Modeling of Enzyme Catalyzed Racemic Reactions and Modification of Enantioselectivity

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Hierdurch erkläre ich, dass ich meine Dissertation selbständig verfasst und die benutzten Hilfsmittel und Quellen sowie gegebenenfalls die zu Hilfeleistungen herangezogenen Institutionen vollständig angegeben habe. Die Dissertation wurde nicht schon als Masterarbeit, Diplomarbeit oder andere Prüfungsarbeit verwendet.

No man is an island,
Entire of itself.
Each is a piece of the continent,
A part of the main.
If a clod be washed away by the sea,
Europe is the less.
As well as if a promontory were.
As well as if a manner of thine own
Or of thine friend's were.
Each man's death diminishes me,
For I am involved in mankind.
Therefore, send not to know
For whom the bell tolls,
It tolls for thee.

John Donne (1572 – 1631)

Dedicated To:

My Mothers; *İnci, Meral, Çiçek, Seyran,*

To My Fathers; *Thomas, Lemi, Haydar, Arif,*

Abstract:

Die Biotechnologie hat sich innerhalb der letzten zwei Jahrzehnte zu einem wichtigen Gebiet der Wissenschaft entwickelt, wobei enzymkatalysierten racemischen Reaktionen eine bedeutende Rolle bei der industriellen Produktion von Verbindungen zukommt. „Chiral switching“ wurde daher ein wesentliches Konzept, um enantiomerenreine Verbindungen zu erhalten. Die Enzymkinetik hat einen wesentlichen Anteil am Verständnis der Reaktionswege, wie auch an der Bestimmung der Reaktionsgeschwindigkeit. Dies ist eine Voraussetzung, um einen neuen Bioreaktor zu entwickeln und/oder die Reaktionsbedingungen zu optimieren.

In der vorliegenden Arbeit wurde zunächst die Enantioselektivität analysiert, wobei das grundlegende Ziel darin besteht, die Enantioselektivität racemischer Reaktionen zu erhöhen. Anhand des Reaktionsmechanismus in der Bulkphase des Reaktionsmediums wurde die Enantioselektivität für eine biomolekulare Reaktion neu bestimmt.

Weiterhin wurde ein grundlegendes mechanistisches Modell aufgestellt und unter der Annahme eines pseudostationären Zustands gelöst. Es wurde ein allgemeines Geschwindigkeitsgesetz für racemische Reaktionen mit der Software Maple aufgestellt. Um das Modell zu prüfen, wurde eine spezifische Reaktion zwischen Isopropyliedeneglycerol und Vinylacetat als Acyldonor als Modellreaktion herangezogen. Es hat sich dabei herausgestellt, daß das Modell problemlos auf diese Modellreaktion angewendet werden konnte. Die Geschwindigkeitskonstanten wurden mit einer zufallsbasierten nichtlinearen Regression (Matlab) simuliert.

Dieses Modell wurde ebenso auf einzelne Enantiomere angewendet. Dabei hat sich herausgestellt, daß die Reaktionsgeschwindigkeit für diesen Fall einem Geschwindigkeitsgesetz nach Michaelis-Menten folgt. Die Michaelis-Menten-Konstanten beider Enantiomere $V_{max,DS}$(0.677 mol/L.h), $K_{m,DS}$ (0.285 mol/L) und $V_{max,LS}$(0.66 mol/L.h) und $K_{m,LS}$ (0.98 mol/L) wurden separat bestimmt.

Ein neuer, allgemeingültiger Ansatz für die Enantioselektivität wurde basierend auf dem mechanistischen Modell für alle Modellsysteme vorgeschlagen. Dieser Ansatz wurde für den irreversiblen Fall verifiziert. Es hat sich dabei herausgestellt, daß die Ergebnisse mit den Literaturdaten zur Enantioselektivität für irreversible Reaktionen übereinstimmen.

Das Modell, welches in dieser Arbeit aufgestellt wurde, kann für jeden Typ racemischer enzymatischer Reaktionen angewendet werden. Wenn der Mechanismus einer Reaktion aufgrund der molekularen Wechselwirkungen bekannt ist, ist es möglich, die Art der Reaktionen zwischen den Intermediaten zu bestimmen. Für irreversible Teilreaktionen können dementsprechend die Geschwindigkeitskonstanten der Rückreaktionen gleich Null gesetzt werden. Das Modell wird hierdurch auf ein Reaktionssystem zugeschnitten und die Koeffizienten können mit Programmen wie Matlab oder Maple berechnet werden. Das auf der Basis dieses Modells berechnete Enantiomerenverhältnis kann angewendet werden, um die Enantioselektivität von Enzymen zu bestimmen.

Abstract :

Along the last two decades, the biotechnology has become a challenging field of science, in which the enzyme catalyzed racemic reactions play an important role in the production of enantiomerically pure industrial compounds. Thus, “chiral switching” became an important concept. The enzyme kinetics significantly takes good part in understanding the behavior of the reaction, as well as determining the rate of reaction, by which it is later possible to design a new bioreactor, and/or to optimize the reaction conditions.

In this work, first the enantioselective ratio (E-value) has been analyzed, while the general aim is to increase the enantioselectivity of racemic reactions. The E value for the two component (bi-bi) reactions was newly suggested according to the overall mechanism in the bulk of the reaction medium.

In addition, a general mechanistic model has been proposed and solved by making a pseudo-steady state assumption. A general rate expression for any racemic reaction has been derived with a Maple software program. A specific reaction between isopropyliedeneglycerol and vinylacetate, as acyl donor, was considered as a case study to test the proposed model. It is obtained that the general model can easily be applied to this specific case, and a rate expression was derived for this specific reaction. The rate constants were simulated with random non-linear regression tool of Matlab software package.

This model has also been applied to single enantiomer, and it is found that the rate of reaction becomes a simple Michaelis- Menten type, and the Michaelis- Menten constants for both enantiomeres $V_{max,DS}$(0.677 mol/L.h), $K_{m,DS}$ (0.285 mol/L) $V_{max,LS}$(0.66 mol/L.h), $K_{m,LS}$ (0.98 mol/L) were separately evaluated.

A new general E- value based on mechanistic model has been suggested for all cases, after derivation of the general rate expression. This new equation was verified for the irreversible case and it is found that it simply turns to be the E-value suggested in the literature for the irreversible conditions.

As a result, the general model proposed in this study can be used for any type of racemic enzymatic reactions. If the mechanism of a reaction can be defined depending on its molecular interactions, then it is possible to determine the type of reactions between intermediates. Accordingly, the reversible rate constants are equated to zero for the irreversible reactions, and the model becomes case specific one, whose coefficients can be calculated by computer programs like Matlab and Maple. The enantiomeric ratio based on mechanistic model can be used in studies to calculate the enantioselectivity of the enyzmes.

Keywords: Racemic reaction, transesterification, enantioselectivity, lipase, kinetic modeling, organic solvent

Die Schlüsselwörter : racemischer Reaktion, Transesterifikation, Enantioselektivität, lipase, kinetische Modellierung, Organische Lösung

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This work is not an end but a new start in my life. Along this work, I have not only completed my PhD, I could drastically declare that I have also studied in school of life. Beyond them all the show has to go on and now this is one of the ends that should have been arrived. Here I am going to gracefully mention and convey my heartily felt feeling and thanks to the secret spirits who were always by me.

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Abbreviations & Nomenculature

B	the number of bootstrap trials
BCL	*Burkholderia cepacia* lipase
CAS	Computer algebra systems
HPR	High Pressure Reactor
IPG	Isopropyledene glycerol
IUBMB	International Union of Biochemistry and Molecular Biology
ODE	Ordinary Differential Equation
OECD	Organization for Economic Co-operation and Development
$scCO_2$	Supercritical Carbon Dioxide
S	Selectivity
SS	the sum of squares
SS_{reg}	the sum of the square of the distances of the points from the fitted line
SS_{tot}	the sum of the square of the distances of the points from the mean values
R^2	the goodness of a fit can be quantified by the
TCI	Technical Chemistry Institute of Hannover University
U	Turnover ratio

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I – INTRODUCTION

“Life itself is the most wonderful fairy tale”

Hans Christian Andersen

1.1. The Brief History of Biotechnology

Biotechnology is an interdisciplinary field of science. Although it is exponentially grown up in the couple of last decades, it is the oldest science/technology, which developed silently via daily life of primitive tribes almost since 10,000 B.C.

The first primitive ancestors of human beings hunted the animals and collected the plants to survive, since they did not invent the appropriate tools and appliances for breeding animals and harvesting crops. As they learned how to tackle with domesticating the animals and to plough for the plants, they went through the crucial transition from nomadic hunter, moving from one hunting area to another, to settled farmer in order to preserve their cultivated crops, which became vital for survival. These primitive ancient farmers found out a way to increase the yield and to improve the taste of crops by selecting seeds from particularly desirable plants, without any knowledge of the natural principles [1].

As primitive wo/man founded their villages, they learnt to live together and also together with their animals and crops. The animals were the carrier of microorganisms, which ferried to human beings introducing them into dieses. They were, by the way, infected by the microbes and viruses as well as they utilized some organisms for their pre-historical biotechnological processes [2].

As a result of residence of wo/men, biotechnology has consequently flourished since prehistoric times. Its history begins when primitive wo/man became to gather and process herbs for medicine; make bread, later improving them for a soft and spongy one rather than a firm one, make beer by fermenting solutions of malt and hops; create many fermented food products using fruit juices for wine, converting milk into yogurt, cheese, and various soy products; create septic systems to deal with their digestive and excretory waste products, and to create vaccines to immunize themselves against diseases. The first farmers realized that different physical traits could be either magnified or lost by hybridizing appropriate pairs of animals, and less or greater genetic variety of plants [2,3,4].

Some important milestones of biotechnology are briefly given at Table 1.1, whereby as clearly seen, it thoroughly ascends after 1980s and becomes a challenging science/technology of 21st century. However behind this drastic growing tendency exits the roots of modern biotechnology dating back a hundred year, to the works of Louis Pasteur, Robert Koch, who based the current science of microbiology, and Gregor Mendel, who first studied the genetic inheritance.

In 1919 a Hungarian engineer Károly Ereky coined the term "biotechnology "for the first time. At that time, the term meant all the lines of work by which products are produced from raw materials with the aid of living organisms. Ereky envisioned a biochemical age similar to the stone and iron ages. The leaders of the "old biotechnology" were engineers and technicians who worked mainly in the specialized industrial plants associated with large scale production of breweries, wineries, tanneries, leather processing, canneries, sugar factories, and otherwise in the production of starch, yeast, alcohol, meat, milk and vegetable oil, *etc.*. From the beginning of the 20th century, in some centers of Europe and in the USA, specialized agricultural engineers started to organize the improvement of agricultural techniques. The mechanization of soil tillage, processes of cultivation, harvesting, transport and preservation, at the same time, use of chemical fertilizers, animal and plant breeding and many other new revolutionary technologies helped to replace the traditional agricultural production systems, like livestock raising, cultivation of cereals, horticulture, *etc.* [5-8].

The modern time of biotechnology started around 1953 as American biochemist James Watson and British biophysicist Francis Crick presented their double-helix model of DNA. In 1973 Stanley Cohen and Herbert Boyer removed a specific gene from one bacterium and put it in other using restriction enzymes, which marked the beginning of recombinant DNA technology or genetic engineering. In 1977 genes were transferred from one organism to bacteria [5,6].

After 1980's, the states, or organizations has cobbled together a cluster of meaningful words and phrases to identify biotechnology. They grasped a frame of reference on which to build decision-making by regarding resource allocation, and the comparative analyses of the academic sector aspects; such as research, training and education and the funding for research. By this accumulation of knowledge, the governments or related associations defined biotechnology according to the application in their own countries.

