several advantages over the other immobilized enzymes, (i) the use of partially purified enzymes, (ii) it allows the combination of two or more enzymes during immobilization. Moreover, the obtained CLEAs can easily be separated by centrifugation (Roessl *et al*., 2010). In CLEAs technology, both purification and immobilization of enzymes are combined into one single operation (R.A. Sheldon, 2007). Although CLEAs are a versatile method, this technology has some bottlenecks, as well. The use of glutaraldehyde led to the conglomeration structure of CLEAs, thus caused mass transfer limitation (Valdés *et al*., 2011). In the case of a low lysine residue

on the enzyme surface, the cross-linking step may be problematic (Amaral-Fonseca *et al*., 2018). Also, cross-linking using micromolecular glutaraldehyde may introduce substrate accessibility problems, especially for macromolecular substrate (Zhen *et al*., 2013b).

Some improvements are required to tackle the problems faced in CLEAs technology when glutaraldehyde is used as a cross-linker. Moreover, a low lysine content in some enzymes may reduce cross-linking efficiency (Guimarães *et al*., 2018a). Lysine residues containing free primary amino groups help in facilitating intermolecular cross-linking between enzymes and cross-linkers (Velasco-Lozano *et al*., 2014). This drawback can be solved by adding polymer containing primary amino acids (polyethyleneimine) or protein feeder (BSA) into the enzyme solution as a source of protein and amino groups to improve the cross-linking (Li *et al*., 2018). CLEAs preparation can be facilitated by the addition of BSA in the case of low protein concentration, or the enzyme activity is susceptible to a high cross-linker concentration (Shah *et al*., 2006; Aytar and Bakir, 2008). A high number of lysine residues on the BSA surface allowed glutaraldehyde to bind to its amino acids and avoid glutaraldehyde to bind amino acids associated with the enzyme active site (Torres *et al*., 2014). The interaction resulted in a less compact structure of CLEAs as it increased the distance between the cross-linker and the amino group of enzymes. Thus, it can improve the catalytic activity with less compact CLEAs structures. The addition of proteic feeder such as BSA retained the enzyme activity of the obtained CLEAs up to 100 % of its initial activity (Shah *et al*., 2006).

Instead of using glutaraldehyde, macromolecular cross-linker is an alternative in CLEAs preparation to overcome the inaccessibility of the macromolecular substrate. Some remarkable approaches that using macromolecular cross-linker proposed in the literature are polyfunctional polymers like pectin, dextran, chitosan, gum arabic and starch polyaldehyde (Rojas *et al.*, 2019: Nadar *et al.*, 2016). For instance, in the cross-linking process, the amino groups of enzyme molecules are bonded to the bifunctional aldehyde group on dialdehyde starch (DAS) via the Schiff base reaction. As the molecular length of dialdehyde starch is higher than enzymes, it cannot get access to all amino groups of enzymes. Thus it can enlarge the spatial structure of CLEAs and

reduced the compactness of CLEAs structure. Moreover, cross-linking using macromolecular cross-linker can reduce substrate diffusional problems by enlarging the pores of CLEAs (Zhen *et al*., 2013b) and lower loss of active site and irreversible immobilization (Mateo *et al*., 2004). However, in some cases, lower aldehyde content or DAS concentration may lead to incomplete cross-linking, while higher DAS concentration may also cause the damage in enzyme active site by excessive aldehyde content. The addition of BSA could protect the enzyme active site and enhanced cross-linking, thus improved the CLEAs activity (Wang *et al*., 2014).

Over the last two decades, many enzymes from different groups such as hydrolases, oxidoreductases, lyase, transferases and isomerases have been successfully used in CLEAs technology (Sheldon, 2019). However, different enzymes have different characteristics that required specific procedures during CLEAs preparation due to the low specificity of the CLEAs process. In the case of L-FOS production, it has been improved by using an immobilization approach to allow reusability of the enzyme in continuous reaction, to get better stability and to reduce operating costs (Liese and Hilterhaus, 2013). Previously, invertase has been covalently immobilized on glutaraldehyde-activated chitosan particles, displays higher reusability for FOSs production and obtained 55.0 % conversion per gram of initial sucrose (Lorenzoni *et al*., 2014). Ganaie *et al*. (2014) achieved 67.8 % (w/w) and 42.8 % (w/w) of FOSs yield by fructosyltransferase-entrapped alginate beads and chitosan beads after 36 h of enzyme-substrate reaction, respectively. Also, CLEAs of inulosucrase from *Lactobacillus reuteri* 121 produced a narrower range of inulin-type FOS than soluble enzyme, which proved that immobilized enzymes showed more specificity toward FOSs synthesis (Charoenwongpaiboon *et al*., 2019). Previous studies have not focused on levanase immobilization via CLEAs or other immobilization methods to improve enzyme stability and efficiency. To the best of our knowledge, this study reports the first immobilization of levanase to improve enzyme efficiency, stability and reusability.

In the present study, a systematic investigation and characterization of the immobilization of rlevblg1 from *Bacillus lehensis* G1 via CLEAs method were performed to enhance enzyme stability, improve operational stability and enhance substrate accessibility. The partially purified rlevblg1 obtained from the expression and purification processes was subjected to CLEAs immobilization.

1.2 Problem Statement

L-FOS has been recognized as a new prebiotic of FOSs due to its beneficial health effects in human gut microbiota. Recombinant levblg1 (rlevblg1) is highly specific towards levan for L-FOS synthesis using enzymatic synthesis method. However, due to the flexibility structures of the free enzyme, the synthesis process does not stand at high temperature, inefficient reusability and low thermal and mechanical stability. Their performances, such as low activity and stability, hindered the reaction process and increased the production cost. Enzyme immobilization is widely applied in enzyme stabilization to increase the operational stability of free enzyme. In the past decade, cross-linked enzyme aggregates (CLEAs) technology has been explored, which exhibits exceptional operational stability, recoverability and volumetric productivities. This carrier-free immobilization involves intermolecular cross-linking of enzyme molecules using cross-linker to form a stable and active CLEAs. However, the CLEAs formation may have some drawbacks if the enzyme contains low number of lysine residues which lead to cross-linking inefficiency. Another crucial problem in CLEAs technology is mass transfer limitation that caused substrate inaccessibility due to the formation of compact cluster of CLEAs.

In this study, the preparation of cross-linked levanase aggregates (CLLAs) was carried out. Co-aggregation strategy using polymers or proteic feeder were applied to improve cross-linking efficiency in the CLLAs formation. Although, CLLAs formation using glutaraldehyde provide high activity and effective cross-linking, substrate diffusion limitation might have occurred when macromolecular substrate is used at high concentration. To solve this challenge, the use of dialdehyde starch represents an attractive candidate for cross-linking the enzymes. Dialdehyde starch is

a polysaccharide derived by mild oxidation from natural starch. Thus, dialdehyde starch-tapioca was developed and was used as a macromolecular cross-linker in CLLAs formation. The preparation of CLLAs using macromolecular cross-linker was investigated to study the effect on immobilization efficiency and activity recovery. Moreover, substrate diffusion analysis was also reported to observe the effectiveness of macromolecular cross-linker in CLLAs preparation. However, due to their soft particle, enzyme leaching analysis and organic solvent tolerance was also determined to justify their stability. Therefore, in this study, the preparation of CLLAs using different cross-linkers were investigated to improve the properties of rlevblg1 as a potential synthetic catalyst for the synthesis of L-FOS.

1.3 Objectives of the Study

The objectives of the research are:

(a) To develop and characterize cross-linked levanase aggregates (CLLAs) with high activity recovery and stability.

(b) To improve, determine and characterize CLLAs development using a macromolecular cross-linker for the synthesis of L-FOS.

1.4 Scope of Study

The scope of this study covers five main parts:

(a) Expression and purification of recombinant levanase (rlevblg1) (Chapter 4).

(b) Preparation of CLLAs formation by manipulating several parameters (precipitants, cross-linkers, enzyme concentration, pH, additives) that affecting enzyme activity and stability using the one factor at a time (O.F.A.T) approach (Chapter 4).

(c) Characterization of the physicochemical, kinetic and thermodynamic properties of resultant CLLAs using glutaraldehyde as cross-linker (CLLAs-GA), and with bovine serum albumin (CLLAs-GA-BSA) (Chapter 4).

(d) Developmental and characterization of dialdehyde starch-tapioca (DAST) used as a macromolecular cross-linker by optimizing several factors such as periodate and starch concentration, temperature, pH and reaction time (Chapter 5).

(e) Screening and characterization of the factors affecting CLLAs formation using DAST as cross-linker by optimizing several parameters (DAST concentration, crosslinking time, agitation speed and BSA concentration) (Chapter 5).

1.5 Significance and Novelty of the Research

Nowadays, enzyme catalyst plays a crucial role in relevant biotechnological processes in the food and chemical industries. At the industrial level, enzyme catalysts should have outstanding characteristics such as smooth handling and operation procedure, high stability, reusability and cost-effective to meet its market demand. In the past decades, numerous works have been committed to improve the development of enzyme catalysts for various applications. However, many biocatalytic processes on an industrial scale unable to perform appropriate due to the enzyme characteristics such as low stability, substrate- and product inhibition, limitations of inefficient recycling and high production cost. In the case of rlevblg1 as the catalyst for L-FOS synthesis, this study represents the first study in demonstrating the immobilization of levanase to overcome free enzyme limitation using CLEAs technique. Enzyme immobilization via the CLEAs technique is a carrier-free immobilized enzyme that has been recognized as a promising technology to obtain robust industrial enzyme catalysts.

Unfortunately, the bottleneck in CLEAs technology hindered its potential as a robust enzyme catalyst, especially when the lysine residue of the enzyme is insufficient to complete a proper cross-linking process. In the present study, the addition of proteic feeder (BSA) is able to improve the cross-linking step during CLEAs formation, which resulted in hyperactivation of CLEAs activity recovery, improved thermal stability and produced higher DP of L-FOS.

On the other hand, the application of dialdehyde starch as a macromolecular cross-linker and natural-based polymer in enzyme immobilization of CLEAs determined their potential of dialdehyde starch as a safer cross-linker than glutaraldehyde. Inadequate research was conducted to study the effect of dialdehyde starch as an enzyme cross-linker, especially in CLEAs strategy. The development of dialdehyde starch using tapioca starch is characterized, and its potential as enzyme cross-linker in CLEAs technology was explored. This study is the first report that demonstrates the use of dialdehyde starch-tapioca as a cross-linker in rlevblg1 immobilization. Also, the first study reported the use of immobilized rlevblg1 in L-FOS synthesis. In this study, the development of immobilized rlevblg1 using different cross-linker in CLLAs formation was investigated to obtain a better idea for the immobilization effect on the reaction process and product formation by immobilized rlevblg1.