Table 1.1. Evolution of Biotechnology timeline

8000BC	Collecting of seeds for replanting. Evidence that Babylonians, Egyptians and Romans used selective breeding practices to improve livestock.
6000BC	Brewing beer, fermenting wine, baking bread with help of yeast.
4000BC	Chinese made yoghurt and cheese with lactic-acid-producing bacteria.
1500	Plant collecting around the world.
1797	Edward Jenner used living microorganisms to protect people from disease.
1800	Nikolai I. Vavilov created comprehensive research on breeding animals.
1856	Gregor Mendel started to work for the recombinant plant genetics.
1859	Charles Darwin hypothesized that animal and plant population adapt over time to best fit the environment.
1864	Louis Pasteur, proved existence of microorganisms, showed that all living things are produced by other living things
1865	Gregor Mendel investigated how traits pass from generation to generation called them actors.
1869	Johann Meischer, isolated DNA from the nuclei of white blood cells
1893	Koch, Pasteur, fermentation process patented
1910	Thomas H. Morgen, proved that genes are carried on chromosomes
1919	Karl Ereky, a Hungarian engineer, first used the word biotechnology
1941	George Beadle, proposed " one gene, one enzyme " hypothesis
1953	James Watson, Francis Crick, determined the double helix structure of DNA
1957	Francis Crick, explained how DNA functions to make proteins
1966	Marshall Nierenberg determined the sequence of three nucleotide bases (a codon) for each of 20 amino acids
1972	Paul Berg, cut sections of viral DNA and bacterial DNA with same restriction enzmye
1973	Herbert Boyer, beginning of genetic engineering
1973	Stanley Cohen produced first recombinant DNA organism
1975	Method for producing monoclonal antibody developed by Kohler and Milstein
1978	Genentech, Inc. used genetic engineering techniques to produce human insulin in *E.coli*.
1980	Modern biotechnology was characterized by recombinant DNA technology. The prokaryote model, *E. coli* is used to produce insulin and other medicine, in human form.
1981	The first gene-synthesizing machine, the first genetically engineered plant, mice are successfully cloned.
1983	The first genetic transformation of plant cells by TI plasmids is performed The first artificial chromosome is synthesized. The first genetic markers for specific inherited dieases are found.
1989	Microorganisms are used to clean up the Exxon Valdez oil spill. The gene responsible for cystic fibrosis is discovered.
1992	FDA approves of the first GM food from Calgene: "flavor saver" tomato
1994	The first breast cancer gene is discovered.
1997	Scottish scientists report cloning a sheep, using DNA from adult sheep cells.
1998	Human skin is produced in vitro. Embryonic stem cells are used to regenerate tissue and create disorders mimicking diseases. The first complete animal genome for the elegans worm is sequenced. A rough draft of the human genome map is produced, showing the locations of more than 30,000 genes
1999	The complete genetic code of the human chromosome is first deciphered.
2000	A rough draft of the human genome is completed by Celera Genomics and the Human Genome Project. Pigs are the next animal cloned by researchers, hopefully to help produce organs for human transplant.
2001	The sequence of the human genome is published in Science and Nature, making it possible for researchers all over the world to begin developing treatments.
2002	Scientists complete the draft sequence of the most important pathogen of rice, a fungus that destroys enough rice to feed 60 million people annually.
2003	Dolly, the cloned sheep that made headlines in 1997, is euthanized after developing progressive lung disease. Dolly was the first successful clone of a mammal.

(*by courtesy* of *North Caroline Biotechnology Center, Biotechnology Industry Organization (BIO) , The National Health Museum, Washington and Biotechnology Institute in Arlington* ; *ref: [5]*)

Although some countries, like USA, Israel, the Nederlands, France, Ireland, New Zealand have stated their own definition. Many countries, like Germany, accepted the common definition of OECD (Organization for Economic Co-operation and Development) [3,9,10]. The provisional single definition of biotechnology of OECD states that,

> *"The application of Science & Technology to living organisms as well as parts, products and models thereof, to alter living or non-living materials for the production of knowledge, goods and services".*

As well as the basic understanding in microbiology, biochemistry and latterly molecular biology drastically exploited in the last couple of decades, the simultaneous application of traditional chemical engineering unit operations in this biological setting is central to making biotechnology processes a reality [10]. Industrial biotechnology applications have led to cleaner processes that produce less waste and use less energy and water in such industrial sectors as chemicals, pulp and paper, textiles, food, energy, metals and minerals. The tremendous production capacity increased year by year, *e.g.* the economical capacity reached $30 billion a year only in some 160 kinds of drugs and vaccines production [5].

In these industrial units, where a reaction takes place, a reactor is certainly the heart of the plant to produce the new product. Thus, in the application of chemical engineering in biology, the chemical reaction engineering and catalysis have been redefined as biotransformation in biotechnology. One definition of biotransformation is stated as; "the carrying out of a chemical reaction by biological systems." This biotransformation is usually carried out by using enzymes, either in a purified or semi-purified state, or whole cells (plant, animal or microbial) that contain the relevant enzyme. The use of enzyme biotransformation rather than traditional chemical conversions allows the use of mild temperatures and conditions, and significantly allows specific reactions to take place, such that a product may consists of single enantiomers [10].

As a result, the kinetic study of a reaction has become important still keeping its some complex biologically secret information in itself for the calculation, design and construction of industrially important reactors.

1.1.1. Application of Biotechnology

Based on the background knowledge of the structure and function of living systems, biotechnology has opened up a new vista for rapid agricultural, industrial and socioeconomic progress. It is highly science based, knowledge intensive, and an interdisciplinary field in which spectacular advances are taking place all over the world. The next step, after the development of the knowledge in biotechnology, was the process development in the industrially large quantity productions of the products. Biotechnological processes still improve and can now compete with other technologies widely used in the chemicals industry; pulp and paper production, textile and leather, food processing, metals and minerals, and energy. The process development also includes the development of media, buffers, reagents, solutions, and assays, biocatalysts and the choice of tools, such as bioreactors and chromatographic equipments for the upstream and/or downstream processing [3,8].

Table1.2: Application fields of Biotechnology [10,11].

Application	Processes
DNA-based	Gene probes, DNA markers, bioinformatics, genomics, pharmacogenetics DNA sequencing/synthesis/amplification, genetic engineering., Micro-array
Biochemistry/Immunology	Vaccines/immune stimulants, drug design & delivery, diagnostic tests, antibiotics, synthesis/sequencing of proteins and peptides, cell receptors/signaling, structural biology, combinatorial chemistry,3-D molecular modeling, biomaterials, microbiology, virology, microbial ecology
Bioprocessing-based	Culturing/manipulation of cells: tissues, embryos, Extractions:, purifications, separations , Fermentation: bio-processing, bio-transformation
Environmental	Bioleaching, bio-pulping, bio-bleaching, bio-desulfurization, bioremediation, bio-filtration, geo-microbiology, bio-weapons, bio-energy; bio-fuels, bio-hydrogen, biomass

With this wide range of its application fields, biotechnology has become a source of economic development and a social providing access to technology on credit and peer markets to especially rural poor entrepreneurs in many developing countries [12].

1.1.2. Sub-fields of Biotechnology

Processes involved in biotechnology are not separately classified as a single industry for purposes of surveys of research, manufacturing, or service firms, since the application and development of modern biotechnologies is relatively recent, and incorporation into statistical accounts requires a specific process and takes some time. More important, as biotechnology is applied to a widening array of industrial applications, developing a single classification category for firms engaged in biotechnology-related activity is proving to be complex and difficult. Biotechnology applications defy attempts to categorize their boundaries that would restrict them to a particular industry [11].

Table 1.3.: Sub-fields of Biotechnology [8, 13 – 15].

Sub-fields of Biotechnology	Application
Red biotechnology	health, medical processes, diagnostic products and services intended for diagnosis and therapy of diseases
Grey or white biotechnology	industrial processes, white biotechnology tends to consume less resources that traditional processes when used to produce industrial goods. Gene-based bio-industries
Blue biotechnology	Aquaculture, coastal and marine biotechnology
Green biotechnology	Agricultural, environmental biotechnology; bio-fuels, bio-fertilizers, bioremediation, geo-microbiology, bio-pesticide, genetically modified animals and plants.

As a result of intersection of many interdisciplinary sciences, the biotechnology has many number of jargon terms in its classification. One of these classifications depends on the sub-fields of application defined by terms of colors.

1.1.3. Systems Biology; a way to enzyme kinetics

Systems biology transmits the information between interdisciplinary sciences using the principles of chemical engineering with the accumulated knowledge of the biotechnology, and combines the mathematical modeling with biological experiments to elucidate a whole picture of complex biological systems in living organisms[16,17]. Thus, systems biology is characterized by synergistic integration of theory, computational modeling, and experiment[18,19].

The mathematical modelling by a set of ordinary differential equations (ODEs) describes the variations in the series of biochemical reactions taking place within the system. As a result, enzyme kinetics is directly relevant to the ODEs, which is built with appropriately parametrized enzyme kinetic rate laws for each of the reactions involved. The model gives information about the derivation of quantitative structure-function relationships for the enzyme in modern enzymology. Structure-based systems biology provides insight into complex enzymatic reactions at a molecular level[20].

In this work, a model for the enantimeric enzyme catalyzed reaction has been suggested and applied for the transesterificaton of isopropyledenglycerol (IPG), as a case study.

1.2. Aim of the Thesis

The systems biology has become a new challenging field to analyze the biologically complex systems in biotechnology. A main good part of this approach is to model the reactions in order to understand the kinetic mechanism and rate expressions. Determination of the reaction rate is significant in design of bioreactors in industry.

Recently, the enantiomeric enzymatic reactions are important in industrial applications, especially in pharmaceutical industry. The separation of one racemate from other is mostly desired. Although various physical methods have been developed to achieve this separation, the study of reaction kinetics is also very important for controlling the production of desired component.

Hence, it is aimed in this work to analyze the concept of enantioselectivity (E), and to model the kinetics of two component reactions, like transesterification reactions in which an acyl donor is used as second component.

The aim of this work is to postulate a general kinetic model for enzyme catalyzed racemic reaction and to calculate the rate expression. Thus a new model was suggested, and a specific *B. Cepacia* lipase catalyzed reaction has been considered as a case study to verify the model. The reaction for case study is the transesterification reaction between isopropyledene–s-glycerol (IPG), which is an important starting synthon for many pharmaceutical components, and vinyl acetate as acyl donor. These reactions were carried out in conventional organic solvent *n*-hexane and in supercritical CO2, as a new solvent suggested by medium engineering approach.

Consequently, the result of this work might be further used to define the mechanism of any enzyme catalyzed racemic two component reaction, and to calculate its rate expression, by which a bioreactor for the production of industrially important products can be designed.

Chapter II. Biocatalysis; Lipase and Medium Engineering

"in the field of observation,
chance only favors the prepared mind"
Louis Pasteur
1854

2.1. Biocatalysis

2.1.1. Enzymes

From the first demonstration of fermentation by Lavoisier in the early 17th century[21], till Kuhne first coined the word " *enzyme* " meaning " *in yeast* " in Greek[22], the enzymes were called "*ferment*" as mentioned in the book writen by Mrs. Fullhame in 1794 [23]. The timeline of enzymology is given on Table 2.1 in detail.

The enzymes are the biochemical catalysts evolved in nature to achieve the speed and coordination of a multitude of chemical reaction necessary to develop and maintain life in all organisms [24]. They are known to catalyze huge number of biochemical reactions[25]. They are a specific group of proteins, as a class of macromolecules that are synthesized by living cells to determine the patterns of chemical transformations, because of their capacity to specifically bind a very wide range of molecules. They catalyze reactions selectively by stabilizing a transition state; and an enzyme determines which one of several potential chemical reactions actually takes place [26].

Enzymes have three distinguishing characteristics as catalysts:

1) They accelerate the rates of reactions.
2) They are selective: the rate of reaction of a particular substance may be accelerated dramatically, while that of a structurally closely related substance is not.
3) They may be subject to regulation: that is, catalytic activity may be strongly influenced by the concentrations of substrates, products, or other species present in solution.

When an organic molecule combines to the surface of an enzyme, it may cause to change its active site configuration. Although the specificity of enzymes can be altered by this way [27], the selectivity of enzymes is the basis for much of their utility in organic synthesis. Enzymes offer the opportunity to carry out highly selective transformations, a feature of great value in working with chiral and polyfunctional molecules [28].

Table 2.1. Timeline of enzymology[29-34].