REFERENCES

Abd Rahman, N. H., Jaafar, N. R., Abdul Murad, A. M., Abu Bakar, F. D., Shamsul Annuar, N. A., and Illias, R. M. (2020). Novel cross-linked enzyme aggregates of levanase from *Bacillus lehensis* G1 for short-chain fructooligosaccharides synthesis : Developmental , physicochemical , kinetic and thermodynamic properties. *International Journal of Biological Macromolecules*, *159*, 577–589.

Abdul Wahab, M. K. H., El-Enshasy, H. A., Bakar, F. D. A., Murad, A. M. A., Jahim, J. M., and Illias, R. M. (2019). Improvement of cross-linking and stability on cross-linked enzyme aggregate (CLEA)-xylanase by protein surface engineering. *Process Biochemistry*, *86*(June), 40–49.

Adebola, O., Corcoran, O., and Morgan, W. A. (2013). Protective effects of prebiotics inulin and lactulose from cytotoxicity and genotoxicity in human colon adenocarcinoma cells. *Food Research International*, *52*(1), 269–274.

Ademakinwa, A. N., Ayinla, Z. A., Omitogun, O. G., and Agboola, F. K. (2018). Preparation, characterization and optimization of cross-linked fructosyltransferase aggregates for the production of prebiotic fructooligosaccharides. *Biotechnologia*, *99*(4), 417–434.

Agyei, D., and He, L. (2015). Evaluation of cross-linked enzyme aggregates of Lactobacillus cell-envelope proteinases, for protein degradation. *Food and Bioproducts Processing*, *94*, 59–69.

Aissaoui, N., Landoulsi, J., Bergaoui, L., Boujday, S., and Lambert, J.-F. (2013). Catalytic activity and thermostability of enzymes immobilized on silanized surface: Influence of the crosslinking agent. *Enzyme and Microbial Technology*, *52*(6), 336–343.

Akkas, T., Zakharyuta, A., Taralp, A., and Ow-Yang, C. W. (2020). Cross-linked enzyme lyophilisates (CLELs) of urease: A new method to immobilize ureases. *Enzyme and Microbial Technology*, *132*(August 2019), 109390.

Álvaro-Benito, M., de Abreu, M., Fernández-Arrojo, L., Plou, F. J., Jiménez-Barbero, J., Ballesteros, A., Polaina, J., and Fernández-Lobato, M. (2007). Characterization of a β-fructofuranosidase from *Schwanniomyces occidentalis* with transfructosylating activity yielding the prebiotic 6-kestose. *Journal of Biotechnology*, *132*(1), 75–81.

Amalin, N., Aziz, A., Safi, A., Yusof, F., and Azmi, A. S. (2020). Involvements of food grade dialdehydic pectin as cross-linker and soy protein as additive in the production of MNP-CLEA-lipase from Hevea brasiliensis. *Journal of Applied Biotechnology and Bioengineering*, *7*(5), 215–223.

Amaral-Fonseca, M., Kopp, W., Giordano, R. de L. C., Fernández-Lafuente, R., and Tardioli, P. W. (2018). Preparation of magnetic cross-linked amyloglucosidase aggregates: Solving some activity problems. *Catalysts*, *8*(11), 1–21.

Arakawa, T., Kita, Y., and Timasheff, S. N. (2007). Protein precipitation and denaturation by dimethyl sulfoxide. *Biophysical Chemistry*, *131*(1–3), 62–70.

Arsenault, A., Cabana, H., and Jones, J. P. (2011). Laccase-Based CLEAs: Chitosan as a Novel Cross-Linking Agent. *Enzyme Research*, *2011*, 1–10.

Ayhan, H., Ayhan, F., and Gülsu, A. (2012). Highly biocompatible enzyme aggregates crosslinked by L-lysine. *Turkish Journal of Biochemistry*, *37*(1), 14–20.

Aytar, B. S., and Bakir, U. (2008). Preparation of cross-linked tyrosinase aggregates. *Process Biochemistry*, *43*, 125–131.

Batista, K. A., Batista, G. L. A., Alves, G. L., and Fernandes, K. F. (2014). Extraction, partial purification and characterization of polyphenol oxidase from *Solanum lycocarpum* fruits. *Journal of Molecular Catalysis B: Enzymatic*, *102*, 211–217.

Bedade, D. K., Muley, A. B., and Singhal, R. S. (2019). Magnetic cross-linked enzyme aggregates of acrylamidase from *Cupriavidus oxalaticus* ICTDB921 for biodegradation of acrylamide from industrial waste water. *Bioresource Technology*, *272*(August 2018), 137–145.

Bekers, M., Laukevics, J., Upite, D., Kaminska, E., Vigants, A., Viesturs, U., Pankova, L., and Danilevics, A. (2002). Fructooligosaccharide and levan producing activity of *Zymomonas mobilis* extracellular levansucrase. *Process Biochemistry*, *38*(5), 701–706.

Biedrzycka, E., and Bielecka, M. (2004). Prebiotic effectiveness of fructans of different degrees of polymerization. *Trends in Food Science and Technology*, *15*(3–4), 170–175.

Bilal, M., Iqbal, H. M. N., Hu, H., Wang, W., and Zhang, X. (2017). Development of horseradish peroxidase-based cross-linked enzyme aggregates and their environmental exploitation for bioremediation purposes. *Journal of Environmental Management*, *188*, 137–143.

Bílková, Z., Slováková, M., Horák, D., Lenfeld, J., and Churáek, J. (2002). Enzymes immobilized on magnetic carriers: Efficient and selective system for protein modification. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, *770*(1–2), 177–181.

Bowes, J. H., and Cater, C. W. (1968). The interaction of aldehydes with collagen. *BBA - Protein Structure*, *168*(2), 341–352.

Brown, D. L., and Glatz, C. E. (1987). Aggregate breakage in protein precipitation. *Chemical Engineering Science*, *42*(7), 1831–1839.

Bruno-Barcena, J. M., and Azcarate-Peril, M. A. (2015). Galacto-oligosaccharides and colorectal cancer: Feeding our intestinal probiome. *Journal of Functional Foods*, *12*, 92–108.

ÇalımLı, M. H., Demirbaş, Ö., Aygün, A., Alma, M. H., Nas, M. S., and Şen, F. (2018). Immobilization kinetics and mechanism of bovine serum albumin on diatomite clay from aqueous solutions. *Applied Water Science*, *8*(7), 1–12.

Candela, M., Maccaferri, S., Turroni, S., Carnevali, P., and Brigidi, P. (2010). Functional intestinal microbiome, new frontiers in prebiotic design. *International Journal of Food Microbiology*, *140*(2–3), 93–101.

Cao, L. (2005). Immobilised enzymes: Science or art? *Current Opinion in Chemical Biology*, *9*(2), 217–226.

Cao, L. (2006a). Introduction: Immobilized Enzymes: Past, Present and Prospects. In *Carrier-bound Immobilized Enzymes: Principles, Application and Design* (pp. 1–52). WILEY-VCH Verlag.

Cao, L. (2006b). Introduction: Immobilized Enzymes: Past, Present and Prospects. In *Carrier-bound Immobilized Enzymes*.

Cao, L., van Langen, L., and Sheldon, R. A. (2003). Immobilised enzymes: Carrier-bound or carrier-free? *Current Opinion in Biotechnology*, *14*(4), 387–394.

Carvalho, A. F. A., Neto, P. de O., da Silva, D. F., and Pastore, G. M. (2013). Xylo-oligosaccharides from lignocellulosic materials: Chemical structure, health benefits and production by chemical and enzymatic hydrolysis. *Food Research International*, *51*(1), 75–85.

Cha, X., Han, S., Yu, J., Zhang, S., Yu, S., Fu, D., Yao, M., Zhang, L., and Feng, G. (2019). Inulin with a low degree of polymerization protects human umbilical vein endothelial cells from hypoxia/reoxygenation-induced injury. *Carbohydrate Polymers*, *216*, 97–106.

Chand, N., Nateri, A. S., Sajedi, R. H., Mahdavi, A., and Rassa, M. (2012). Enzymatic desizing of cotton fabric using a Ca2+-independent α-amylase with acidic pH profile. *Journal of Molecular Catalysis B: Enzymatic*, *83*, 46–50.

Chapman, J., Ismail, A. E., and Dinu, C. Z. (2018). Industrial Applications of Enzymes : Recent Advances , Techniques , and Outlooks. *Catalysts*, *8*(238), 20–29.

Charoenwongpaiboon, T., Pichyangkura, R., Field, R. A., and Prousoontorn, M. H. (2019). Preparation of Cross-Linked Enzyme Aggregates (CLEAs) of an Inulosucrase Mutant for the Enzymatic Synthesis of Inulin-Type Fructooligosaccharides. *Catalysts*, *9*, 641–652.

Chaudhary, A., Gupta, L. K., Gupta, J. K., and Banerjee, U. C. (1996). Purification and properties of levanase from *Rhodotorula* sp. *Journal of Biotechnology*, *46*(1), 55–61.

Chávez, G., Hatti-Kaul, R., Sheldon, R. A., and Mamo, G. (2013). Baeyer-Villiger oxidation with peracid generated in situ by CaLB-CLEA catalyzed perhydrolysis. *Journal of Molecular Catalysis B: Enzymatic*, *89*, 67–72.

Chen, S. C., Sheu, D. C., and Duan, K. J. (2014). Production of fructooligosaccharides using β-fructofuranosidase immobilized onto chitosan-coated magnetic nanoparticles. *Journal of the Taiwan Institute of Chemical Engineers*, *45*(4), 1105–1110.

Chen, Z., Wang, Y., Liu, W., Wang, J., and Chen, H. (2017). A novel cross-linked enzyme aggregates (CLEAs) of papain and neutrase-production, partial characterization and application. *International Journal of Biological Macromolecules*, *95*, 650–657.

Cheng, T., Duan, K., and Sheu, D. (2005). Immobilization of b -fructofuranosidase from *Aspergillus japonicus* on chitosan using tris (hydroxymethyl) phosphine or glutaraldehyde as a coupling agent. *Biotechnology Letter*, *27*, 335–338.

Chiang, C. J., Lee, W. C., Sheu, D. C., and Duan, K. J. (1997). Immobilization of β-fructofuranosidases from *Aspergillus* on methacrylamide-based polymeric beads for production of fructooligosaccharides. *Biotechnology Progress*, *13*(5), 577–582.

Chung, Y. C., Hsu, C. K., Ko, C. Y., and Chan, Y. C. (2007). Dietary intake of xylooligosaccharides improves the intestinal microbiota, fecal moisture, and pH value in the elderly. *Nutrition Research*, *27*(12), 756–761.

Cruz, J., Barbosa, O., Rodrigues, R. C., Fernandez-Lafuente, R., Torres, R., and Ortiz, C. (2012). Optimized preparation of CALB-CLEAs by response surface methodology: The necessity to employ a feeder to have an effective crosslinking. *Journal of Molecular Catalysis B: Enzymatic*, *80*, 7–14.

Cui, J. D., and Jia, S. R. (2015). Optimization protocols and improved strategies of cross-linked enzyme aggregates technology: current development and future challenges. *Critical Reviews in Biotechnology*, *35*(1), 15–28.