1752	Reamur	demonstrated the solvent power of the gastric juice of birds
1783	Spallanzani	had extended the Reamur'sstudies to humans and other species
1794	Mrs. Elizabeth Fulhame	First suggestion of catalysis in her book " *An Essay on Combustion* "
1833	Jöns Jacob Berzelius	recognized that a natural catalyst, an amylase that causes the hydrolysis of starch
1833	Payen & Peroz	alcohol precipitate of barley holds heat labile components (proteins) that converts starch to sugars
1836	Scwann	isolated the enzyme pepsin from gastric juice
1836	Jöns Jacob Berzelius	The study of catalysis, coined the term of catalysis
1878	F.W. Kühne	coins term 'enzyme Greek "in yeast"
1880	Charles Adolph Wurtz	Papain appears to form an insoluble compound with fibrin prior to hydrolysis; complex formation
1890	Cornelius O'Sullivan and Frederick William Tompson	The activity of invertase with temperature in absence and presence of sucrose
1892	Adrian John Brown	A study of rate of fermentation of sucrose
1894	Emil Fischer	the specificity of enzyme action: Key-Lock hypothesis
1897	Büchner	The discovery of cell-free fermentation and zymase
1898	Ducleaux	uses suffix "ASE" for enzyme names
1900	E. Fischer	stereospecificty of enzymes is discovered
1901	Victor Henri	Anticipated the enzyme actions
1901	C. Eijkmann	The first lipolytic activity
1902	Adrian John Brown	A study of existence of enzyme-substrate complex
1907	Bodenstein	The rate expression between H_2 and Br_2
1911	Micahelis and Davicdson	The effect of pH on enzyme active site
1913	Michaelis-Menten	At lower concentrations the rate becomes proportional to the concentration of substrate
1923	Hartridge and Roughton	Flow method in which the solutions are forced together very rapidly
1925	Georg Edward Briggs and John Burdon Sonderson Haldane	Improved the M-M assumptions in kinetics
1926	Sumner	The first enzyme crystallized and shown to be a protein
1940	Brittion Chance	Stopped-flow method
1941	John Alfred Valentine Butler	The first kinetic studies with a pure enzyme; molecular kinetics
1954	Eigen	The relaxation method for fast intermediates
1956	King and Altman	The kinetics methods for the complex reactions
1960s	Werner Arber	The discovery of restriction enzymes, cutting DNA helices
1964	Garfinkel and Hess	The computer studies of methabolic pathways
1965	Monod,Wyman,Changeux	Allostericity of enzymes
1973	Laidler and Bunting	The kinetic studies with different enzymes
1973	Kascer and Burns	The methabolic control analysis
1982	Chen and Sih	The first definition of E for the enantioselectivity of one component reactions

2.1.2. Enzyme Nomenculature

The naming of enzymes was not systematic in its early days. Enzymes were given trivial names that often meant little or were ambiguous. Many different enzymes were given the same name and, conversely, several names were given to the same enzyme, leading to much confusion. In general, the suffix "-ase " was added to the name of the substrate[35]. A system of enzyme nomenclature is comprehensive, consistent and at the same time easy to use. The common names for the most enzymes derive from their most distinctive characteristic: their ability to catalyze a specific chemical reaction[36]. Each enzyme has been given a four digit number by the Enzyme Commission of the International Union of Biochemistry. The first three digits relate to the reaction catalyzed by the enzyme and the final one is required if several enzymes with different protein structures catalyze the same reaction[37].

Name of enzyme (EC W.X.Y.Z)], where

EC – Enzyme Commission number system
W – indicates the reaction catalyzed (1 to 6)
X – indicates the general substrate or group involved
Y – indicates the specific substrate or coenzyme
Z – the serial numer of the enzyme

Table 2.2. Classification of enzymes [36-38].

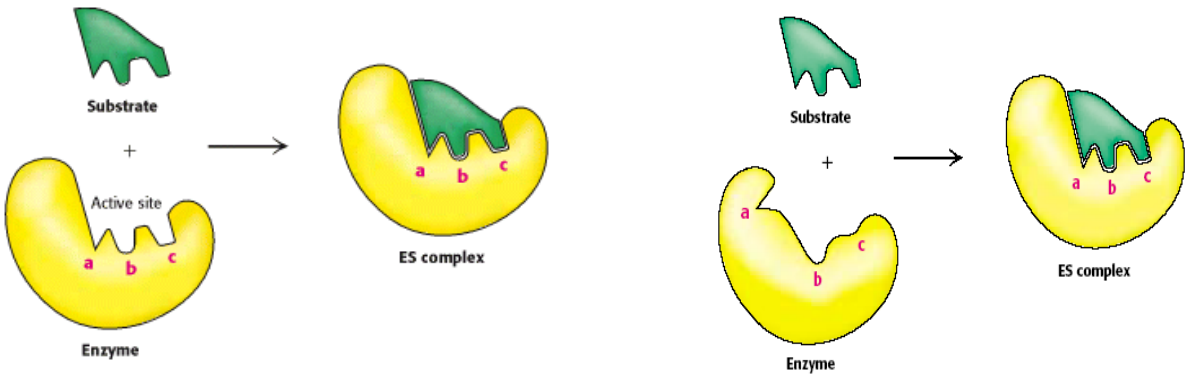
Enzyme Comission number	Enzyme type	The type of reaction catalysed
EC 1	Oxidoreductases	Transfer of H_2 or O_2 atom or electrons form one substrate to other.
EC 2	Transferases	transfer of groups such as methyl or glycosyl groups from a donor molecule to an acceptor molecule.
EC 3	Hydrolases	the hydrolytic cleavage of C-C, C-N, P-O, and certain other bonds, including hydride bonds.
EC 4	Lyases	cleavage of C-C, C-O, C-N, and other bonds by elimination, leaving double bonds, and also add groups to double bonds.
EC 5	Isomerases	geometric or structural changes within a single molecule.
EC 6	Ligases	joining together of two molecules, coupled to the hydrolysis of a pyrophosphoryl group in ATP or a similar nucleoside triphosphate.

Accordingly to the above mentioned way of nomenculature, the enzyme lipase utilized in this work is named as EC 3.1.1.3. From the table Table 2.2, EC3 corresponds to hydrolases. Following the nomenclature, EC 3.1 demonstrates that enzyme acts on ester bonds, as well as EC 3.1.1 is the representation for carboxylic ester hydrolases. The more specific revealing about type of enzyme is given by the serial number. As a result, lipase [EC.3.1.1.3] is the

enzyme with a full name of triacylglycerol lipase defined by International Union of Biochemistry and Molecular Biology (IUBMB). Evetually, lipases could be defined as the enzymes (triacylglycerol acylhydrolases, (EC 3.1.1.3) catalyzing the hydrolysis and the synthesis of esters formed from glycerol and long-chain fatty acids [39].

2.1.3. Specificity

Since the enzymes are remarkable catalysts, which tremendously accelerate reactions under mild temperature and pressure conditions[40], one of their drastic properties is the specificity. The active site and specificity play a major role along the course of reaction. Many substrates are capable of undergoing a variety of different biochemical reactions, although the enzyme preferably catalyses only one of these reactions[41], depending on the three dimensional structure of their amino acid sequence. Thus this property is known as the specificity, which is the most distinctive feature of enzyme based catalysis. The specitify of an enzyme, as illustrated in Fig 2.1, was postulated as two analogies which are very well explained in literature. The first analogy is the key and lock model postulated by Emil Fischer in 1984[42-45], and this rigid analogy was improved by Daniel Koshland in 1958 as induced fit model for certain enzymes, which do not obey previos model[46-49].



(a) (b)

Fig 2.1. Enyzme models (a) Lock and Key Model, b) Induced-fit model [26].

Complementary shape, charge and hydrophilic/hydrophobic characteristics of enzymes and substrates are responsible for the specificity. Enzymes can also show impressive levels of stereospecificity, regioselectivity and chemoselectivity[50]. A few enzymes exhibit absolute

specificity; they will catalyze only one particular reaction. Other enzymes will be specific for a particular type of chemical bond or functional group[51].

Enzymes are usually impressively specific in their action. The specificity toward substrate is sometimes almost absolute. For example in this work, the lipase tends to catalyse D-IPG more than L-IPG.

2.1.4. Chirality

Natural optical activity is perhaps the most well known property [52]. With the use of polarized light, first in 1815, Jean-Baptiste Biot stuided the the optical rotation of organic chiral liquids[53,54], and later in 1848, Louis Pasteur detected that the molecular structure of the tartaric acid crystals had a mirror image. He postulated the dissymmetric forces of nature according to the prevalence of only one of the two mirror-image enantiomers among natural organic products[55, 56]. As a result of these experiments, it was demonstrated that the same arrangement of the same atoms gave two different molecules, called racemates, which is derived from the Latin word " ***racemus*** "meaning "for a bunch of grape", since the tartaric acid had been isolated from wine[57].

A chiral molecule is one that cannot be superimposed on its mirror image with the two mirror image forms referred to as enantiomers (**see Fig. 2.2.**). One isomer of a pair rotates plane polarized light in a clockwise direction and is known as *dextrorotatory* ***(D).*** The other pair rotates plane polarized light in a counterclockwise direction and is termed *levorotatory* ***(L)*** [65,66].

The chiral asymmetry and optical activity have been considered with a principal criterion for the early stages life[58,59], although the chirality is universal property as detected on the indigenous enantiomeric excesses of L-amino acids in the *Murchison* meteorite fallen Australia in 1969[60].

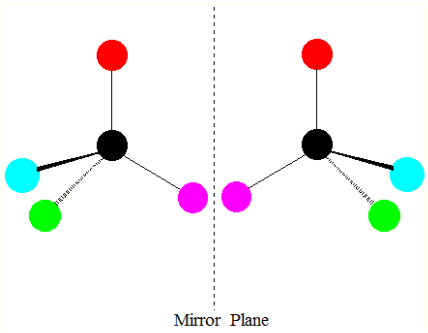


Fig 2.2. The two enantiomers

It is generally presumed that chirality was needed in prebiotic times so proteins could have their well defined structures[61]. The organic constituents of all living organisms on Earth are optically pure while they are

always composed of one enantiomer from a chiral pair of molecules, as also found in our terrestrial life[62]. The symmetry aspects of optical activity had been called *dissymmetry*, which has been replaced by the word chirality, derived from the Greek work "**chiros**" meaning 'hand' in the nomenclature of stereochemistry[63]. The concept of chirality is inextricably linked to that of enantiomers (from the Greek ***enantios*** opposite, ***meras,*** part)[64].

A mixture of equal amounts of the two enantiomers is said to be a racemic mixture. In this work, the reagent used in the transesterifictation reaction is *rac*-IPG.

An enantiomer can be best identified on the basis of their absolute configuration or their optical rotation in which it rotates the plane of plane polarized light. Nomenculature of chirality can be expressed conventionally in three ways, given on Table 2.3.[67].

Table 2.3. Nomenculature in Chirality

Forms	**Latin Affixes & Names**	**Direction of polarity of light**
D/L-form -	Dextro- = right ; Laevo or levo- = left	Clockwise ;Counterclockwise
R/S-form	Rectus = right ; Sinister = left	Clockwise ; Counterclockwise
(+/-)- form	+/– system for optical rotation	(+) clockwise ;(-) counterclockwise
Racemic (racemus)	for a bunch of grape	

Among these notations, the *R*/*S* system has no fixed relation to the (+)/(−) system. The *R*/*S* system does not involve a reference molecule [68], and also has no fixed relation to the D/L system. This means that an *R* isomer can be either dextrorotatory (D) or levorotatory (L), depending on its exact substituents. For example, in 1973, the dermested beetle pheromone, which was levorotatory, was shown to be the (R)-enantiomer, because the synthetic (S)-isomer was dextrorotatory[69]. Hence, the D/L system remains in common use in certain areas of biochemistry. For this reason, the L/D nomenculature will be used along this work according to its common use.

2.2. *Burkholderia cepacia vs Pseudomonas Cepacia*

The enzyme utilized in this work is isolated from previously called *Pseudomonas cepacia* which is later taxonomically renamed as *Burkholderia cepacia*, whose lipases were extensively studied and the most widely used during the past two decades for industrial use in biotechnology [70,71]. The genus *Pseudomonas* comprises a large assemblage of bacteria widely distributed in nature in a great variety of natural and artificial habitats[72]. *B. cepacia* can be found in infected plants, animals, humans, and in a variety of soil and rhizosphere environments; playgrounds, athletic fields, parks, hiking trails, residential yards, and gardens[73]. It was first described by Walter Burkholder in 1949, when he determined it to be the cause of bacterial rot of onion bulbs. It was originally named *P. cepacia,* which comes from Latin "*cepa*" (onion)[74], and Pseudomonas comes form (greek "*pseudo*" = false, Latin "*monas*" = single unit) as well[75]. The name *P. cepacia* was later changed to its current name *B. cepacia*, which refers to a complex of nine closely related *cepacia* species in the genus *Burkholderia*[76].

B. cepacia is an important gram-negative bacterial pathogen in patients with cystic fibrosis[77]. It may cause premature death in these patients. The species are recognized with seemingly increasing frequency as nosocomial pathogens[78] for which reservoirs, modes of transmission, and host factors predisposing to infection are still being defined[79]. *B. cepacia* has been registered, since 1992, as a microbial pesticide by the US Environmental Protection Agency[80]. This bacteria is motile by means of monotrichous or multitrichous polar flagella. Cell dimensions are generally between 0.5-1.0 μ by 1.5-4 μ[81]. Many produce a water-soluble green fluorescent pigment, and, while others are nonchromogenic[82]. The cells have a straight curved rod shape and no spores[72].

The pathogen has undergone several taxonomic reclassifications in accordance with the Bacteriologic Code[83]. Most confusion surrounding this species name was initially due to its transfer from the genus *Pseudomonas* to the newly described genus *Burkholderia* in 1992[74]. In order to get rid of any confusion, the recent name *B. Cepacia* will be preferred after now along this work, instead of *P. Cepacia*, which had been used in previous works at Technical Chemistry Institute (TCI).