Cui, J. D., Li, L. L., and Zhao, Y. M. (2014). Simple technique for preparing stable and recyclable cross-linked enzyme aggregates with crude-pored microspherical silica core. *Industrial and Engineering Chemistry Research*, *53*(42), 16176–16182.

Cui, J. D., Liu, R. L., and Li, L. B. (2016). A facile technique to prepare cross-linked enzyme aggregates of bovine pancreatic lipase using bovine serum albumin as an additive. *Korean Journal of Chemical Engineering*, *33*(2), 610–615.

Cui, J. D., Sun, L. M., and Li, L. L. (2013). A simple technique of preparing stable cleas of phenylalanine ammonia lyase using Co-aggregation with starch and bovine serum albumin. *Applied Biochemistry and Biotechnology*, *170*(8), 1827–1837.

Cui, Jian dong, Zhao, Y., Feng, Y., Lin, T., Zhong, C., Tan, Z., and Jia, S. (2017). Encapsulation of spherical cross-linked phenylalanine ammonia lyase aggregates in mesoporous biosilica. In *Journal of Agricultural and Food Chemistry* (Vol. 65, Issue 3).

Cui, Jiandong, Jia, S., Liang, L., Zhao, Y., and Feng, Y. (2015). Mesoporous CLEAs-silica composite microparticles with high activity and enhanced stability. *Scientific Reports*, *5*, 1–13.

Cui, Jiandong, Zhao, Y., Feng, Y., Lin, T., Zhong, C., Tan, Z., and Jia, S. (2017). Encapsulation of spherical cross-linked phenylalanine ammonia lyase aggregates in mesoporous biosilica. In *Journal of Agricultural and Food Chemistry* (Vol. 65, Issue 3).

Dahech, I., Ayed, H. Ben, Belghith, K. S., Belghith, H., and Mejdoub, H. (2013). Microbial production of levanase for specific hydrolysis of levan. *International Journal of Biological Macromolecules*, *60*, 128–133.

Dahili, L. A., Kelemen-Horváth, I., and Feczkó, T. (2015). 2,4-Dichlorophenol removal by purified horseradish peroxidase enzyme and crude extract from horseradish immobilized to nano spray dried ethyl cellulose particles. *Process Biochemistry*, *50*(11), 1835–1842.

Dalal, S., Kapoor, M., and Gupta, M. N. (2007). Preparation and characterization of combi-CLEAs catalyzing multiple non-cascade reactions. *Journal of Molecular Catalysis B: Enzymatic*, *44*(3–4), 128–132.

Datta, S., Christena, L. R., and Rajaram, Y. R. S. (2013). Enzyme immobilization: an overview on techniques and support materials. *3 Biotech*, *3*(1), 1–9.

Davids, T., Schmidt, M., Böttcher, D., and Bornscheuer, U. T. (2013). Strategies for the discovery and engineering of enzymes for biocatalysis. *Current Opinion in Chemical Biology*, *17*(2), 215–220.

Davis, B. G. (2003). Chemical modification of biocatalysts. *Current Opinion in Biotechnology*, *14*(4), 379–386.

Dawlee, S., Sugandhi, A., Balakrishnan, B., Labarre, D., and Jayakrishnan, A. (2005). Oxidized chondroitin sulfate-cross-linked gelatin matrixes: A new class of hydrogels. *Biomacromolecules*, *6*(4), 2040–2048.

de Oliveira, R. L., da Silva, M. F., da Silva, S. P., de Araújo, A. C. V., Cavalcanti, J. V. F. L., Converti, A., and Porto, T. S. (2020). Fructo-oligosaccharides production by an *Aspergillus aculeatus* commercial enzyme preparation with fructosyltransferase activity covalently immobilized on Fe3O4–chitosan-magnetic nanoparticles. *International Journal of Biological Macromolecules*, *150*, 922–929.

Demirjian, D. C., Morís-Varas, F., and Cassidy, C. S. (2001). Enzymes from extremophiles. *Current Opinion in Chemical Biology*, *5*(2), 144–151.

Dijkstra, Z. J., Merchant, R., and Keurentjes, J. T. F. (2007). Stability and activity of enzyme aggregates of Calb in supercritical CO2. *Journal of Supercritical Fluids*, *41*(1), 102–108.

Dong, T., Zhao, L., Huang, Y., and Tan, X. (2010a). Preparation of cross-linked aggregates of aminoacylase from *Aspergillus melleus* by using bovine serum albumin as an inert additive. *Bioresource Technology*, *101*(16), 6569–6571.

Doraiswamy, N., Sarathi, M., and Pennathur, G. (2019). Cross-linked esterase aggregates (CLEAs) using nanoparticles as immobilization matrix. *Preparative Biochemistry and Biotechnology*, *49*(3), 270–278.

Dou, Y., Huang, X., Zhang, B., He, M., Yin, G., and Cui, Y. (2015). Preparation and characterization of a dialdehyde starch crosslinked feather keratin film for food packaging application. *RSC Adv.*, *5*(34), 27168–27174.

Dwevedi, A. (2016). Enzyme immobilization: Advances in industry, agriculture, medicine, and the environment. *Enzyme Immobilization: Advances in Industry, Agriculture, Medicine, and the Environment*, 1–132.

Emregul, E., Sungur, S., and Akbulut, U. (2006). Polyacrylamide–gelatine carrier system used for invertase immobilization. *Food Chemistry*, *97*(4), 591–597.

Ernest, V., Nirmala, M. J., Gajalakshmi, S., Mukherjee, A., and Chandrasekaran, N. (2013). Biophysical investigation of alpha amylase conjugated silver nanoparticles proves structural changes besides increasing its enzyme activity. *Journal of Bionanoscience*, *7*(3), 271–275.

Ernits, K., Eek, P., Lukk, T., Visnapuu, T., and Alamäe, T. (2019). First crystal structure of an endo-levanase - the BT1760 from a human gut commensal Bacteroides thetaiotaomicron. *Scientific Reports*, *9*(1), 8443.

Eş, I., Vieira, J. D. G., and Amaral, A. C. (2015). Principles, techniques, and applications of biocatalyst immobilization for industrial application. *Applied Microbiology and Biotechnology*, *99*(5), 2065–2082.

Fattah, S. S. bt A. (2018). *Kejuruteraan protein endo-levanase recombinan daripada Bacillus lehensis G1 bagi meningkatkan produk khusus*. Universiti Teknologi Malaysia.

Fernandez-Lopez, L., Pedrero, S. G., Lopez-Carrobles, N., Gorines, B. C., Virgen-Ortíz, J. J., and Fernandez-Lafuente, R. (2017). Effect of protein load on stability of immobilized enzymes. *Enzyme and Microbial Technology*, *98*, 18–25.

Fernandez Silva, M., Golunski, S. M., Rigo, D., Mossi, V., Kunh, G., Di Luccio, M., de oliveira, J. vladimir, Tres, M. V., De Oliveira, D., and Treichel, H. (2013). Fructooligosaccharide production using inulinase of *Aspergillus niger* after treatment with fluid pressurized in two different means. *III Iberoamerican Conference on Supercritical Fluids Cartagena de Indias (Colombia),* 1–7.

Förster-Fromme, K., Schuster-Wolff-Bühring, R., Hartwig, A., Holder, A., Schwiertz, A., Bischoff, S. C., and Hinrichs, J. (2011). A new enzymatically produced 1-lactulose: A pilot study to test the bifidogenic effects. *International Dairy Journal*, *21*(12), 940–948.

Ganaie, M. A., Rawat, H. K., Wani, O. A., Gupta, U. S., and Kango, N. (2014). Immobilization of fructosyltransferase by chitosan and alginate for efficient production of fructooligosaccharides. *Process Biochemistry*, *49*(5), 840–844.

Garcia-Galan, C., Berenguer-Murcia, Á., Fernandez-Lafuente, R., and Rodrigues, R. C. (2011). Potential of different enzyme immobilization strategies to improve enzyme performance. *Advanced Synthesis and Catalysis*, *353*(16), 2885–2904.

Glassford, S. E., Byrne, B., and Kazarian, S. G. (2013). Recent applications of ATR FTIR spectroscopy and imaging to proteins. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics*, *1834*(12), 2849–2858.

Goetze, D., Foletto, E. F., da Silva, H. B., Silveira, V. C. C., Dal Magro, L., and Rodrigues, R. C. (2017). Effect of feather meal as proteic feeder on combi-CLEAs preparation for grape juice clarification. *Process Biochemistry*, *62*(May), 122–127.

González-Garcinuño, Á., Tabernero, A., Sánchez-Álvarez, J. M., Galán, M. A., and Martin del Valle, E. M. (2017). Effect of bacteria type and sucrose concentration on levan yield and its molecular weight. *Microbial Cell Factories*, *16*(1), 1–11.

Górecka, E., and Jastrzębska, M. (2011). Review article: Immobilization techniques and biopolymer carriers. *Biotechnology and Food Science*, *75*(1), 65–86.

Granza, A. G., Travalini, A. P., Farias, F. O., Colman, T. A. D., Schnitzler, E., and Demiate, I. M. (2015). Effects of acetylation and acetylation–hydroxypropylation (dual-modification) on the properties of starch from Carioca bean (Phaseolus vulgaris L.). *Journal of Thermal Analysis and Calorimetry*, *119*(1), 769–777.

Guajardo, N., Ahumada, K., Domínguez de María, P., and Schrebler, R. A. (2019). Remarkable stability of *Candida antarctica* lipase B immobilized via cross-linking aggregates (CLEA) in deep eutectic solvents. *Biocatalysis and Biotransformation*, *37*(2), 106–114.

Gualandi, A., Calogero, F., Potenti, S., and Cozzi, P. G. (2019). Al(Salen) metal complexes in stereoselective catalysis. *Molecules*, *24*(9).

Guauque Torres, M. P., Foresti, M. L., and Ferreira, M. L. (2014). CLEAs of *Candida antarctica* lipase B (CALB) with a bovine serum albumin (BSA) cofeeder core: Study of their catalytic activity. *Biochemical Engineering Journal*, *90*, 36–43.

Guimarães, J. R., de Lima Camargo Giordano, R., Fernandez-Lafuente, R., and Tardioli, P. W. (2018a). Evaluation of strategies to produce highly porous cross-linked aggregates of porcine pancreas lipase with magnetic properties. *Molecules*, *23*(11), 13–16.

Guimarães, J. R., de Lima Camargo Giordano, R., Fernandez-Lafuente, R., and Tardioli, P. W. (2018b). Evaluation of strategies to produce highly porous cross-linked aggregates of porcine pancreas lipase with magnetic properties. *Molecules*, *23*(11), 4–5.

Guisan, M., Rodriguez, V., Resell, C. M., Soler, G., Bastida, A., Blanco, R. M., Fernindez-lafuente, R., and Garcia-junceda, E. (1997). Stabilization of Immobilized Enzymes by Chemical with Polyfunctional Macromolecules. In *Immobilization of Enzyme and Cells: Vol. I* (pp. 289–298). Humana Press. Inc.

Guo, J., Ge, L., Li, X., Mu, C., and Li, D. (2014). Periodate oxidation of xanthan gum and its crosslinking effects on gelatin-based edible films. *Food Hydrocolloids*, *39*, 243–250.

Gupta, M. N., Perwez, M., and Sardar, M. (2020). Protein crosslinking: Uses in chemistry, biology and biotechnology. *Biocatalysis and Biotransformation*, *38*(3), 178–201.

Gürdaş, S., Güleç, H. A., and Mutlu, M. (2012). Immobilization of *Aspergillus oryzae* β-Galactosidase onto Duolite A568 Resin via Simple Adsorption Mechanism. *Food and Bioprocess Technology*, *5*(3), 904–911.

Guzik, U., Hupert-Kocurek, K., and Wojcieszyńska, D. (2014). Immobilization as a strategy for improving enzyme properties-application to oxidoreductases. *Molecules (Basel, Switzerland)*, *19*(7), 8995–9018.

Harish Prashanth, K. V., and Tharanathan, R. N. (2007). Chitin/chitosan: modifications and their unlimited application potential-an overview. *Trends in Food Science and Technology*, *18*(3), 117–131.

Hassani, T., Ba, S., and Cabana, H. (2013). Formation of enzyme polymer engineered structure for laccase and cross-linked laccase aggregates stabilization. *Bioresource Technology*, *128*, 640–645.

Heck, T., Faccio, G., Richter, M., and Thöny-Meyer, L. (2013). Enzyme-catalyzed protein crosslinking. *Applied Microbiology and Biotechnology*, *97*(2), 461–475.

Hero, J. S., Morales, A. H., Perotti, N. I., Romero, C. M., and Martinez, M. A. (2020). Improved development in magnetic Xyl-CLEAs technology for biotransformation of agro-industrial by-products through the use of a novel macromolecular cross-linker. *Reactive and Functional Polymers*, *154*(June), 104676.

Hilal, N., Nigmatullin, R., and Alpatova, A. (2004). Immobilization of cross-linked lipase aggregates within microporous polymeric membranes. *Journal of Membrane Science*, *238*(1–2), 131–141.

Homaei, A. A., Sariri, R., Vianello, F., and Stevanato, R. (2013). Enzyme immobilization: An update. *Journal of Chemical Biology*, *6*(4), 185–205.

Hongbo, T., Yanping, L., Wen, Z., and Siqing, D. (2016). Synthesis, Optimization, Property, Characterization, and Application of Dialdehyde Cross-Linking Guar Gum. *International Journal of Polymer Science*, *2016*, 1–14.

Hormigo, D., García-Hidalgo, J., Acebal, C., De la Mata, I., and Arroyo, M. (2012). Preparation and characterization of cross-linked enzyme aggregates (CLEAs) of recombinant poly-3-hydroxybutyrate depolymerase from *Streptomyces exfoliatus*. *Bioresource Technology*, *115*, 177–182.

Hu, X., Liu, L., Chen, D., Wang, Y., Zhang, J., and Shao, L. (2017). Co-expression of the recombined alcohol dehydrogenase and glucose dehydrogenase and cross-linked enzyme aggregates stabilization. *Bioresource Technology*, *224*, 531–535.

Hwang, E. T., and Gu, M. B. (2013). Enzyme stabilization by nano/microsized hybrid materials. *Engineering in Life Sciences*, *13*(1), 49–61.

Ibrahim, O. O. (2018). *Functional Oligosaccharides: Chemicals Structure, Manufacturing, Health Benefits, Applications and Regulations*. 65–76.

Igarashi, T., Takahashi, M., Yamamoto, A., Etoh, Y., And Takamori, K. (1987). Purification and characterization of ginsenoside-α-L-rhamnosidase. *American Society for Microbiology*, *55*(12), 3001–3005.

Illias, R. M., Fen, T. S., Abdulrashid, N. A., Yusoff, W. M. W., Hamid, A. A., Hassan, O., and Kamaruddin, K. (2002). Cyclodextrin Glucanotransferase producing alkalophilic *Bacillus* sp. G1: Its cultural condition and Partial Characterization of the enzyme. *Pakistan Journal of Biological Science*, *5*(6), 688–692.

Ito, H., Takemura, N., Sonoyama, K., Kawagishi, H., Topping, D. L., Conlon, M. A., and Morita, T. (2011). Degree of polymerization of inulin-type fructans differentially affects number of lactic acid bacteria, intestinal immune functions, and immunoglobulin A secretion in the rat cecum. *Journal of Agricultural and Food Chemistry*, *59*(10), 5771–5778.

Iyer, P. V., and Ananthanarayan, L. (2008). Enzyme stability and stabilization-Aqueous and non-aqueous environment. *Process Biochemistry*, *43*(10), 1019–1032.

Jadhav, S. B., and Singhal, R. S. (2013). Polysaccharide conjugated laccase for the dye decolorization and reusability of effluent in textile industry. *International Biodeterioration and Biodegradation*, *85*, 271–277.

Jaiswal, K. S., and Rathod, V. K. (2020). Green synthesis of amyl levulinate using lipase in the solvent free system: Optimization, mechanism and thermodynamics studies. *Catalysis Today*.

Jathore, N. R., Bule, M. V., Tilay, A. V., and Annapure, U. S. (2012). Microbial levan from Pseudomonas fluorescens: Characterization and medium optimization for enhanced production. *Food Science and Biotechnology*, *21*(4), 1045–1053.

Jin, W., Xu, Y., and Yu, X. W. (2019). Preparation of lipase cross-linked enzyme aggregates in octyl-modified mesocellular foams. *International Journal of Biological Macromolecules*, *130*, 342–347.

Jung, D., Paradiso, M., and Hartmann, M. (2009). Formation of cross-linked glucose oxidase aggregates in mesocellular foams. *Journal of Materials Science*, *44*(24), 6747–6753.

Kang, S. K., Lee, S. O., Lim, Y. S., Jang, K. L., and Lee, T. H. (1998). Purification and characterization of a novel levanoctaose-producing levanase from *Pseudomonas* strain K-52. *Biotechnology and Applied Biochemistry*, *27*(2), 159–166.

Karam, E. A., Abdel Wahab, W. A., Saleh, S. A. A., Hassan, M. E., Kansoh, A. L., and Esawy, M. A. (2017). Production, immobilization and thermodynamic studies of free and immobilized *Aspergillus awamori* amylase. *International Journal of Biological Macromolecules*, *102*, 694–703.

Kartal, F., and Kilinc, A. (2012). Crosslinked aggregates of Rhizopus oryzae lipase as industrial biocatalysts: preparation, optimization, characterization, and application for enantioselective resolution reactions. *Biotechnology Progress*, *28*(4), 937–945.

Kasperowicz, A., Pristaš, P., Piknová, M., Javorský, P., Guczyńska, W., Michałovski, T., and Kwiatkowska, E. (2010). Fructanolytic and saccharolytic enzymes of *Treponema zioleckii* strain kT. *Anaerobe*, *16*(4), 387–392.

Kaul, P., Stolz, A., and Banerjee, U. C. (2007). Cross-linked amorphous nitrilase aggregates for enantioselective nitrile hydrolysis. *Advanced Synthesis and Catalysis*, *349*(13), 2167–2176.

Kim, I. Y., Seo, S. J., Moon, H. S., Yoo, M. K., Park, I. Y., Kim, B. C., and Cho, C. S. (2008). Chitosan and its derivatives for tissue engineering applications. *Biotechnology Advances*, *26*(1), 1–21.

Kiosseoglou, A., Doxastakis, G., Alevisopoulos, S., and Kasapis, S. (1999). Physical characterization of thermally induced networks of lupin protein isolates prepared by isoelectric precipitation and dialysis. *International Journal of Food Science and Technology*, *34*(3), 253–263.

Krajewska, B. (2004). Application of chitin- and chitosan-based materials for enzyme immobilizations: A review. *Enzyme and Microbial Technology*, *35*(2–3), 126–139.

Kulkarni, N. H., Muley, A. B., Bedade, D. K., and Singhal, R. S. (2020). Cross-linked enzyme aggregates of arylamidase from *Cupriavidus oxalaticus* ICTDB921: process optimization, characterization, and application for mitigation of acrylamide in industrial wastewater. *Bioprocess and Biosystems Engineering*, *43*(3), 457–471.

Kumar, P., and Dubey, K. K. (2019). Current Perspectives and Future Strategies for Fructooligosaccharides Production Through Membrane Bioreactor. In *Applied Microbiology and Bioengineering*. Elsevier Inc.

Li, A. B., Kluge, J. A., Guziewicz, N. A., Omenetto, F. G., and Kaplan, D. L. (2015). Silk-based stabilization of biomacromolecules. *Journal of Controlled Release : Official Journal of the Controlled Release Society*, *219*, 416–430.

Li, S., Su, Y., Liu, Y., Sun, L., Yu, M., and Wu, Y. (2016). Preparation and characterization of cross-linked enzyme aggregates (CLEAs) of recombinant thermostable alkylsulfatase (SdsAP) from *Pseudomonas* sp. S9. *Process Biochemistry*, *51*(12), 2084–2089.

Li, X., Yu, Z., Bian, Z., Xu, J., Zhang, L., and Qiao, M. (2018). Physiochemical characterization of α-amylase as crosslinked enzyme aggregates. *Catalysts*, *8*(8).

Liao, Q., Du, X., Jiang, W., Tong, Y., Zhao, Z., Fang, R., Feng, J., and Tang, L. (2018). Cross-linked enzyme aggregates (CLEAs) of halohydrin dehalogenase from *Agrobacterium radiobacter* AD1: Preparation, characterization and application as a biocatalyst. *Journal of Biotechnology*, *272–273*, 48–55.

Liese, A., and Hilterhaus, L. (2013). Evaluation of immobilized enzymes for industrial applications. *Chemical Society Reviews*, *42*(15), 6236–6249.

Lim, Y. S., Kang, S. K., Lee, S. O., Lee, J. D., and Lee, T. H. (1998). Purification and characterization of a levanase from *Streptomyces* sp. 366L. *Journal of Biotechnology*, *61*, 33–41.

Liu, X., Wang, L., Song, X., Song, H., Zhao, J. R., and Wang, S. (2012). A kinetic model for oxidative degradation of bagasse pulp fiber by sodium periodate. *Carbohydrate Polymers*, *90*(1), 218–223.

Liu, Y., Guo, Y. L., Chen, D. W., Peng, C., and Yan, Y. J. (2011). Conformation and Activity of Sol-Gels Encapsulated Cross-Linked Enzyme Aggregates of Lipase from *Burkholderia cepacia*, *Advanced Materials Research*, *291–294*, 614–620.