2.3. Lipases

In 1856, Claude Bernard first discovered the lipase in pancreatic juice as an enzyme that hydrolysed insoluble oil droplets and converted them to soluble products[84]. Later in 1889, J. Reynolds Green showed that germinating seeds of the castor-oil plant contain an enzyme which is capable of hydrolyzing castor oil[85]. Consequently, it was detected that the fats are hydrolysed under the influence of lipases[86], which were well defined in kinetic terms, based on the phenomenon of interfacial activation, in 1958[87].

According to the IUBMB, lipases (EC 3.1.1.3) are defined in the class of hydrolyses[88-90], a detailed list of names is given in App.I., catalyzing the hydrolysis of carboxylic acid esters[91-96]. All hydrolases belong to main class 3 of the Enzyme Commission Nomenclature System and there are 1065 different EC numbers for hydrolases classified, recently[97]. Lipases are ubiquitous enzymes which play an important role in lipid metabolism. They are a versatile group of biocatalysts, which apparently hydrolyse insoluble triglycerides and other water insoluble highly lipophilic carboxylic acid esters by tailoring into the concomitant production of free fatty acids and glycerol during digestion as shown in Fig.2.3.[98-101].

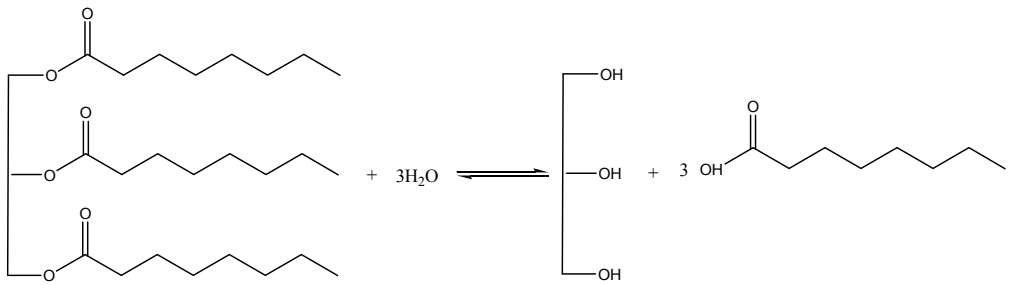


Fig.2.3. Lipase catalysed hydrolysis of a triglyceride

Lipases are versatile biocatalysts. In addition to their hydrolytic activity on triglycerides, they can also catalyze other reactions such as esterification, interesterification, acidolysis, alcoholysis, and aminolysis (Fig.2.4.)[102, 103].

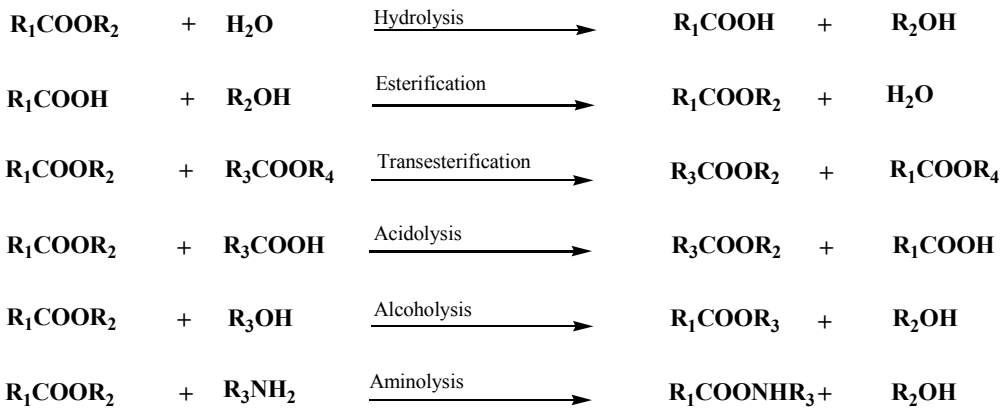


Fig. 2.4. Different type of reactions catalysed by lipase[102, 104].

2.3.1. Sources of lipases

Use of industrial enzymes allows the technologist to develop the gentle and efficient processes [84]. The source of the lipase is important; because their properties differ greatly depending on their origins [105]. Although lipases are ubiquitous throughout the Earth's flora and fauna, they are found more abundantly in the plants, pancreas of mammals, and the microbial flora comprising bacteria, fungi, and yeast. In the field of biotechnology, much attention has been paid to the use of lipases of microbial origin, and commercially available lipases are usually derived from microorganisms. Since the advent of genetic engineering techniques, an increasing number of lipases are being commercially manufactured from recombinant bacteria and yeasts [39,92,106-107].

Commercially available triacylglycerol lipases are produced from 34 different sources, including 18 from fungi and 7 from bacteria [108]. Among more than 30 commercially available lipases [92, 109] are frequently used in enantioselective acylation of alcohols and amines [110] or in esterification of carboxylic acids and hydrolysis of their esters [111-112], *Burkholderia cepacia* lipase (BCL) is the one of most thoroughly studied [113]. In this work also BCL has been used and its properties have been considered.

2.3.2. Industrial applications of lipases

With the increased awareness of environment and cost issues, biotechnology drastically gains various advantages over conventional technologies [84]. Because of their biotechnological potential, lipolytic enzymes have an increasing enormous attention for a variety of biotechnological applications [114]. Especially, lipases have a rapid and steadily increasing usage as catalysts for industrial chemical reactions, since these biocatalysts act under extremely mild conditions with good regio- and stereo-selectivity in catalysis. They are active, highly stable in the solvents, and have a wide range of substrate specificity. They are generally environmentally benign, and produce few by-products [115-118]. Since lipases constitute the most important group of biocatalysts for biotechnological applications, their optimisation of industrially relevant properties can be achieved by directed evolution [119], which is nowadays widely used for the optimization of diverse enzyme properties like stability, high product purity, regioselectivity and in particular, enantioselectivity [120-121].

In order to increase the activity and stability of lipases and at the same time to facilitate their recovery, many immobilization techniques have been studied [122], e.g., on hydrophilic supports [123], hollow membrane [124], adsorption on solid supports, encapsulation and entrapment within the membrane. Recently, the entrapment of lipases in hydrophobic sol-gel materials with formation of heterogeneous biocatalysts having significantly enhanced the enzyme activities [125]. Furthermore, these will then determine desirable bioreactor design (batch, stirred tank, membrane reactor, column and plug-flow)[126].

The main application fields for lipases include dairy, diagnostics, oil processing, and biotransformations. Recently, special emphasis is lying on the production of chiral chemicals, which serve as basic building blocks in the production of active pharmaceuticals, agrochemical intermediates, food ingredients, and pesticides or insecticides [127,128]. They are also utilized, with general interest, in many other industrial applications, e.g., detergent formulations, oil and fats, baking, organic synthesis, hard surface cleaning, and paper industry [129]. These enzymes find promising applications in organic chemical processing, synthesis of biosurfactants, the oleochemical industry, the dairy industry, nutrition, and cosmetics [108]. As well as lipases have become of commercial importance as constituents of synthetic targets [130], their versatility makes

them the enzymes of choice for potential applications in the leather and textile industries [102]. Furthermore, novel biotechnological applications have been successfully established using lipases for the synthesis of biopolymers and biodiesel, the production of enantiopure pharmaceuticals, agrochemicals, and flavour compounds [119].

One of the very important application is the synthesis of enantiomerically pure chiral compounds for the drugs produced in either pharmaceutical or agricultural industry for living organisms [128,131]. The increasing awareness of the importance of chirality in the context of biological activity has stimulated a growing demand for efficient methods for industrial synthesis of pure enantiomers in synthesis of the drugs [39]. Therefore, the kinetic study of enantiomeric reaction has become important to understand and control the production of desired racemate, as considered in the main topic of this work.

2.4. Medium Engineering for Lipases

The enzyme activity and the enantioselectivity are the important characteristics of enzymes. The biocatalyst itself and the reaction conditions can influence the measured enantioselectivity[132]. The choice of the optimal reaction conditions for an enzyme is a critical factor, since the enzyme activity and stability are greatly influenced by the reaction media[133]. Recently, the substrate specificity, enantioselectivity, prochiral selectivity, regioselectivity, and chemoselectivity of enzymes have been found dramatically to be dependent on the nature of the solvent[134]. Three approaches of using biocatalyst engineering, medium (solvent) engineering and substrate engineering have been proposed to improve the enzyme activity and enantioselectivity[135]. Medium engineering is a relevant technique to improve the efficiency and stability of enzymes with the selection of convenient medium[136]. As previously mentioned, the media for the transesterification reaction used in this work was performed in organic solvent, *n*-hexane, and supercritical CO_2 medium, the both medium was explained in detail while the other solvents were explained briefly.

2.4.1. Aqueous Medium

Water plays a central role in enzymatic catalysis and is a ubiquitous component in all biological processes such as biosynthesis, photosynthesis, metabolism and catabolism [137]. Historically, the enzymatic catalysis has been carried out primarily in aqueous systems, because the water is the natural medium of the enzymes [138]. It was previously difficult to visualize enzymes catalyzing reactions in the absence of water [139]. The enzyme maintains its native three dimensional conformations and its natural activity in water [140]. The amount of water associated with the enzyme molecules is controlled by the water activity [141-142]. A high hydration level leads to a more flexible enzyme[143-146], which is in some way directly related to the enantioselectivity of the reaction[133,147]. The rates of enzyme-catalyzed reactions are in many cases strongly dependent on the water activity of the reaction medium. The picture is more complex regarding the effect on enzyme enantioselectivity [148]. It is reported that lowering the water affinity (or water content), decreased[149-152], increased[153-157] or not affected [149, 158-160] the enantioselectivity.

2.4.2. Organic Media

The use of water as natural solvent in enzyme catalysis is often a problem because the most of the substrates are insoluble in water [161,162]. The water itself may become a reactant and frequently gives rise to unwanted side reactions. It sometimes degrades common organic reagents and shifts the equilibrium towards the reverse reaction [163-165]. In water, the transesterification reaction would not be possible because of the domination of the hydrolysis reaction [137]. Beyond all these circumstances, the thermodynamic equilibrium of many processes is unfavorable in water, and product recovery is sometimes difficult from this medium. In principle, the most of these problems might be overcome by switching the reaction media from water to organic solvent [94, 128, 166, 167] or monophasic / biphasic mixture of water and organic solvent. In organic solvents, the hydrolysis of esters is negligible, and optically pure esters can be produced; *e.g.*, in addition to the transesterification reactions, lipases can also catalyze intramolecular esterification [137].

Although the experiments to place enzymes in systems other than aqueous media began from the end of the nineteenth century[138,168], it was shown that enzymes are functional in both aqueous

and organic media in last two decades[137, 144]. The detection of the enzyme activity in an organic solvent has opened a totally new horizon for enzyme technologists[169], and greatly expanded the potential scope and economic impact of biocatalysis[170]. Studies of biocatalysts in non-aqueous environments have led to a deeper understanding of how enzymes function in unnatural surroundings as well as in water[171]. Later, it was determined that the activity, specificity, and stability of the catalyst depend on the type of the solvent[94, 172-175]. Especially, lipase catalyzed esterification reactions in organic solvents was often more enantioselective than the corresponding hydrolytic reactions in water[176]. Due to decreased enzyme flexibility in organic solvents, an increase in enzyme enantioselectivity has been observed[177]. Enzyme activity in organic solvents depends dramatically on such parameters as water activity, substrate-product salvation, pH control, enzyme form and the nature of the solvent. It became apparent that enzyme activity was higher in hydrophobic solvents than in hydrophilic ones[178], because the latter have a greater tendency to strip tightly bound water from the enzyme molecules[94,132].

However, it is very well documented that enzymes are denatured or highly inactivated in the presence of organic solvents. The specific catalytic activity of enzymes, that is stable in non-aqueous environments, are generally lower than those in aqueous systems [179-181]. Improvement of enzyme activity in non-aqueous solvents has been of great significance in the field of non-aqueous enzymology. This observation has led to numerous attempts to improve enzyme activity and stability in non-aqueous media, using strategies including: entrapment in water-in-oil microemulsions [182,183], utilization of solid enzymes (suspended in organic solvents) [172, 184,185], surfactant -coating and bioimprinting [186], immobilization on appropriate insoluble supports [187-193] to offer several advantages those include: reusability, rapid termination of reactions, low cost product formation, and ease of separation [194]. Another diversely used method is the enzyme-containing reverse micelle.systems, which has become an attractive proposition when substrates and/or products are hydrophobic and low water content is desired. This is often the case with lipase reactions, since the solubility of the substrate, e.g. a triglyceride, is markedly improved in an organic solvent [195]. Reverse micelles offer a unique possibility to overcome problems caused by medium heterogeneity [196]. Hovewer, an important problem for application of the lipase in reversed micelles is the deactivation of the enzyme [197].