Liu, Z., Fatehi, P., Sadeghi, S., and Ni, Y. (2011). Application of hemicelluloses precipitated via ethanol treatment of pre-hydrolysis liquor in high-yield pulp. *Bioresource Technology*, *102*(20), 9613–9618.

Logan, S. R. (1977). Arrhenius activation energy of reactions that are almost diffusion-controlled. *Journal of the Chemical Society, Faraday Transactions 1: Physical Chemistry in Condensed Phases*, *73*, 592–595.

López-Gallego, F., Betancor, L., Hidalgo, A., Alonso, N., Fernández-Lafuente, R., and Guisán, J. M. (2005). Co-aggregation of enzymes and polyethyleneimine: A simple method to prepare stable and immobilized derivatives of glutaryl acylase. *Biomacromolecules*, *6*(4), 1839–1842.

Lôpez-Serrano, P., Cao, L., Van Rantwijk, F., and Sheldon, R. A. (2002). Cross-linked enzyme aggregates with enhanced activity: Application to lipases. *Biotechnology Letters*, *24*(16), 1379–1383.

Lorenzoni, A. S. G., Aydos, L. F., Klein, M. P., Rodrigues, R. C., and Hertz, P. F. (2014). Fructooligosaccharides synthesis by highly stable immobilized β-fructofuranosidase from *Aspergillus aculeatus*. *Carbohydrate Polymers*, *103*(1), 193–197.

Lu, S., Wang, X., Lu, Q., Hu, X., Uppal, N., Omenetto, F. G., and Kaplan, D. L. (2009). Stabilization of Enzymes in Silk Films. *Biomacromolecules*, *10*(5), 1032–1042.

Lucena, G. N., Santos, C. C. dos, Pinto, G. C., Piazza, R. D., Guedes, W. N., Jafelicci Júnior, M., de Paula, A. V, and Marques, R. F. C. (2020). Synthesis and characterization of magnetic cross-linked enzyme aggregate and its evaluation of the alternating magnetic field (AMF) effects in the catalytic activity. *Journal of Magnetism and Magnetic Materials*, *516*, 167326.

Lv, Y., Long, Z., Song, C., Dai, L., He, H., and Wang, P. (2013). Preparation of Dialdehyde Chitosan and its Application in Green Synthesis of Silver Nanoparticles. *Bioresources.Com*, *8*(4), 6161–6172.

Mafra, A. C. O., Kopp, W., Beltrame, M. B., de Lima Camargo Giordano, R., de Arruda Ribeiro, M. P., and Tardioli, P. W. (2016). Diffusion effects of bovine serum albumin on cross-linked aggregates of catalase. *Journal of Molecular Catalysis B: Enzymatic*, *133*, 107–116.

Mahmod, S. S., Yusof, F., Jami, M. S., and Khanahmadi, S. (2016). Optimizing the preparation conditions and characterization of a stable and recyclable cross-linked enzyme aggregate (CLEA)-protease. *Bioresources and Bioprocessing*, *3*(1).

Maldonado-Valderrama, J., and Patino, J. M. R. (2010). Interfacial rheology of protein-surfactant mixtures. *Current Opinion in Colloid and Interface Science*, *15*(4), 271–282.

Martínez-Moñino, A. B., Zapata-Pérez, R., García-Saura, A. G., Cabanes, J., and Sánchez-Ferrer, Á. (2017). A new cross-linked enzyme aggregate biocatalyst for NAD+-booster production. *RSC Advances*, *7*(23), 14272–14278.

Martins, G. N., Ureta, M. M., Tymczyszyn, E. E., Castilho, P. C., and Gomez-Zavaglia, A. (2019). Technological aspects of the production of fructo and galacto-oligosaccharides. Enzymatic synthesis and hydrolysis. *Frontiers in Nutrition*, *6*(May).

Martins, M., Azoia, N., Silva, C., and Cavaco-Paulo, A. (2015). Stabilization of enzymes in micro-emulsions for ultrasound processes. *Biochemical Engineering Journal*, *93*, 115–118.

Marx, S. P., Winkler, S., and Hartmeier, W. (2000). Fermentation of levan-oligosaccharides by different bifidobacteria. *FEMS Microbiology Letters*, *182*(1), 163–169.

Matamá, T., Araújo, R., Gübitz, G. M., Casal, M., and Cavaco-Paulo, A. (2010). Functionalization of cellulose acetate fibers with engineered cutinases. *Biotechnology Progress*, *26*(3), 636–643.

Matella, N. J., Dolan, K. D., and Lee, Y. S. (2006). Comparison of galactooligosaccharide production in free-enzyme ultrafiltration and in immobilized-enzyme systems. *Journal of Food Science*, *71*(7), 363–368.

Mateo, C., Palomo, J. M., Fernandez-Lorente, G., Guisan, J. M., and Fernandez-Lafuente, R. (2007). Improvement of enzyme activity, stability and selectivity via immobilization techniques. *Enzyme and Microbial Technology*, *40*(6), 1451–1463.

Mateo, C., Palomo, J. M., Van Langen, L. M., Van Rantwijk, F., and Sheldon, R. A. (2004). A New, Mild Cross-Linking Methodology to Prepare Cross-Linked Enzyme Aggregates. *Biotechnology and Bioengineering*, *86*(3), 273–276.

Mateo, C., Pessela, B. C. C., Fuentes, M., Torres, R., Betancor, L., Hidalgo, A., Fernandez-Lorente, G., Fernandez-Lafuente, R., and Guisan, J. M. (2020). Stabilization of Multimeric Enzymes via Immobilization and Further Cross-Linking with Aldehyde-Dextran. *Methods in Molecular Biology (Clifton, N.J.)*, *2100*, 175–187.

Matijošyte, I., Arends, I. W. C. E., de Vries, S., and Sheldon, R. A. (2010). Preparation and use of cross-linked enzyme aggregates (CLEAs) of laccases. *Journal of Molecular Catalysis B: Enzymatic*, *62*(2), 142–148.

Maugard, T., Gaunt, D., Legoy, M. D., and Besson, T. (2003). Microwave-assisted synthesis of galacto-oligosaccharides from lactose with immobilized β-galactosidase from *Kluyveromyces lactis*. *Biotechnology Letters*, *25*(8), 623–629.

Mayer, J., Kranz, B., and Fischer, L. (2010). Continuous production of lactulose by immobilized thermostable β-glycosidase from *Pyrococcus furiosus*. *Journal of Biotechnology*, *145*(4), 387–393.

Menéndez, C., Hernández, L., Selman, G., Mendoza, M. F., Hevia, P., Sotolongo, M., and Arrieta, J. G. (2002). Molecular cloning and expression in Escherichia coli of an exo-levanase gene from the endophytic bacterium *Gluconacetobacter diazotrophicus* SRT4. *Current Microbiology*, *45*(1), 5–12.

Meyer, T. S. M., Miguel, A. S. M., Fernandez, D. E. R., and Ortiz, G. M. D. (2016). Biotechnological Production of Oligosaccharides — Applications in the Food Industry. In *Food Production and Industry: Vol. Chapter 2* (pp. 25–77).

Michel, D. (2018). A probabilistic rate theory connecting kinetics to thermodynamics. *Physica A: Statistical Mechanics and Its Applications*, *503*, 26–44.

Migneault, I., Dartiguenave, C., Bertrand, M. J., and Waldron, K. C. (2004). Glutaraldehyde: Behavior in aqueous solution, reaction with proteins, and application to enzyme crosslinking. *BioTechniques*, *37*(5), 790–802.

Miller, G. L. (1959). Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. *Analytical Chemistry*, *31*(3), 426–428.

Moehlenbrock, M. J., and Minteer, S. D. (2017). Introduction to the Field of Enzyme Immobilization and Stabilization BT. In S. D. Minteer (Ed.), *Enzyme Stabilization and Immobilization: Methods and Protocols* (pp. 1–7). Springer New York.

Mohamad, N. R., Marzuki, N. H. C., Buang, N. A., Huyop, F., and Wahab, R. A. (2015). An overview of technologies for immobilization of enzymes and surface analysis techniques for immobilized enzymes. *Biotechnology and Biotechnological Equipment*, *29*(2), 205–220.

Moosavi-Nasab, M., Layegh, B., Aminlari, L., and Hashemi, M. B. (2010). Microbial Production of Levan using Date Syrup and Investigation of Its Properties. *World Academy of Science, Engineering and Technology, International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering*, *4*, 603–609.

Muley, A. B., Chaudhari, S. A., and Singhal, R. S. (2017). Non-covalent conjugation of cutinase from *Fusarium* sp. ICT SAC1 with pectin for enhanced stability: Process minutiae, kinetics, thermodynamics and structural study. *International Journal of Biological Macromolecules*, *102*, 729–740.

Muley, A. B., Mulchandani, K. H., and Singhal, R. S. (2019). Immobilization of enzymes on iron oxide magnetic nanoparticles : Synthesis , characterization , kinetics and thermodynamics. In *Nanoarmoring of Enzymes with Carbon Nanotubes and Magnetic Nanoparticles* (1st ed.). Elsevier Inc.

Murakami, H., Kuramoto, T., Mizutani, K., Nakano, H., and Kitahata, S. (1992). Purification and Some Properties of a New Levanase from *Bacillus* sp. No. 71. *Bioscience, Biotechnology and Biochemistry*, *56*(4), 608–613.

Mussatto, S. I., and Mancilha, I. M. (2007). Non-digestible oligosaccharides: A review. *Carbohydrate Polymers*, *68*(3), 587–597.

Nadar, S. S., Muley, A. B., Ladole, M. R., and Joshi, P. U. (2016). Macromolecular cross-linked enzyme aggregates (M-CLEAs) of α-amylase. *International Journal of Biological Macromolecules*, *84*, 69–78.

Nair, A. R., and Chellapan, G. (2019). Improving operational stability of thermostable *Pythium myriotylum* secretory serine protease by preparation of cross-linked enzyme aggregates (CLEAs) serine protease by preparation of cross-linked enzyme aggregates (CLEAs). *Preparative Biochemistry and Biotechnology*, *0*(0), 1–10.

Nawawi, N. N., Hashim, Z., Manas, N. H. A., Azelee, N. I. W., and Illias, R. M. (2020). A porous-cross linked enzyme aggregates of maltogenic amylase from *Bacillus lehensis* G1: Robust biocatalyst with improved stability and substrate diffusion. *International Journal of Biological Macromolecules*, *148*, 1222–1231.

Oluwasina, O. O., Falola, T., Wahab, O. J., and Idahagbon, N. B. (2017). *Research Article Enhancement of Physical and Mechanical Properties of*. 1–26.

Öztürk, B. (2001). Immobilization of Lipase from *Candida rugosa* on Hydrophobic and Hydrophilic Supports. In *Immobilization of Lipase from Candida rugosa on Hydrophobic and Hydrophilic Supports*.

Park, H. J., Uhm, K. N., and Kim, H. K. (2010). Biotransformation of amides to acids using a co-cross-linked enzyme aggregate of *Rhodococcus erythropolis* amidase. *Journal of Microbiology and Biotechnology*, *20*(2), 325–331.