As a summary of all above mentioned acknowledgement about enzymatic catalysis in organic solvents, it can be simply revealed that enzymes significantly broadens conventional aqueous-based biocatalysis [92, 163, 198- 202]. The advantages and disadvantages of biocatalysis in organic solvents are given on Table 2.4 in detail.

2.4.3. Solvent free Systems (SFS)

An alternative media to organic solvents is the possibility of avoiding the use of any reaction medium [203]. The SFS is simply a mixture of reactants without any organic solvent added [204]. The choice of solventless or specific non-organic solvent reaction medium will depend on several issues; including selectivity, stereochemistry, yield, waste, viscosity, ease of recycling, energy usage, and ease of isolation of products, competing reactions, and heat of reaction [205]. The SFS, which is a simple mixture of reactants, offers greater safety, increased reactant concentrations and high volumetric productivity [204,206]. A system, that presents the major advantage of the absence of solvents, facilitates downstream processing. Moreover, the elimination of solvents from the production step offers significant cost saving [207], and the environmental impact is minimum, since there is no solvent in the reaction system [163].

Table 2.4.: The Advantages and Disadvatages of Organic Solvents as Reaction Medium

Advantages (*ref*: 208, 209)	**Disadvantages (*ref*: 167, 180, 209-213)**
i- The product and biocatalyst recovery from systems are relatively easy, with better overall yield. ii- The solubility of non-polar substrates and products increases in the organic solvent, which markedly speeds up overall reaction rates. iii- The denaturation of enzymes and the substrate or product inhibition are minimized. iv- The undesirable side reactions are largely suppressed and the unwanted water-dependent side reactions are possibily minimized. v- The immobilization of enzymes may not be required. vi- The thermodynamic equilibrium shifts to favor synthesis over hydrolysis, allowing reactions usually not favored in aqueous solutions (*e.g.*,transesterification, thioesterification, aminolysis). vii- The enantioselectivity of the reaction drastically changes when one organic solvent is changed to another. viii- The microbial contamination can be eliminated in the reaction mixture.	i- Solvent toxicity is a problem for many applications ii- Some organic solvents employed are too expensive to allow profitable commercial scale up iii- Many organic solvents are known to inactivate or to denature biocatalysts iv- Organic solvents effect the dehydration of polar groups, which also reduces the ionic and charged form of these groups v- The substrate conversion rate can be reduced because of a more rigid protein molecule; this rigidity has the double effect of increasing resistance to thermal vibrations and reducing the enzyme-substrate interaction vi- They may be flammable, volatile, which makes the separation more difficult and the hazard of environmental pollution increases due to solvent loss. vii- Mass transfer limitations

2.4.4. Ionic liquids (ILs)

Besides the organic solvents and supercritical fluids, the ionic liquids, ILs have been used as clean and green solvents not only for the enzymatic reactions in a wide variety of biochemical processes [214-217], but also as extremely excellent solvents or catalysts for a wide range of polar and non-polar organic, inorganic, and polymeric compounds [218-226]. In the last years ILs have gained much attention, because they can be used for all types of reactions [224, 225, 227- 230]. In contrast to molten salts, ILs are the organic salts in liquid phase at/below room temperature [227, 229, 231-233]. They are entirely composed of ions [225], normally consisting of a bulky asymmetric organic cation and a wide variety of polyatomic inorganic anion [234]. In the future, solvents will be designed to control chemistry [235]. In this aspect, by changing the structure of anion or the R group of the cation, or both [236,237], (ILs) can be tuned for each specific enzymatic reaction system [238].

2.4.4.1. Lipases in Ionic Liquids

After the first report on enzyme catalysis in ILs [239, 240], the potential use of lipase catalysis in (ILs) [241-244] for improvement of enantioselectivity were immediately followed [245,246]. Lipase-catalysed reactions in (ILs) have some potential advantages besides environmental ones, including increased stability, high activity, high reaction rates, high thermal and operational stability, the regioselectivity [229,247-251], and the enhancement of enantioselectivity [252-259]. It is also determined that the stable microbial lipases, like *Burkholderia cepacia* lipase (BcL), are catalytically quite active in the (ILs) [238, 260-263]. (ILs) offer an excellent media for many lipase-catalyzed trans/esterification reactions with an increased activity and specificity[222, 262, 264-268].

Nowadays, a new class of intensified processes comes into perspective, by combining ionic liquids and supercritical carbon dioxide ($scCO_2$) as new study for the enzymatic reactions [269-276].

2.5. SUPERCRITICAL FLUIDS (scF)

Recently, the most of the biotechnologically important materials are produced by enzymatic biotransformations on industrial scale [277], while the biocatalysts are generally non-toxic and produce less or almost no by-products [278]. The reaction media is known to be important, because they can change the activity, specificity and stereoselectivity of an enzyme along the course of the reaction [94]. Prevoiusly, all reactions in bioprosesses were essentially performed either in water or occasionally in organic solvents [277]. Although the aqueous medium is an environmentally benign and cheap, it is not a good solvent especially for the hydrolysis reactions using hydrolytic enzymes [173,279-291]. Thus, the reactions using hydrolytic enzymes have been usually taken place in non-aqueous organic solvents, *e.g.* lipase catalyzed esterification, transesterification reactions [292- 296].

As briefly mentioned in previous sections, the different solvent engineering methods have been searched to increase the efficiency and the yield of the enzymatic reactions by studying wide range of variuos reaction media. In this work, two solvents have been utilized for the kinetic studies. The first one is the widely used organic solvent *n*-hexane for lipase catalysed transesterification [297-303]. Unfortunately the use of *n*-hexane is under greater scrutiny due to increasing government restrictions and consumer concerns regarding the safe use of organic solvents [304]. The second solvent is the carbondioxide in its supercritical phase ($scCO_2$) [153, 279, 280, 299, 300, 305- 311] within which the reactions might be considered as solventless [286].

Using scF as medium has several additional advantages as given in detail on Table 2.5. It can diffuse through solids like a gas, and dissolve materials like a liquid because of its low viscosity [312-315]. Supercritical fluids have been used as solvents for organic synthesis as well as for extraction and chromatography by taking advantage of both their gas-like low viscosities and high diffusivities and their liquid-like solubilizing power [316-320]. They have low surface tension allowing dissolved reactants to penetrate easily throughout macro- and micro-porous materials [321]. These unique transport properties enhance the mass transfer rates of substrates to active sites of enzymes [277, 316, 322-324]. Moreover, its properties, such as density, dielectric constant, diffusivity, viscosity, solubility and the partition coefficientcan be tuned by adjusting the pressure and temperature, which clearly distinguishes the supercritical fluids from conventional solvents [316-320, 325-328]. By tuning, it is possible to eliminate transport limitations on reaction rates [316].

Table 2.5.: Advantages and disadvantages of $scCO_2$ [ref: 313, 315, 320, 323, 325, 337-354]

Advantages:	Disadvantages
● green chemistry ● cheap ● abundant and available in high purity ● better product uniformity ● environmentally benign ● high reaction rate and conversion ● improved selectivity ● inert ● non-flammable, ● energy reductions ● ease of product recovery ● reduction in side reactions ● non-cancerogenic, ● non-mutagenic, ● non-flammable ● non-toxic ● non-corrosive ● higher diffusivities and no mass transfer limitations ● thermodynamically stable ● low temperature for heat labile materials ● the control of dissolving power by tuning the pressure and/or temperature ● easy extract recovery ● high boiling components are extracted at relatively low temperatures ● the size, shape and morphology control of the material ● similar solvating power as organic solvents ● low viscosity ● low surface tension ● no microbial contamination in bioreactor ● the solubilization of hydrophobic chemicals ● elimination of side reactions ● enzyme stability ● gaseous under ambient conditions ● moderate critical conditions to facilitate process design	●CO_2 is a potent inhibitor of the reaction ●technological difficulties related to high pressure operations ● high capital investment for equipment ● poor solvent for high molecular weight or hydrophilic molecules because of its very low dielectric constant and polarizability per volume ● scF technology requires sensitive process control ● compression of solvent requires elaborate recycling measures to reduce energy costs ● the phase transitions in the critical region is rather complex and difficult to measure and predict. ● enzymes are insoluble in sSCF, and therefore recovery is straightforward and immobilization is unnecessary ● $scCO_2$ could strip essential water from the enzyme microenvironment, causing enzyme deactivation. ● the hydrophobic nature of supercritical fluids hydrophylic reactants and products can not be dissolved. ● the reactivity of $scCO_2$ with amines to form carbamide may affect the stability and activity of enzymes by reaction with amino acid residues ● CO_2 reacts with water to form carbonic acid, which will lead to a drop in pH in non-buffered systems ● complex character of the phase equilibrium in systems

Especially from an industrial point of view, the scFs have also a great advantage of that the different products are obtained to a high level of purity upon de-pressurisation without the need for the extensive separation processes [321]. By this way, the reaction mixture and the

final product are freely separated from residual solvent [329,330]. The adjustable solvent power of the fluid allows the design of a production process with integrated downstream separation of products and un-reacted substrates [277]. One of the basic difficulties in using scFs is that the operation should be carried out in high pressure equipments. For optimal equipment design, the fundamental aspects involve phase behavior and solubility as well as density and interfacial tension. In addition, transport properties, including viscosity, thermal conductivity and diffusion coefficients are needed [304]. Safety aspects of any reactor should also be considered during design [322]. There are several reports on batch processes [331-333], and continuous processes [279, 334-336].

Although the supercritical properties were first observed in 1822 by Baron Charles Cagniard de la Tour, its industrial application first appeared in 1978 as given on brief historical timetable of scFs (Table 2.6.). In the past twenty years, there has been a sudden expansion of the "critical fluid" technology platform with respect to using or combining multiple types of unit operations and compressed fluids in both their sub- and supercritical states [346]. Supercritical fluids (scF) have been focused on as a new reaction medium [316, 319, 352]. The thermophysical properties of many compounds, and their solubility parameters are very well listed in literature [320, 324, 349]. One of the interesting point on these data is that the solubility parameter for *n*-hexane and CO_2 are 7.325 and 7.118 $(cal/cm^3)^{0.5}$ respectively [355]. This shows that $scCO_2$ showed approximately equal polarity and solubility as *n*-hexane, and can be used instead of this conventional organic solvent [280,351,356]. Consequently, the enzymatic reactions under ($scCO_2$) have become one of the most useful processes [283, 316, 319, 352, 357, 358-361]. A brief list of works on the enzymatic reactions in $scCO_2$ are given on Table 2.7. Among other scFs, supercritical carbon dioxide ($scCO_2$) has found a widespread usage because of that it is non-toxic, inflammable, relatively inert, abundant and inexpensive, stabile in radioactive applications, ease of separation from substrates and products, low viscosity, high diffusivity and ambient critical temperature (T_c = 31.0 °C). $scCO_2$ has recently attracted attention as an environmentally friendly solvent for extraction, chemical reactions and chromatography [319, 322, 378]. Its low critical temperature allows the heat sensitive materials to be processed without denaturation [379], and it has also been proved that the enzymes have stability, activity and specificity as weel as tunable enantioselectivity in $scCO_2$ [284,285]. The limitation for $scCO_2$ usage is that it can only dissolve the non-polar solid compounds [289], because of the non-polarity of carbon dioxide, which preferentially dissolves hydrophobic compounds [313, 346].