Park, J. M., Kim, M., Park, H. S., Jang, A., Min, J., and Kim, Y. H. (2012). Immobilization of lysozyme-CLEA onto electrospun chitosan nanofiber for effective antibacterial applications. *International Journal of Biological Macromolecules*, *54*(1), 37–43.

Park, J. M., Kim, M., Park, H. S., Jang, A., Min, J., and Kim, Y. H. (2013). Immobilization of lysozyme-CLEA onto electrospun chitosan nanofiber for effective antibacterial applications. *International Journal of Biological Macromolecules*, *54*(1), 37–43.

Park, J. M., Kim, M., Park, J. Y., Lee, D. H., Lee, K. H., Min, J., and Kim, Y. H. (2010). Immobilization of the cross-linked para-nitrobenzyl esterase of *Bacillus subtilis* aggregates onto magnetic beads. *Process Biochemistry*, *45*(2), 259–263.

Patel, S., and Goyal, A. (2011). Functional oligosaccharides: Production, properties and applications. *World Journal of Microbiology and Biotechnology*, *27*(5), 1119–1128.

Pellis, A., Cantone, S., Ebert, C., and Gardossi, L. (2018). Evolving biocatalysis to meet bioeconomy challenges and opportunities. *New Biotechnology*, *40*, 154–169.

Pervez, S., Nawaz, M. A., Shahid, F., Aman, A., Tauseef, I., and Qader, S. A. U. (2019). Characterization of cross-linked amyloglucosidase aggregates from *Aspergillus fumigatus* KIBGE-IB33 for continuous production of glucose. *International Journal of Biological Macromolecules*, *135*, 1252–1260.

Poli, A., Kazak, H., Gürleyendağ, B., Tommonaro, G., Pieretti, G., Öner, E. T., and Nicolaus, B. (2009). High level synthesis of levan by a novel *Halomonas* species growing on defined media. *Carbohydrate Polymers*, *78*(4), 651–657.

Porras-Domínguez, J. R., Ávila-Fernández, Á., Miranda-Molina, A., Rodríguez-Alegría, M. E., and Munguía, A. L. (2015). *Bacillus subtilis* 168 levansucrase (SacB) activity affects average levan molecular weight. *Carbohydrate Polymers*, *132*, 338–344.

Porras-Domínguez, J. R., Ávila-Fernández, Á., Rodríguez-Alegría, M. E., Miranda-Molina, A., Escalante, A., González-Cervantes, R., Olvera, C., and López Munguía, A. (2014). Levan-type FOS production using a *Bacillus licheniformis* endolevanase. *Process Biochemistry*, *49*(5), 783–790.

Porras-domínguez, J. R., Rodríguez-alegría, M. E., and Ávila-fernández, Á. (2017). Levan-type fructooligosaccharides synthesis by a levansucrase-endolevanase fusion enzyme (LevB 1 SacB). *Carbohydrate Polymers*, *177*(July), 40–48.

Possiel, C., Ortiz-Soto, M. E., Ertl, J., Münch, A., Vogel, A., Schmiedel, R., and Seibel, J. (2019). Exploring the sequence variability of polymerization-involved residues in the production of levan- and inulin-type fructooligosaccharides with a levansucrase. *Scientific Reports*, *9*(1), 1–11.

Quigley, M. E., Hudson, G. J., and Englyst, H. N. (1999). *Determination of resistant short-chain carbohydrates (non-digestible oligosaccharides) using gas ± liquid chromatography*. *65*(20315), 381–390.

Rahman, M. A., Culsum, U., Kumar, A., Gao, H., and Hu, N. (2016). Immobilization of a novel cold active esterase onto Fe_3O_4~cellulose nano-composite enhances catalytic properties. *International Journal of Biological Macromolecules*, *87*, 488–497.

Rajan, A., and Emilia Abraham, T. (2008). Studies on crystallization and cross-linking of lipase for biocatalysis. *Bioprocess and Biosystems Engineering*, *31*(2), 87–94.

Rastall, G. R., and Gibson, R. A. (2006). Prebiotics: Development and Application. In G. R. G. and R. A. Rastall (Ed.), *International Journal of Dairy Technology* (Vol. 61, Issue 3). John Wiley & Sons, Ltd.

Razib, M. S. M., Rahman, R. N. Z. R. A., Shariff, F. M., and Ali, M. S. M. (2020). Biochemical and structural characterization of cross-linked enzyme aggregates (CLEAs) of organic solvent tolerant protease. *Catalysts*, *10*(1).

Rehman, S., Bhatti, H. N., Bilal, M., and Asgher, M. (2016). Cross-linked enzyme aggregates (CLEAs) of *Pencilluim notatum* lipase enzyme with improved activity, stability and reusability characteristics. *International Journal of Biological Macromolecules*, *91*, 1161–1169.

Reshmi, R., and Sugunan, S. (2013). Improved biochemical characteristics of crosslinked β-glucosidase on nanoporous silica foams. *Journal of Molecular Catalysis B: Enzymatic*, *85*, 111–118.

Ritter, D. W., Newton, J. M., and McShane, M. J. (2014). Modification of PEGylated enzyme with glutaraldehyde can enhance stability while avoiding intermolecular crosslinking. *RSC Advances*, *4*(53), 28036–28040.

Roberfroid, M. (2007). Inulin-type fructans - Functional Food Ingredients. In *The Journal of Nutrition* (pp. 2493s-2502s). ASN.

Roessl, U., Nahálka, J., and Nidetzky, B. (2010). Carrier-free immobilized enzymes for biocatalysis. *Biotechnology Letters*, *32*(3), 341–350.

Rojas, M. J., Amaral-fonseca, M., Zanin, G. M., Fernandez-lafuente, R., Lima, R. De, and Giordano, C. (2019). Preparation of Crosslinked Enzyme Aggregates of a Crosslinking Agent. *Catalysts*, *9*, 120.

Romano, N., Sciammaro, L., Mobili, P., Puppo, M. C., and Gomez-Zavaglia, A. (2019). Flour from mature *Prosopis nigra* pods as suitable substrate for the synthesis of prebiotic fructo-oligosaccharides and stabilization of dehydrated Lactobacillus delbrueckii subsp. bulgaricus. *Food Research International*, *121*(October), 561–567.

Rotticci, D., Norin, T., and Hult, K. (2000). Mass Transport Limitations Reduce the Effective Stereospecificity in Enzyme-Catalyzed Kinetic Resolution. *Organic Letters*, *2*(10), 1373–1376.

Roy, J. J., and Abraham, T. E. (2006). Preparation and characterization of cross-linked enzyme crystals of laccase. *Journal of Molecular Catalysis B: Enzymatic*, *38*(1), 31–36.

Saad, N., Delattre, C., Urdaci, M., Schmitter, J. M., and Bressollier, P. (2013). An overview of the last advances in probiotic and prebiotic field. *LWT - Food Science and Technology*, *50*(1), 1–16.

Sabater-Molina, M., Larque, E., Torrella, F., and Zamora, S. (2009). Dietary fructooligosaccharides. *Ovid MEDLINE(R)Journal of Physiology & Biochemistry*, *65*(3), 315–328.

Salisu, A. A., Musa, H., Abba, H., and Kogo, A. A. (2013). Preparation and characterization of dialdehyde starch and its' cross-linking with copper (II) ion. *Journal of Chemical and Pharmaceutical Research*, *5*(5), 153–158.

Samaniuk, J. R., Tim Scott, C., Root, T. W., and Klingenberg, D. J. (2011). The effect of high intensity mixing on the enzymatic hydrolysis of concentrated cellulose fiber suspensions. *Bioresource Technology*, *102*(6), 4489–4494.

Sambrook, J., and Russell, D. W. (2001). Molecular cloning: A laboratory manual. In *Cold Spring Harbor Laboratory Press, New York.* (Vol. 1).

Sánchez-Martínez, M. J., Soto-Jover, S., Antolinos, V., Martínez-Hernández, G. B., and López-Gómez, A. (2020). Manufacturing of Short-Chain Fructooligosaccharides: from Laboratory to Industrial Scale. *Food Engineering Reviews*, *12*(2), 149–172.

Sangeetha, K., and Emilia Abraham, T. (2008). Preparation and characterization of cross-linked enzyme aggregates (CLEA) of Subtilisin for controlled release applications. *International Journal of Biological Macromolecules*, *43*(3), 314–319.

Sangeetha, P. T., Ramesh, M. N., and Prapulla, S. G. (2005). Recent trends in the microbial production, analysis and application of Fructooligosaccharides. *Trends in Food Science and Technology*, *16*(10), 442–457.

Sangmanee, S., Nakapong, S., Pichyangkura, R., and Kuttiyawong, K. (2016). Levan-type fructooligosaccharide production using *Bacillus licheniformis* RN-01 levansucrase Y246S immobilized on chitosan beads. *Songklanakarin Journal of Science and Technology*, *38*(3), 295–303.

Sangwan, V., Tomar, S. K., Ali, B., Singh, R. R. B., and Singh, A. K. (2015). Galactooligosaccharides reduce infection caused by *Listeria monocytogenes* and modulate IgG and IgA levels in mice. *International Dairy Journal*, *41*, 58–63.

Sanjay, G., and Sugunan, S. (2005). Glucoamylase immobilized on montmorillonite: Synthesis, characterization and starch hydrolysis activity in a fixed bed reactor. *Catalysis Communications*, *6*(8), 525–530.

Sarangapani, P. S., Hudson, S. D., Jones, R. L., Douglas, J. F., and Pathak, J. A. (2015). Critical examination of the colloidal particle model of globular proteins. *Biophysical Journal*, *108*(3), 724–737.

Schoevaart, R., Wolbers, M. W., Golubovic, M., Ottens, M., Kieboom, A. P. G., Van Rantwijk, F., Van Der Wielen, L. A. M., and Sheldon, R. A. (2004). Preparation, optimization, and structures, of cross-linked enzyme aggregates (CLEAs). *Biotechnology and Bioengineering*, *87*(6), 754–762.

Shaarani, S. M., Jahim, J. M., Rahman, R. A., Idris, A., Murad, A. M. A., and Illias, R. M. (2016). Silanized maghemite for cross-linked enzyme aggregates of recombinant xylanase from Trichoderma reesei. *Journal of Molecular Catalysis B: Enzymatic*, *133*, 65–76.

Shah, S., Sharma, A., and Gupta, M. N. (2006). Preparation of cross-linked enzyme aggregates by using bovine serum albumin as a proteic feeder. *Analytical Biochemistry*, *351*(2), 207–213.

Shao, W.-X., Mo, X.-Y., and Li, L. (2008). Preparation and Property of Magnetic Cross-Linked Nuclease P1 Aggregations. *Hsi-An Chiao Tung Ta Hsueh*, *42*(8), 1035–1039.

Sheldon, R.A. (2007). Cross-linked enzyme aggregates (CLEA®s): stable and recyclable biocatalysts. *Biochemical Society Transactions*, *35*(6), 1583–1587.