Table 2.6.:Brief history of Supercritical Fluids (ref : 284, 295, 323, 330, 349, 380- 394)

Year	Scientist / Company	Work / Process
1822	Baron Charles Cagniard de la Tour	Discovery of the critical point of a substance
1861	Gore	The first publications about phase behavior and solvent characteristics of near-critical, liquid carbon dioxide
1879	Hannay and Hogarth	The ability of a supercritical fluid to dissolve low-vapor-pressure solid materials
1822	Baron Carniard de la Tour	Discovery of the critical point of a substance
1861	Gore	Solvent properties of compressed CO_2 detected
1869	Dr. Thomas Andrews	A lecture about investigation of phase behavior of CO_2
1879	Amagat	A method for compressing gases to -400 bar using mercury columns extending to the bottom of a mine shaft
1879	Hannay & Hogart	Several solids were extracted with CO_2
1881	Ramsay	Dissolved eosin in CO_2
1891	Cailletet	Generation of high pressures with a mercury column
1896	Villard	Publicationf of a review of supercritical fluid solubility phenomena
1906	Buchner	The experimental data base of high pressure SCF-solute mixtures
1913	Ipatiev & Rutala	Homogeneous catalysis in SCFs
1915	Prins	Explored the solubility of naphthalene in both supercritical ethane and carbon dioxide
1940	Pilat, S. and Godlewicz	Patent for the fractionation of mineral oils using $scCO_2$
1954	Francis	An extensive, quantitative study on the solvent properties of liquid CO; with hundreds of compounds
1962	Klesper , ***et.al.***,	Discovery of supercritical fluids chromatography
1978	Kaffe Hag A.G	The first decaffeination of green coffee beans plant in Bremen
1980	Carlton and United Breweries	The liquid CO2 extration for hop flavours in Melbourne
1980	Bart Raiser Co	The first hop extraction plant in Wolnzach
1980	SKW Trostberg	Tea decaffeination Munchmunster
1984	Tom & Debenetti	RESS (rapid Expansion of supercritical solutions) Process
1985	Randolph et al., Nakamura	The first reports on biotransformations in supercritical fluids
1986	Nakamura	The use supercritical carbon dioxide as a reaction media
1992-1995	Kamat	Improved reaction rates, control of selectivities by pressure
1992	Marty	The kinetic behavior of an enzymatic reaction in scCO2 compared to that in a conventional organic solvent was studied.
2002	Thomas Swan & Co. Ltd.	Operating a 800 t/a of heterogeneously catalyzed reactions in $scCO_2$

Table 2.7.:Enzymatic reactions in $scCO_2$

Enzymatic Reaction in $scCO_2$	**References**
oxidation	362, 363, 364, 365
hydrolysis	290, 348, 366 – 369
esterification	321, 328, 341, 348, 358, 359
interesterification	338, 370, 371, 372, 373
transesterification	281, 329, 332, 374-377
enantioselective synthesis	278, 285, 327, 330

The simple addition of a polar co-solvent, such as methanol and ethanol, greatly enhances the solubility of polar compounds in $scCO_2$ [322, 378] due to specific intermolecular interactions between the co-solvent and specific components of a mixture, since the solubility of all mixture components is enhanced due to the density effect [304].

2.5.1. Physicochemical properties of the supercritical fluids

A fluid is considered to be supercritical at a temperature and pressure above its thermodynamical critical point [395]. In the supercritical environment only one phase exists. The fluid is neither a gas nor a liquid, and combines properties of gases and liquids [349, 396]. Thus, a supercritical fluid (scF) is characterized by physical and thermal properties that are between those of the pure liquid and gas (Table 2.8.). In this phase, the fluid density is a strong function of the temperature and pressure. The diffusivity of scF is much higher than for a liquid and scF readily penetrates porous and fibrous solids [173, 325, 352]. The high diffusion rates in scFs cause the rapid mass transfer, which may be further enhanced by natural convection [397]. The dimensionless parameters in scFs were studied in detail [398-401], and the natural convection is expected to be a function of the Grashof number Gr, and the overall mass transfer may then be correlated as Sh = f(Re, Sc, Gr), where Sh is the Sherwood number, a dimensionless number useful for correlating the mass transfer coefficient, k_c.(eqn 2.1.).

$$\frac{Sh}{ScGr^{1/4}} = 1.692\left(\frac{Re}{Gr^{1/2}}\right)^{0.356} \qquad (2.1.)$$

A maximum in the mass transfer coefficient near the critical point, correlated with a maximum in the Grashof number defined as in the following correlation for mass transfer in SCFs [402, 403].

The schematic phase diagram for pure carbon dioxide (Fig. 2.5) shows the projection on the P-T plane of the P-V-T surface, where carbon dioxide exists as a gas, liquid, solid or as a scF.

The solid-gas coexistence line, the triple point, and the solid-liquid and liquid-gas coexistence curves are shown [295]. The gas–liquid coexistence curve is known as the boiling curve. Moving upwards along the boiling curve with ascending temperature and pressure, the liquid becomes less dense due to thermal expansion and the gas becomes very dense as the pressure rises. This situation is well obversed by the video taken by Schwabe *et.al.* at Technical Chemistry Institute of Hannover University (Fig. 2.6).

Table 2.8.: Comparison of Physical Properties of Liquids, Gases and scFs,
(Kinematic viscosity ν=η/ρ), (ref : 313, 324, 349, 352, 355)

Physical Properties		Gas (normal cond.)	Supercritical fluids (critical point)	Liquid (normal cond.)
Density	ρ / kg m^{-3}	0.6–2	200–500	600–1600
Dynamic viscosity	η / mPa s	0.01–0.3	0.01–0.03	0.2–3
Kinematic viscosity	ν / 10^{-6} m^2 s^{-1}	5–500	0.02–0.1	0.1–5
Diffusion coefficient	D / 10^{-6} m^2 s^{-1}	10–40	0.07	0.0002–0.002

On f and g pictures of Fig. 2.6., it is quite clear how the surface become weak and the interface between gas and liquid disappears, then the boiling curve comes to an end at the critical point, where a liquid and gas phase in equilibrium converge into a single phase [379]. The critical point for CO_2 occurs at a pressure of critical point P_c 7.39 MPa and a temperature of 304.1 K of a pure substance. Thus, above the critical temperature a gas cannot be liquefied by pressure. At slightly above the critical temperature, in the vicinity of the critical pressure, the line is almost vertical. A small increase in pressure causes a large increase in the density of the supercritical phase.

The phase distinction was observed by setting up a vessel with a window, through which the meniscus between liquid and gas can be seen to disappear as the critical point is reached (Fig. 2.6.). Photograph (a) shows a two phase liquid–gas system, with a clearly defined meniscus. As the temperature and pressure of the system are increased, the meniscus becomes weak (photograph b). This is due to the decrease in the difference between the densities of the two

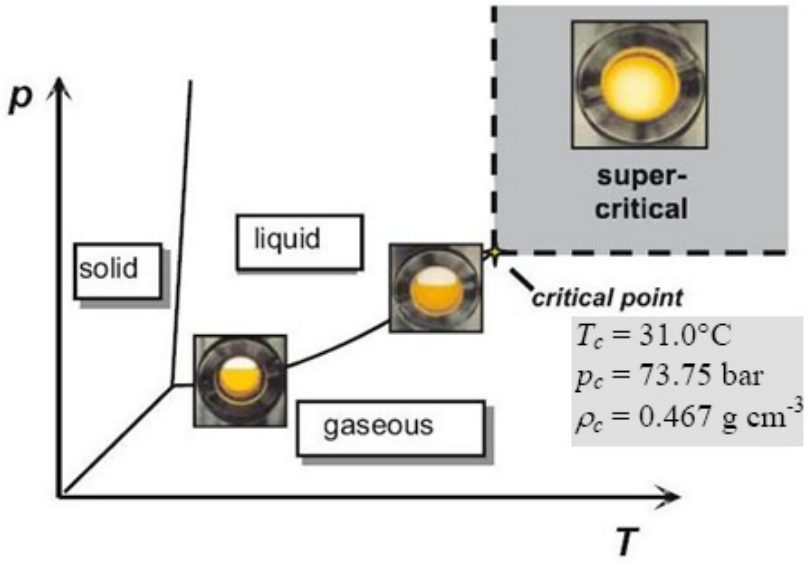


Fig. 2.5.: Schematic phase diagram of CO_2 (with the courtesy of ref. 404, 405)

phases. As the pressure is increased more, the interfacial forces seem to be strong not to separate the two phases (d). Near the critical point, the interfacial surface forces beome more weak (f, g) and, finally, in photograph (i), no meniscus is present and the system is now a single homogeneous scF [309]. Fig. 2.6 also supports this situation by the pictures taken from video at TCI by Schwabe *et.al.*

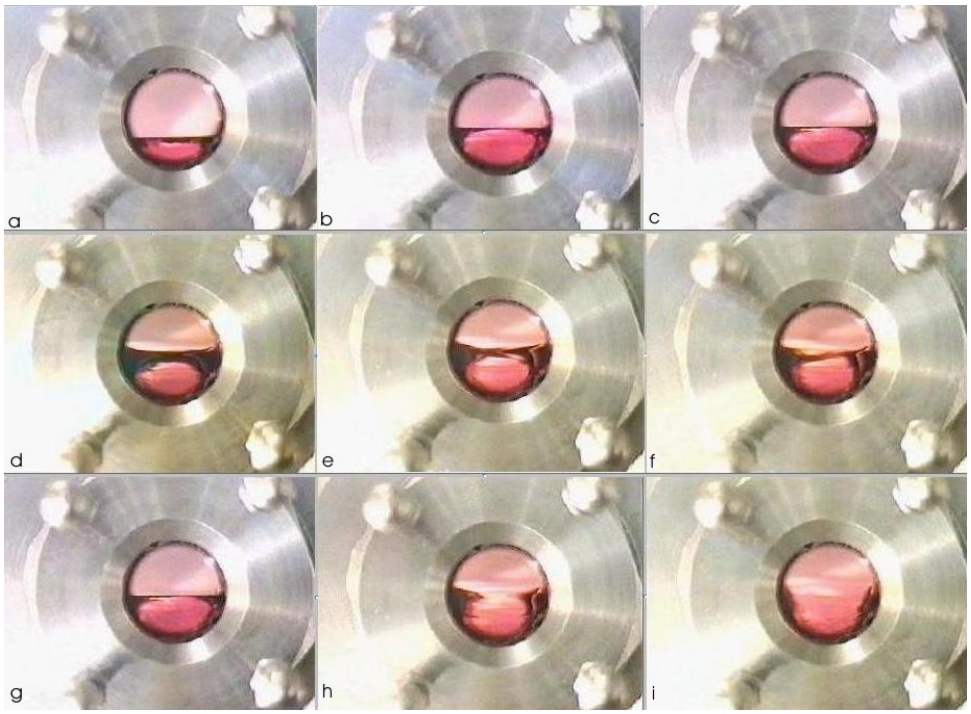


Fig. 2.6.: Pictural demonstration of phase diagram photographed in TCI, by Schwabe. *et.al.*,.

2.5.2. Applications Using Supercritical CO2

The special properties of supercritical fluids bring certain advantages to chemical separation processes. Several applications ,given on Table 2.9, have been fully developed and commercialized.

Table 2.9.: Application of $scCO_2$ in Industry

Industry	Process	References
Cleaning	Dry-cleaning of garments, usageof CO_2 instead of PERC (perchloroethylene), cleaning of equipments and microparticles	221, 313, 379, 406-413
Cosmetics	Cosmetic compounds extracted from herbs	414 - 419
Energy	Biofuel, biodiesel, biorefinery, nuclear power plants, the destruction of toxic waste, refrigeration,	420 - 428
Environmental	Soil remedation, waste treatment, killing and inactivatiom of microorganisms	353, 429 - 440
Food and flavouring	Extraction or syhthesis of edible and aromatical compounds, lipid extraction, cholesterol lowering, pasteurization, cheese maturation	441 – 447
Nanotechnology	Tuneable pore sizes; high surface areas aerogel, drug delivery systems, catalyst for fuel cell, controlled crystal growth, semiconductor fabrication, bio-medical materials, polymeric nanocomposite foams, sensors, nanoparticle formation	448 – 458
Petrochemistry	The destillation residue of the crude oil, regeneration of used oils and lubricants,plastic recycle, heterogeneous catalysts preparation	459 – 471
Pharmaceutical & medical	Controlled or targeted drug delivery, biomedical devices, stem cell, producing of active ingradients from herbal plants, elimination of residual solvents from the products.	354, 448, 472 - 480
Polymer	Biodegradable polymers ; design for a specific particle shape and size ; plastic recycling ; Encapsulation ; Plastic degradation ; Thin film formation ; Plastic foaming	355, 481 – 492
Supercritical fluid chromatography	Chiral separations; analysis of high molecular weight hydrocarbons	392, 493 – 498
Textile	Dyeing, scouring, bleaching, leather cleaning	337, 499 - 508
Tissue Engineering	3D scaffholds preparation, foamed structure formation, sterilization,	476, 509 - 524

2.5.3. Lipases in $scCO_2$

In the last two decades, the lipase-catalyzed reactions in $scCO_2$ have been reported by numerous investigators[332, 333, 342, 525-532]. Enzymes in scFs can be used in their native form (powder, reversed micelles, liquid, etc.) [533] or used as immobilized enzymes on a carrier (resin, sol–gel matrix, etc.) [289, 326, 534-542]. They can be used as crosslinked enzyme crystals, crosslinked enzyme aggregates, and lipid coated enzymes. It is thus very difficult to predict the stability and activity of an enzyme in any scF [277]. Methods to enhance the activity of enzymes in $scCO_2$ are studied much in detail [543]. The use of lipases in non conventional SCFs, has been proposed to enhance not only the activity, but also the utility of enzymes in anhydrous environments, the stability and enantioselectivity of the enzyme [329, 544- 549].

2.5.3.1. The effect of pressure on Lipase

Change in pressure of $scCO_2$ influences its density-dependent physical properties such as partition coefficient, dielectric constant and Hildebrandt solubility parameters (eqn 2.2) [550], that indirectly regulate the activity, specificity and stability of enzymes [551].

$$\delta = 1.25 P_c^{1/2} \left[\frac{\rho}{\rho_{liq}}\right] \qquad (2.2)$$

The pressure has direct and indirect effects on enzyme activity in scFs. Directly, it my lead to denaturation as a result of sudden depressurization, which influences the residual enzyme activity by unfolding of the enzyme [313,552]. The enzyme activity and selectivity are affected indirectly. Many ezymes are usually stable under high pressure [342], and pressurizing usually does not play an important role in changing enzyme activity. Hovewer, sometimes local changes may occur in the enzyme structue, causing an altered activity with a shift of the active state [277,553]. The effect of pressure on the efficiency of the reaction and enantioselectivity is indeed noteworthy. When the pressure of $scCO_2$ was changed, the density of $scCO_2$ does change, as given by Hildbrand solubility parameter equation (eqn 2.2) [286]. Pressure is also likely to have an indirect affect on the rate of the reaction [313], by changing the concentrations of reactants and products in solution because the partitioning of reaction components between the two phases depends on pressure [554]. Large changes in the scF

density as a result of increasing pressure altered the interaction between CO_2 and the enzyme to progressively affect the enzyme conformation and, therefore, the enantioselectivity of the reaction [286].

2.5.3.2. The effect of temperature on Lipase

Temperature is one of the most important reaction parameter, which influences enzyme activity much more than pressure [313].Temperature significantly affects enzyme catalysis in $scCO_2$. This effect is strongly related to the enzyme activity, fluid density and stability and to the CO_2 solvating power [555]. In scCO2 the increase in temperature increases the solubility and decreases the density. The lower density and viscosity of the $scCO_2$ results in an increase in the mass transfer rate of the substrates and products. The reactants also easily surpass the activation energy barrier of the transition state at high temperature [556], consequently an in reaction rates is observed [557,558]. Beyond a very high temperature, the conversion decreased slightly probably caused by the vibration and movement of the enzyme molecule, which would affect the hydrogen bonds and other bonds in the lipase structure. Hence, the enzyme molecule will unfold and alter its tertiary and quaternary structure (three-dimensional conformation). Consequently the enzyme is denatured and results in the reduction of catalytic power [559]. Denaturation also affects the differential partition of substrates between the vapour and the liquid phase in contact with the enzyme phase [560].

Chapter III. Enantioselectivity and Mechanistic Kinetics Modeling

A mathematical model is neither a hypothesis nor a theory.
Unlike scientific hypotheses,
a model is not verifiable directly by an experiment.
For all models are both true and false…
The validation of a model is not that it is "true"
but that it generates good testable hypotheses relevant to important problems. "
(R. Levins, Am. Scientist.54:421-31, 1966)

3. Enantioselectivity and Mechanistic Kinetics Modeling

In this chapter, the concept of enantioselectivity, depending on the racemic reaction kinetics, is reviewed and an enantiomeric ratio (E-value) for two components case (bi-bi ordered reaction) is newly suggested. In addition to enantioselectivity concept, a general mechanistic kinetic model for the enzyme catalyzed enantiomeric reaction is postulated. In the following chapters, a specific transesterification reaction is considered as a case study to check the validity of this mechanistic model.

The chemical engineers usually design and built a reactor to increase capacity or selectivity at minimum cost. For multiple reactions, not only the conversion but also the selectivity (S), defined as the ratio of the desired product per all products by eqn. 3.1, is investigated and controlled to produce the desired product and its yield. In fact, the selectivity is frequently much more important than conversion because the increase in the conversion can be achieved by using a larger reactor, a lower flow rate, or a higher temperature, but poor selectivity necessarily requires consumption of more reactant for a given amount of desired product, and separation of reactants [561].

$$S_i = \frac{C_{P_{desired}}}{C_{A_o} - C_A} = \frac{Y}{X} \qquad (3.1)$$

where Y is the yield and X is the conversion. In order to understand the kinetic model, the basic rate definition can be taken into account as defined by Levenspiel [562].

For a single phase reaction below,

$$aA + bB \longrightarrow rR + sS$$

the determination of rate expression, for the design of suitable reactor in industry, is required, and defined by the following equation.

$$-r_A = -\frac{1}{V}\frac{dN_A}{dt} = \frac{(amount\ of\ A\ dissapearing)}{(volume)(time)}, \quad \left[\frac{mol}{m^3.s}\right] \qquad (3.2)$$

The rates of reaction of all materials are related by

$$-\frac{r_A}{a} = -\frac{r_B}{b} = \frac{r_R}{r} = \frac{r_S}{s} \tag{3.3}$$

This stoichiometric relation is important to calculate any immeasurable component via measurable ones. The understanding of the kinetic mechanism in a reaction is not only very important for design of a new reactor, but also to operate the system at optimum reaction conditions. Especially, for the racemic substrates, the concept of enantioselectivity plays a significant role during the enzymatic reactions, because enzyme selectively reacts more with one of the desired racemates.

3.1. Enantioselectivity

Enantiopure chiral compounds are frequently needed as building blocks in the pharmaceutical industry. To achieve such compounds, enzymes can be used for the kinetic resolution of racemates [563]. These enzymes do not always show satisfying performance in terms of activity, stability and most importantly enantioselectivity. These properties can be optimized by protein engineering techniques, by alteration of the substrates or the reaction system (medium engineering as detailed in previous chapter). The molecular basis for enantioselectivity is usually expressed as an E-value, which is the ratio of the activity towards each enantiomer[564]. Especially in the absence of structural data, predictions for how enantioselectivity will change are almost impossible [565].

The kinetic resolution is also important in the resolution of racemates. Kinetic resolution of racemic compounds is by far the most common transformation, in which the enzyme discriminates between the two enantiomers of racemic mixture, so that one enantiomer is readily transferred to the product faster than the other with a chiral entity [94,163,199, 200].

A high E-value for a given substrate-enzyme pair is crucial for the success of a kinetic resolution, since a high E-value ensures not only a high (ee), but also a high yield[566]. In 1974, the enantiomeric relation was defined for a photodecomposition of a racemate of a racemic reaction by circularly polarized light[567]. A similar equation was derived for the chemocatalytic racemic reaction[568]. Later, in 1982, Chen *et.al.* showed that this relation also holds for

enzymatic catalysis with E as the selectivity parameter[564]; thus, similar equations and parameters are used to describe enantioselective photochemical, chemical, and enzymatic catalysis.

3.1.1. E-value for uni-uni irreversible reactions

The basic enzymatic example is the irreversible conversion of a single racemic substrate, LS/DS, into a single chiral product, LP/DP, via a substrate-enzyme complex in a homogeneous batch reactor, in the absence of side reactions.

$$LS + E \xrightarrow{k_1} LP + E$$

$$DS + E \xrightarrow{k_2} DP + E$$

As the reaction rate for each single reaction is written;

$$-\frac{d[LS]}{dt} = k_1[E][LS] \quad (3.4)$$

$$-\frac{d[DS]}{dt} = k_2[E][DS] \quad (3.5)$$

The above equations were obtained, according to the definition of selectivity by eqn. 3.1 it the following form is derived;

$$S = \frac{d[LS]}{d[DS]} = \left(\frac{k_1}{k_2}\right)\frac{[LS]}{[DS]} \quad (3.6)$$

Integrating the above equation gives the result derived by Chen *et.al.*,[564].

$$\left(\frac{k_1}{k_2}\right) = E = \frac{ln\left[\frac{LS}{LS_o}\right]}{ln\left[\frac{DS}{DS_o}\right]} \quad (3.7)$$

This is the enantioselectivity value (E –value) for irreversible uni-uni racemic reactions.

3.1.2. E-value for uni-uni reversible reactions

Later, the model given in section 3.1.1., was extended to reversible reactions. If the resolution reaction is reversible, also the equilibrium constant K has to be taken into account[566]. The reaction used is a uni- uni reversible racemic reaction[569], and the scheme is as follows

$$LS + E \underset{k_2}{\overset{k_1}{\rightleftharpoons}} LP + E$$

$$DS + E \underset{k_4}{\overset{k_3}{\rightleftharpoons}} DP + E$$

Where $k_2 \neq k_4 \neq 0$ (3.8)

The rate of disappearance of LS and DS B are defined as

$$-\frac{d[LS]}{dt} = k_1[E][LS] - k_2[E][LP] \quad (3.9)$$

$$-\frac{d[DS]}{dt} = k_3[E][DS] - k_4[E][DP] \quad (3.10)$$

Again by using the definition of selectivity by eqn 3.1, and defining the concentration terms

$$\begin{cases} [LP] = [LS_0] - [LS] \\ [DP] = [DS_o] - [DS] \end{cases} \quad (3.11)$$

are obtained. From the equilibrium constans

$$K = \frac{k_2}{k_1} = \frac{k_4}{k_3} \Rightarrow k_2 = Kk_1 \Rightarrow k_4 = Kk_3 \quad (3.12)$$

and by substitution of LP and DP concentration equalities (eqn. 3.11)

$$\frac{d[LS]}{d[DS]} = \frac{k_1[LS] - Kk_1([LS_o] - [LS])}{k_3[DS] - Kk_3([DS_o] - [DS])} \quad (3.13)$$

equation (3.13) is obtained.

Rearranging this equation, the final definition of enantioselectivity is derived.

$$E = \frac{\ln\left[1 - (1+K)\left(1 - \frac{[LS]}{[LS_0]}\right)\right]}{\ln\left[1 - (1+K)\left(1 - \frac{[DS]}{[DS_0]}\right)\right]} \quad (3.14)$$

This is the equation derived to define the enantioselectivity for reversible uni-uni racemic reactions[570]. When $k_2 = k_4 = 0$, equation (3.14) reduces to homocompetitive equation (3.7) for the irreversible case[564]. These equations show that the enantioselectivity of enzyme-catalyzed synthesis depends on the complex interaction of both kinetic (E) and thermodynamic (K) constant.

When the enzyme shows no absolute enantioselectivity, optimization of the yield or enantiomeric excess (ee) of the resolution process requires kinetic knowledge of the enzyme reaction. Usually, the model of Chen *et.al.* is assumed to be valid. However, some deviations from this model were detected during the study of the lipase –catalyzed hydrolysis in which the determination of E-value according to Chen and coworkers[564,569] may not be conclusive[571]. Thus, a formal analysis of the kinetic studies of complicated enzymatic resolution processes were suggested by Staathof *et.al.*[572-575].

3.1.3. E-value for two component (Bi-Bi) reactions

The kinetics of the two pairs of reactions was simplified to irreversible first-order kinetics, when the reversible reaction does not exit. For the bi-bi reactions, such a simplification is not possible because equilibria are involved; thus, each substrate or product will influence the reaction rate. Therefore, the analysis of such kinetics for resolution reactions[573] was extended to a kinetic model for the tandem resolution reaction [574].

On kinetic grounds, different mechanism for bi-bi reactions can be suggested, *e.g.* the ping-pong mechanism. The type of mechanisms also leads to different relations for the ratio of reaction rates of enantiomers in a racemic mixture. Therefore, at a certain degree of conversion, the enantiomeric excess may depend on the mechanism, and consequently on the kinetic parameters involved [572].

Equation (3.14) [569] is not recommended in any of the bi-bi cases. When the wrong model is used, an appearance of E-value will be found which may differ from the real value. Then incorrect conclusions may be drawn about the enantioselectivity of the enzyme.

Leaving the enzyme-complex intermediates and mechanism to be considered in the following section, a new general approach was studied in this work to analyze the two component racemic reactions (bi – bi ordered reaction), according to the general chemical reaction engineering approach for the overall reaction rate in bulk medium.

3.1.3.1. **E-value for irreversible Mutli component reactions**

In this reaction type, it is assumed that enzyme reacts with *n* different excess components (C_i) within the reactor as well as racemic solution, LS and DS, and the multi products P_i., shown as follows;

$$E + LS + C_1 + C_2 + \ldots\ldots\ldots + C_n \longrightarrow E + LP + P_1 + P_2 + \ldots\ldots\ldots + P_n$$

$$E + DS + C_1 + C_2 + \ldots\ldots\ldots + C_n \longrightarrow E + DP + P_1 + P_2 + \ldots\ldots\ldots + P_n$$

The overall rate expression for such reaction is written as

$$\begin{cases} -\frac{d[LS]}{dt} = k_1[LS][E]\prod_{i=1}^{n}[C_i] \\ -\frac{d[DS]}{dt} = k_2[DS][E]\prod_{i=1}^{n}[C_i] \end{cases} \tag{3.15}$$

Because the total enzyme concentration [E] during the reaction remains constant, it may not be considered in the ratio of the rate expression, in equation (3.16).

$$\frac{d[LS]}{d[DS]} = \frac{k_1[LS]\prod_{i=1}^{n}[C_i]_i}{k_2[DS]\prod_{i=1}^{n}[C_i]} \tag{3.16}$$

Since $\prod_{i=1}^{n} C_i$ term cancels each other for the calculation of E- value, the equation (3.16) reduces to equation (3.7) in all cases. In this work, it was demonstrated that the equation suggested by Chen *et.al.* can be applicable for any type of overall enzyme reaction if it is irreversible, where it can also be said that K_{eq} does not exist.