Sheldon, Roger A. (2011a). Characteristic features and biotechnological applications of cross-linked enzyme aggregates (CLEAs). *Applied Microbiology and Biotechnology*, *92*(3), 467–477.

Sheldon, Roger A. (2011b). Cross-Linked Enzyme Aggregates as Industrial Biocatalysts Roger. *Organic Process Research and Development*, *15*, 213–223.

Sheldon, Roger A. (2019). CLEAs, combi-cleas and 'smart' magnetic cleas: Biocatalysis in a bio-based economy. *Catalysts*, *9*(3), 1–31.

Shen, Q., Yang, R., Hua, X., Ye, F., Wang, H., Zhao, W., and Wang, K. (2012). Enzymatic synthesis and identification of oligosaccharides obtained by transgalactosylation of lactose in the presence of fructose using β-galactosidase from Kluyveromyces lactis. *Food Chemistry*, *135*(3), 1547–1554.

Shi, J., Chi-Tang, H., and Fereidoon, S. (2010). *Functional Foods of the East*. CRC Press, Taylor & Francis Group.

Silbir, S., Dagbagli, S., Yegin, S., Baysal, T., and Goksungur, Y. (2014). Levan production by *Zymomonas mobilis* in batch and continuous fermentation systems. *Carbohydrate Polymers*, *99*, 454–461.

Silva, C., Martins, M., Jing, S., Fu, J., and Cavaco-Paulo, A. (2017). Practical insights on enzyme stabilization. *Critical Reviews in Biotechnology*, *0*(0), 1–16.

Silva, J. D. C., de França, P. R. L., Converti, A., and Porto, T. S. (2019). Pectin hydrolysis in cashew apple juice by *Aspergillus aculeatus* URM4953 polygalacturonase covalently-immobilized on calcium alginate beads: A kinetic and thermodynamic study. *International Journal of Biological Macromolecules*, *126*, 820–827.

Silva, M. F., Rigo, D., Mossi, V., Golunski, S., De Oliveira Kuhn, G., Di Luccio, M., Dallago, R., De Oliveira, D., Oliveira, J. V., and Treichel, H. (2013). Enzymatic synthesis of fructooligosaccharides by inulinases from *Aspergillus niger* and *Kluyveromyces marxianus* NRRL Y-7571 in aqueous-organic medium. *Food Chemistry*, *138*(1), 148–153.

Singh, R. K., Zhang, Y.-W., Nguyen, N.-P.-T., Jeya, M., and Lee, J.-K. (2011). Covalent immobilization of β-1,4-glucosidase from *Agaricus arvensis* onto functionalized silicon oxide nanoparticles. *Applied Microbiology and Biotechnology*, *89*(2), 337–344.

Soleimani, M., Khani, A., and Najafzadeh, K. (2012). α-Amylase immobilization on the silica nanoparticles for cleaning performance towards starch soils in laundry detergents. *Journal of Molecular Catalysis B: Enzymatic*, *74*(1–2), 1–5.

Srikanth, R., Reddy, C. H. S. S. S., Siddartha, G., Ramaiah, M. J., and Uppuluri, K. B. (2015). Review on production, characterization and applications of microbial levan. *Carbohydrate Polymers*, *120*, 102–114.

Stepankova, V., Bidmanova, S., Koudelakova, T., Prokop, Z., Chaloupkova, R., Damborsky, J., and V. Stepankova, Sarka Bidmanova, Tana Koudelakova, Zbynek Prokop, R. C. and J. D. (2013). Strategies for Stabilization of Enzymes in Organic Solvents. *ACS Catalysis*, *3*(12), 2823–2836.

Studier, F. W. (2005). Protein production by auto-induction in high density shaking cultures. *Protein Expression and Purification*, *41*(1), 207–234.

Subhedar, P. B., and Gogate, P. R. (2014). Enhancing the activity of cellulase enzyme using ultrasonic irradiations. *Journal of Molecular Catalysis B: Enzymatic*, *101*(December 2018), 108–114.

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.

Sun, B., Hou, Q., Liu, Z., and Ni, Y. (2015). Sodium periodate oxidation of cellulose nanocrystal and its application as a paper wet strength additive. *Cellulose*, *22*(2), 1135–1146.

Sunil, K. (1987). *A Crosslinked Preparation of E . coil*. *16*.

Talekar, S., Ghodake, V., Ghotage, T., Rathod, P., Deshmukh, P., Nadar, S., Mulla, M., and Ladole, M. (2012). Novel magnetic cross-linked enzyme aggregates (magnetic CLEAs) of alpha amylase. *Bioresource Technology*, *123*, 542–547.

Talekar, S., Joshi, A., Joshi, G., Kamat, P., Haripurkar, R., and Kambale, S. (2013). Parameters in preparation and characterization of cross linked enzyme aggregates (CLEAs). *RSC Advances*, *3*(31), 12485–12511.

Talekar, S., Nadar, S., Joshi, A., and Joshi, G. (2014). Pectin cross-linked enzyme aggregates (pectin-CLEAs) of glucoamylase. *RSC Advances*, *4*(103), 59444–59453.

Talekar, S., Shah, V., Patil, S., and Nimbalkar, M. (2012). Porous cross linked enzyme aggregates (p-CLEAs) of *Saccharomyces cerevisiae* invertase. *Catalysis Science and Technology*, *2*(8), 1575–1579.

Talekar, S., Waingade, S., Gaikwad, V., Patil, S., and Nagavekar, N. (2012). Preparation and characterization of cross linked enzyme aggregates (CLEAs) of Bacillus amyloliquefaciens alpha amylase. *Journal of Biochemical Technology*, *3*(4), 349–353.

Tanaka, K., Yamaguchi, F., and Kusui, S. (1983). Action of levan Fructotransferase of Arthrobacter ureafaciens on Levan and Phlein. *Agricultural and Biological Chemistry*, *53*(5), 1203–1211.

Tang, H., Lv, X., Li, Y., Li, Q., and Liu, X. (2020). Dialdehyde Oxidation of Cross - Linked Waxy Corn Starch : Optimization , Property and Characterization. *Arabian Journal for Science and Engineering*, *0123456789*, 1–10.

Tavares, A. P. M., Silva, C. G., Dražić, G., Silva, A. M. T., Loureiro, J. M., and Faria, J. L. (2015). Laccase immobilization over multi-walled carbon nanotubes: Kinetic, thermodynamic and stability studies. *Journal of Colloid and Interface Science*, *454*, 52–60.

Thakrar, F. J., and Singh, S. P. (2019). Catalytic, thermodynamic and structural properties of an immobilized and highly thermostable alkaline protease from a haloalkaliphilic actinobacteria, Nocardiopsis alba TATA-5. *Bioresource Technology*, *278*, 150–158.

Tischer, W., and Kasche, V. (1999). Immobilized enzymes: crystals or carriers? *Trends in Biotechnology*, *17*(8), 326–335.

Tomasik, P., and Tomasik, P. (2020). Probiotics, Non-dairy prebiotics and postbiotics in nutrition. *Applied Science*, *10*, 1470–1484.

Torres, D. P. M., Gonçalves, M. do P. F., Teixeira, J. A., and Rodrigues, L. R. (2010). Galacto-Oligosaccharides: Production, properties, applications, and significance as prebiotics. *Comprehensive Reviews in Food Science and Food Safety*, *9*(5), 438–454.

Trindade, L. V., Desagiacomo, C., Polizeli, M. de L. T. de M., Damasio, A. R. de L., Lima, A. M. F., Gomes, E., and Bonilla-Rodriguez, G. O. (2016). Biochemical Characterization, Thermal Stability, and Partial Sequence of a Novel Exo-Polygalacturonase from the *Thermophilic Fungus Rhizomucor pusillus* A13.36 Obtained by Submerged Cultivation. *BioMed Research International*, *2016*, 8653583.

Tummalapalli, M., and Gupta, B. (2015). A UV-Vis spectrophotometric method for the estimation of aldehyde groups in periodate-oxidized polysaccharides using 2,4-dinitrophenyl hydrazine. *Journal of Carbohydrate Chemistry*, *34*(6), 338–348.

Ureta, M. M., Romano, N., Kakisu, E., and Gómez-Zavaglia, A. (2019). Synthesis of fructo-oligosaccharides using grape must and sucrose as raw materials. *Food Research International*, *123*(April), 166–171.

Vaidya, B. K., Kumar, S. S., Golegaonkar, S. B., and Nene, S. N. (2012). Preparation of Cross-Linked Enzyme Aggregates of Trehalose Synthase via Co-aggregation with Polyethyleneimine. *Journal of Molecular Catalysis B: Enzymatic*, *74*, 184–191.

Vaidya, B. K., Kuwar, S. S., Golegaonkar, S. B., and Nene, S. N. (2012). Preparation of cross-linked enzyme aggregates of l-aminoacylase via co-aggregation with polyethyleneimine. *Journal of Molecular Catalysis B: Enzymatic*, *74*(3–4), 184–191.

Valdés, E. C., Soto, L. W., and Arcaya, G. A. (2011). Influence of the pH of glutaraldehyde and the use of dextran aldehyde on the preparation of cross-linked enzyme aggregates (CLEAs) of lipase from *Burkholderia cepacia*. *Electronic Journal of Biotechnology*, *14*(3), 1–7.

Varma, A. J., and Kulkarni, M. P. (2002). Oxidation of cellulose under controlled conditions. *Polymer Degradation and Stability*, *77*(1), 25–27.

Veenashri, B. R., and Muralikrishna, G. (2011). In vitro anti-oxidant activity of xylo-oligosaccharides derived from cereal and millet brans - A comparative study. *Food Chemistry*, *126*(3), 1475–1481.

Velasco-Lozano, S., López-Gallego, F., Mateos-Díaz, J. C., and Favela-Torres, E. (2016). Cross-linked enzyme aggregates (CLEA) in enzyme improvement – a review. *Biocatalysis*, *1*(1), 166–177.

Velasco-Lozano, S., López-Gallego, F., Vázquez-Duhalt, R., Mateos-Díaz, J. C., Guisán, J. M., and Favela-Torres, E. (2014). Carrier-free immobilization of lipase from candida rugosa with polyethyleneimines by carboxyl-activated cross-linking. *Biomacromolecules*, *15*(5), 1896–1903.

Venema, K. (2012). Intestinal fermentation of lactose and prebiotic lactose derivatives, including human milk oligosaccharides. *International Dairy Journal*, *22*(2), 123–140.

Verma, R., Kumar, A., and Kumar, S. (2019). Synthesis and characterization of cross-linked enzyme aggregates (CLEAs) of thermostable xylanase from *Geobacillus thermodenitrificans* X1. *Process Biochemistry*, *80*, 72–79.

Vinoth Kumar, V., Prem Kumar, M. P., Thiruvenkadaravi, K. V., Baskaralingam, P., Senthil Kumar, P., and Sivanesan, S. (2012). Preparation and characterization of porous cross linked laccase aggregates for the decolorization of triphenyl methane and reactive dyes. *Bioresource Technology*, *119*, 28–34.

Vršanská, M., Voběrková, S., Jiménez Jiménez, A. M., Strmiska, V., and Adam, V. (2018a). Preparation and optimisation of cross-linked enzyme aggregates using native isolate white rot fungi *Trametes versicolor* and *Fomes fomentarius* for the decolourisation of synthetic dyes. *International Journal of Environmental Research and Public Health*, *15*(1), 1–15.

Vršanská, M., Voběrková, S., Jiménez Jiménez, A. M., Strmiska, V., and Adam, V. (2018b). Preparation and optimisation of cross-linked enzyme aggregates using native isolate white rot fungi *Trametes versicolor* and *Fomes fomentarius* for the decolourisation of synthetic dyes. *International Journal of Environmental Research and Public Health*, *15*(1), 1–15.

Wang, K., Gao, Y., Wang, Z., and Meng, G. (2014). Cross-Linked Enzyme Aggregates of b-Galactosidase from Different Source by Dialdehyde Starch as Cross-Linker. *Proceedings of the 2012 International Conference on Applied Biotechnology*, 1733–1739.

Wang, Mengfan, Jia, C., Qi, W., Yu, Q., Peng, X., Su, R., and He, Z. (2010). Porous-CLEAs of papain: Application to enzymatic hydrolysis of macromolecules. *Bioresource Technology*, *102*(3), 3541–3545.

Wang, Mengfan, Qi, W., Jia, C., Ren, Y., Su, R., and He, Z. (2011). Enhancement of activity of cross-linked enzyme aggregates by a sugar-assisted precipitation strategy: Technical development and molecular mechanism. *Journal of Biotechnology*, *156*(1), 30–38.

Wang, Mingming, Wang, H., Feng, Y., Xu, Q., Admassu, H., Yang, R., and Hua, X. (2018a). Preparation and Characterization of Sugar-Assisted Cross-Linked Enzyme Aggregates (CLEAs) of Recombinant Cellobiose 2-epimerase from *Caldicellulosiruptor saccharolyticus* (CsCE). *Journal of Agricultural and Food Chemistry*, *66*(29), 7712–7721.

Wilson, L., Fernández-Lorente, G., Fernández-Lafuente, R., Illanes, A., Guisán, J. M., and Palomo, J. M. (2006). CLEAs of lipases and poly-ionic polymers: A simple way of preparing stable biocatalysts with improved properties. *Enzyme and Microbial Technology*, *39*(4), 750–755.

Wilson, L., Illanes, A., Abián, O., Pessela, B. C. C., Fernández-Lafuente, R., and Guisán, J. M. (2004). Co-aggregation of penicillin G acylase and polyionic polymers: An easy methodology to prepare enzyme biocatalysts stable in organic media. *Biomacromolecules*, *5*(3), 852–857.

Wingfield, P. (1998). Protein Precipitation Using Ammonium Sulfate. *Current Protocols in Protein Science*, A.3F.1-A.3F.8.

Wojeicchowski, J. P., de Siqueira, G. L. de A., Lacerda, L. G., Schnitzler, E., and Demiate, I. M. (2018). Physicochemical, structural and thermal properties of oxidized, acetylated and dual-modified common bean (Phaseolus vulgaris L.) starch. *Food Science and Technology*, *38*(2), 318–327.

Wongsagon, R., Shobsngob, S., and Varavinit, S. (2005). Preparation and physicochemical properties of dialdehyde tapioca starch. *Starch/Staerke*, *57*(3–4), 166–172.

Wu, J., Wang, J.-L., Li, M.-H., Lin, J.-P., and Wei, D.-Z. (2010). Optimization of immobilization for selective oxidation of benzyl alcohol by *Gluconobacter oxydans* using response surface methodology. *Bioresource Technology*, *101*(23), 8936–8941.

Xie, X., Li, B., Wu, Z., Dong, S., and Li, L. (2012). Preparation of cross-linked cellulase aggregates onto magnetic chitosan microspheres. *Advanced Materials Research*, *550–553*(August), 1566–1571.

Xu, D. Y., Yang, Y., and Yang, Z. (2011). Activity and stability of cross-linked tyrosinase aggregates in aqueous and nonaqueous media. *Journal of Biotechnology*, *152*(1–2), 30–36.

Xu, D. Y., and Yang, Z. (2013). Cross-linked tyrosinase aggregates for elimination of phenolic compounds from wastewater. *Chemosphere*, *92*(4), 391–398.

Yamaguchi, H., Miyazaki, M., Asanomi, Y., and Maeda, H. (2011). Poly-lysine supported cross-linked enzyme aggregates with efficient enzymatic activity and high operational stability. *Catalysis Science & Technology*, *1*(7), 1256.

Yang, D., Fan, J., Cao, F., Deng, Z., Pojman, J. A., and Ji, L. (2019). Immobilization adjusted clock reaction in the urea-urease-H+ reaction system. *RSC Advances*, *9*, 3514–3519.

Yang, Xinyi, Chen, Y., Yao, S., Qian, J., Guo, H., and Cai, X. (2019). Preparation of immobilized lipase on magnetic nanoparticles dialdehyde starch. *Carbohydrate Polymers*, *218*, 324–332.

Yang, Xu'e, Zheng, P., Ni, Y., and Sun, Z. (2012). Highly efficient biosynthesis of sucrose-6-acetate with cross-linked aggregates of Lipozyme TL 100 L. *Journal of Biotechnology*, *161*(1), 27–33.

Yanjun, J., Qi, W., Wenqin, W., Liya, Z and Jing, G. (2012). Preparation of Immobilized Lipase through Combination of Cross-Linked Enzyme Aggregates and Biomimetic Silicification. *Chinese Journal of Catalysis*, *33*(5), 857–862.

Yi, X., Zhang, S., and Ju, B. (2014). Preparation of water-soluble oxidized starch with high carbonyl content by sodium hypochlorite. *Starch/Staerke*, *66*, 115–123.

Yu, H. W., Chen, H., Wang, X., Yang, Y. Y., and Ching, C. B. (2006). Cross-linked enzyme aggregates (CLEAs) with controlled particles: Application to *Candida rugosa* lipase. *Journal of Molecular Catalysis B: Enzymatic*, *43*(1–4), 124–127.

Zerva, A., Antonopoulou, I., Enman, J., Iancu, L., Rova, U., and Christakopoulos, P. (2018). Cross-linked enzyme aggregates of feruloyl esterase preparations from *Thermothelomyces thermophila* and *Talaromyces wortmannii*. *Catalysts*, *8*(5), 1–2.

Zerva, A., Pentari, C., and Topakas, E. (2020). Crosslinked enzyme aggregates (CLEAs) of laccases from *Pleurotus citrinopileatus* induced in olive oil mill wastewater (OOMW). *Molecules*, *25*(9), 7–9.

Zhang, J., Li, M., and Zhang, Y. (2018). Enhancing the thermostability of recombinant cyclodextrin glucanotransferase via optimized stabilizer. *Process Biochemistry*, *67*(January), 64–70.

Zhang, Q., Zha, X., Zhou, N., and Tian, Y. (2016). Preparation of crosslinked enzyme aggregates (CLEAs) of acid urease with urethanase activity and their application. *Journal of Basic Microbiology*, *56*(4), 422–431.

Zhang, R., Yip, V. L. Y., and Withers, S. G. (2010). Mechanisms of enzymatic glycosyl transfer. *Comprehensive Natural Products II: Chemistry and Biology*, *8*, 385–422.

Zhang, W. W., Yang, X. L., Jia, J. Q., Wang, N., Hu, C. L., and Yu, X. Q. (2015). Surfactant-activated magnetic cross-linked enzyme aggregates (magnetic CLEAs) of *Thermomyces lanuginosus* lipase for biodiesel production. *Journal of Molecular Catalysis B: Enzymatic*, *115*, 83–89.

Zhang, W., Xu, W., Ni, D., Dai, Q., Guang, C., Zhang, T., and Mu, W. (2019). An overview of levan-degrading enzyme from microbes. In *Applied Microbiology and Biotechnology* (Vol. 103, Issue 19, pp. 7891–7902). Applied Microbiology and Biotechnology.

Zhao, H., Olubajo, O., Song, Z., Sims, A. L., Person, T. E., Lawal, R. A., and Holley, L. A. (2006). Effect of kosmotropicity of ionic liquids on the enzyme stability in aqueous solutions. *Bioorganic Chemistry*, *34*(1), 15–25.

Zhen, Q., Wang, M., Qi, W., Su, R., and He, Z. (2013a). Preparation of beta-mannanase CLEAs using macromolecular cross-linkers. *Catalysis Science & Technology*, *3*, 1937–1941.

Zhen, Q., Wang, M., Qi, W., Su, R., and He, Z. (2013b). Preparation of β-mannanase CLEAs using macromolecular cross-linkers. *Catalysis Science & Technology*, *3*(8), 1937.

Zheng, G. W., Yu, H. L., Li, C. X., Pan, J., and Xu, J. H. (2011). Immobilization of *Bacillus subtilis* esterase by simple cross-linking for enzymatic resolution of dl-menthyl acetate. *Journal of Molecular Catalysis B: Enzymatic*, *70*(3–4), 138–143.

Zhong, D., Pal, S. K., and Zewail, A. H. (2011). Biological water: A critique. *Chemical Physics Letters*, *503*(1–3), 1–11.

Zhou, L., Mou, H., Gao, J., Ma, L., He, Y., and Jiang, Y. (2017). Preparation of cross-linked enzyme aggregates of nitrile hydratase ES-NHT-118 from E. coli by macromolecular cross-linking agent. *Chinese Journal of Chemical Engineering*, *25*(4), 487–492.

Ziegler-Borowska, M., Wegrzynowska-Drzymalska, K., Chelminiak-Dudkiewicz, D., Kowalonek, J., and Kaczmarek, H. (2018). Photochemical reactions in dialdehyde starch. *Molecules*, *23*(12).

Zuo, Y., Liu, W., Xiao, J., Zhao, X., Zhu, Y., and Wu, Y. (2017). Preparation and characterization of dialdehyde starch by one-step acid hydrolysis and oxidation. *International Journal of Biological Macromolecules*, *103*, 1257–1264.

LIST OF PUBLICATION

Journal with Impact Factor

1) **Abd Rahman, N. H**., Jaafar, N. R., Abdul Murad, A. M., Abu Bakar, F. D., Shamsul Annuar, N. A., and Illias, R. M. (2020). Novel cross-linked enzyme aggregates of levanase from *Bacillus lehensis* G1 for short-chain fructooligosaccharide synthesis : Developmental , physicochemical , kinetic and thermodynamic properties. *International Journal of Biological Macromolecules*, *159*, 577–589. https://doi.org/10.1016/j.ijbiomac.2020.04.262. **(Q1, IF: 5.162).**