3.1.3.2. **E-value for reversible two component (bi-bi) reactions**

If overall reaction is reversible as shown in the flowing scheme, the overall consumption of excess component (C) (*e.g.* acyl donor for transesterification) is dependent on both of the

$$E + LS + C \underset{k_2}{\overset{k_1}{\rightleftharpoons}} E + LP + P$$

$$E + DS + C \underset{k_4}{\overset{k_3}{\rightleftharpoons}} E + DP + P$$

racemates, and as one of the racemates used fast it is indirectly diluted in the slow reacting racemate in the time course of reaction. The excess component C and product P are defined as;

$$[LP] = [LS]_o - [LS] \tag{3.17}$$

$$[DP] = [DS]_o - [DS] \tag{3.18}$$

$$[P] = [LP] + [DP] \tag{3.19}$$

$$[C] = [C]_o - [LP] - [DP] \tag{3.20}$$

in such kind of reactions. By writing the overall rate expression for each racemates, the following terms are obtained.

$$\begin{cases} -\frac{d[LS]}{dt} = k_1[LS][C][E] - k_2[LP][P][E] \\ -\frac{d[DS]}{dt} = k_3[DS][C][E] - k_4[DP][P][E] \end{cases} \tag{3.21}$$

Since the overall enzyme concentration at any time within the reaction course is to be constant, they cancel each other in equation (3.21) and it reduces to

$$\frac{d[LS]}{d[DS]} = \frac{k_1[LS][C] - k_2[LP][P]}{k_3[DS][C] - k_4[DP][P]} \tag{3.22}$$

Substituting the concentration values in equations (3.17 to 3.20) into equation (3.22) in a Maple worksheet and arranging this expression (Appendix 2), the general definition for E-value is derived as;

$$\frac{d[LS]}{d[DS]} = E\,\frac{(\alpha_1[LS]^2+\alpha_2[LS]+\alpha_1[LS][DS]+\alpha_3[DS]-\alpha_4)}{(\alpha_1[DS]^2+\alpha_5[DS]+\alpha_1[LS][DS]+\alpha_6[LS]-\alpha_7)} \tag{3.23}$$

Where the constants α values are given on the Table 3.1.

Table 3.1. Kinetic constat for E-value

$\alpha_1 = (1-K)$
$\alpha_2 = C_o - 2K[LS_o] - [DS_o] - [LS_o] + K[DS_o]$
$\alpha_3 = K[LS_o]$
$\alpha_4 = K[LS_o]^2 + K[LS_o][DS_o]$
$\alpha_5 = C_o + 2K[DS_o] - [DS_o] - [LS_o] + K[LS_o]$
$\alpha_6 = K[DS_o]$
$\alpha_7 = K[DS_o]^2 + K[DS_o][LS_o]$

When equilibrium constant K (defined in equation 3.12) was equated to zero in Maple worksheet, this general E-value given by equation (3.23) reduces to the simple irreversible case,

$$\frac{\mathrm{d[LS]}}{\mathrm{d[DS]}} = \mathrm{E}\,\frac{\mathrm{[LS]}}{\mathrm{[DS]}} \quad , \tag{3.24}$$

which is same as the E-value suggested by Chen *et.al.*[564].

When the general E-value equation, suggested in this work, was mathematicallay analyzed, it is thoroughly seen that numerator and denominator is a function of both enantiomers. In this case, this general differential equation becomes a close function, whose solution has not been achieved by the known simpe calculus tools. It seems to be first order Abel types of differential equation. The general form of the first order Abel's second kind non-linear differential equation is defined in mathematics as [576-578].

$$\frac{du}{dx} = \frac{f_2(x)u^2+f_1(x)u+f_o(x)}{g_1(x)u+g_o(x)} \tag{3.25}$$

Hence, the equation (3.23) is a non-linear differential equation, which is said to be Abel's differential equation of the second kind, whose solution can be considered as in the field of applied mathematics.

In order to determine the E values, either molecular basis or mechanistic model is studied. The molecular acknowledgment of how the enzyme behaves selectively was studied by several scientists. Tomic *et.al.* studied the molecular enantioselectivity for *Burkholderia cepacia* lipase (BCL) at the active site of this enzyme, and they have defined which amino acids are responsible for the selectivity [579-582]. In addition to the molecular basis, the E values must be the result of changes in the structures of the transition state intermediates of the elementary steps [575]. In the following section, a general mechanistic model is postulated for the enantiomeric kinetic resolution of racemic reactions. The E-value is surely the ratio of the rates on the left and right loops of the scheme suggested.

3.2. A General Kinetic Model for the Enantiomeric Reactions

Esters are one of the most important natural flavor fragrances traditionally extracted from plant materials or direct biosynthesis by fermentation[583]. Many compounds which are very difficult to obtain can be synthesized by the transesterification reaction easily[584]. Chemical conversion of the oil to its corresponding fatty ester is called transesterification, which is the process of exchanging the alkoxy group of an ester compound by another alcohol (Fig. 2.4.) [585]. This process is an important one in conventional and modern industry as well as scientific study, especially in modern fine chemical process. Although numerous studies on transesterification of primary and secondary alcohols by lipases have been published[586-589], however there are actually few papers dealing with the kinetics of reactions[590-593].

Commercial implementation of lipase-catalyzed processes requires a determination of the rate expressions that describe the performance of the reactor in terms of the major factors that control the reaction rate (e.g., temperature, enzyme loading, concentrations of substrates, etc.)[594]. This actually provides information in terms of reaction kinetics and parameters. By the thorough investigation of the kinetic mechanism involved, the determination of the corresponding parameters becomes a key step in process development[590].

The most investigations of the kinetics of lipase mediated reactions have focused on model systems that contain only a few different chemical species and/or enzyme complexes that lump several species into an appropriate representative surrogate. Several different mechanisms have been proposed in the scientific literature to explain lipase-catalyzed reactions like[595-599].

- irversible consecutive pseudo first-order reactions
- a generalized Michaelis-Menten mechanism
- a uni-bi mechanism
- an ordered bi-bi mechanism

Although above mentioned cases have been investigated for the specific reaction types., a general kinetic study taking into account the reversibility and enantioselectivity of the reaction at the same time has not been presented. Berendsen *et.al.*, studied a detailed model developed for reversible ping-pong bi-bi models by taking into account both reversibility and competitive inhibition[590], and Xiong *et.al.*, studied one cycle of the reaction but not both reaction simultaneously[207]. However, these models are specific for the studied reactions.

Thus, a most general model for the enantioselective enzyme reactions has been developed in this work, where it is the both enantiomers are effective simultaneously with the system. Before explaining the details of how to build the model, the methods of the kinetic analysis should be overlooked briefly by three different methods whouse details are given in the relative reference [600].

a) Derivation by determinants;

   The most rigorous method is to solve a series of simultaneous linear equations by determinants or by repetitive and judicial substitution of one equation into another.

b) King Altman Method [32] ;

   It is operationally a graphical method, although it is based on the determinant method. In order to implement this method, the polygonal form of the chemical mechanism is drawn. All of the subforms of this figure in which all of the enzyme forms are connected, but which contain no closed polygons, are taken into account.

c) Derivation by inspection,

   This is the most rapid and the most easily implemented method. It can be described rather briefly “net-rate-constant” method. In this method, the steady-state approximation specifies that the rates of all steps are equal to each other, and the rate is also equal to the initial velocity.

3.2.1. Construction of a General Model for an Enantioselective Reaction

The objective of this work is to develop a general mechanistic model for the two substrate (bi-bi ordered) reaction sequence treating both enantiomers as competing substrates. This new mathematical model is proposed to describe the enantioselective biocatalysis. The kinetic resolution of chiral reactions is a popular method, which represents conversion of primarily one of the two enantiomers of a racemic substrate, yielding either the substrate or the product in high enantiomeric purity. The unknown kinetic parameters can be estimated by nonlinear regression analysis from the experimental observations of selected experiments in any specific reaction types. In this work, the transesterification of isopropylidene glycerol (IPG) has been taken into account as a case- study for the verification of the model proposed. The combination of steady-state enzyme kinetics and mathematical modeling is frequently faced with data and the set of reasonable mathematical models[600].

The kinetic mechanism of an enantioselective reaction is a ping-pong bi–bi pattern[593,601]. A kinetic model based on reversible ping-pong bi–bi mechanism, taking into account the competition of both substrate enantiomers for the active site, was applied to describe the kinetic behavior of such kind of reactions, e.g., lipase catalyzed transesterification.

The following assumption were taken into account in this model[524, 600, 602],

- Even sized enzyme particles are uniformly distributed inside the medium
- Enzyme deactivation is not taken into account.
- The concentrations of all enzyme forms are constant during the measurement.
- The inhibition of products was negligible.
- Mass transfer resistance is negligible.
- All other reaction conditions are constant during measurement.

The spontaneous enantioselective reaction below was set as a parallel reaction for the following King-Altman scheme.

$$E + LS + C \underset{k_2}{\overset{k_1}{\rightleftharpoons}} E + LP + P$$

$$E + DS + C \underset{k_4}{\overset{k_3}{\rightleftharpoons}} E + DP + P$$

Derivation of rate equations for complex enzymatic reactions is a tedious task because of requirements of manipulation with massive algebraic expressions. A number of methods were proposed to derive rate equations for such enzymatic systems. The well known and commonly used one is King Altman method which mainly depends on schematic representation of the reactions[32]. But in order to apply this method to state the rate equations for the systems, it requires the system to be a closed one. Modern techniques of computer algebra systems perform the symbolic computations to be tackled with previously insoluble problems[603].

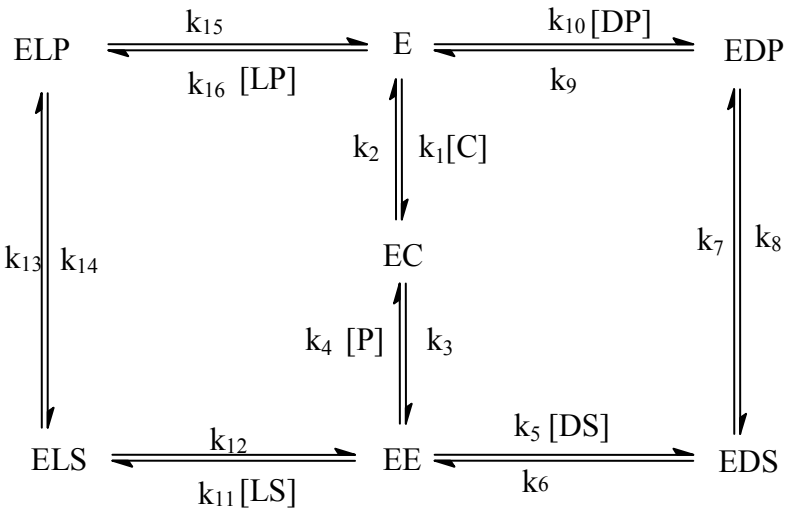


Fig.3.1. Mechanism of the enantioselective reaction (King – Altman scheme)

In this model enzyme E first reacts with the excess component C and the by-product P is produced. In trans/esterification reactions the excess component is usually the acyl donor component and the enzyme-acyl complex EC is formed. Then, the enzyme complex EC surrogates to EE complex by relaxation reaction. In addition, for the kinetic resolution of a racemic mixture, the competition of both enantiomers for the same active site should be considered. The relaxed EE complex is the key component to select any one of the racemates, which is more suitable to the active site of the enzyme. The both racemates compete to bind the active site of the EE complex. Consequently, two parallel pathways exist for the decomposition of the acyl-enzyme intermediate. When DS combines to EE, the right side of the loop on model is functional, and the same is valid for the LS component on the left loop of model. Depending on which component is combined to enzyme, EDS and ELS complexes formed and they soon get the relaxation reactions to form EDP and ELP complexes, respectively. In the final step, the enzyme releases the product DP and LP and becomes free in the reaction medium to react with next excess component.

A general kinetic resolution has been derived for this model, where all steps are considered as reversible. The benefit of this model is that the model can later be adapted any type of enantioselective reaction whose intermediates will be determined by their own chemical mechanism. Any corresponding mechanism is then obtained by equating the reversible k values to zero value. With the above mechanism and assumptions, the model equations can be written according to the each reaction path given as follows:

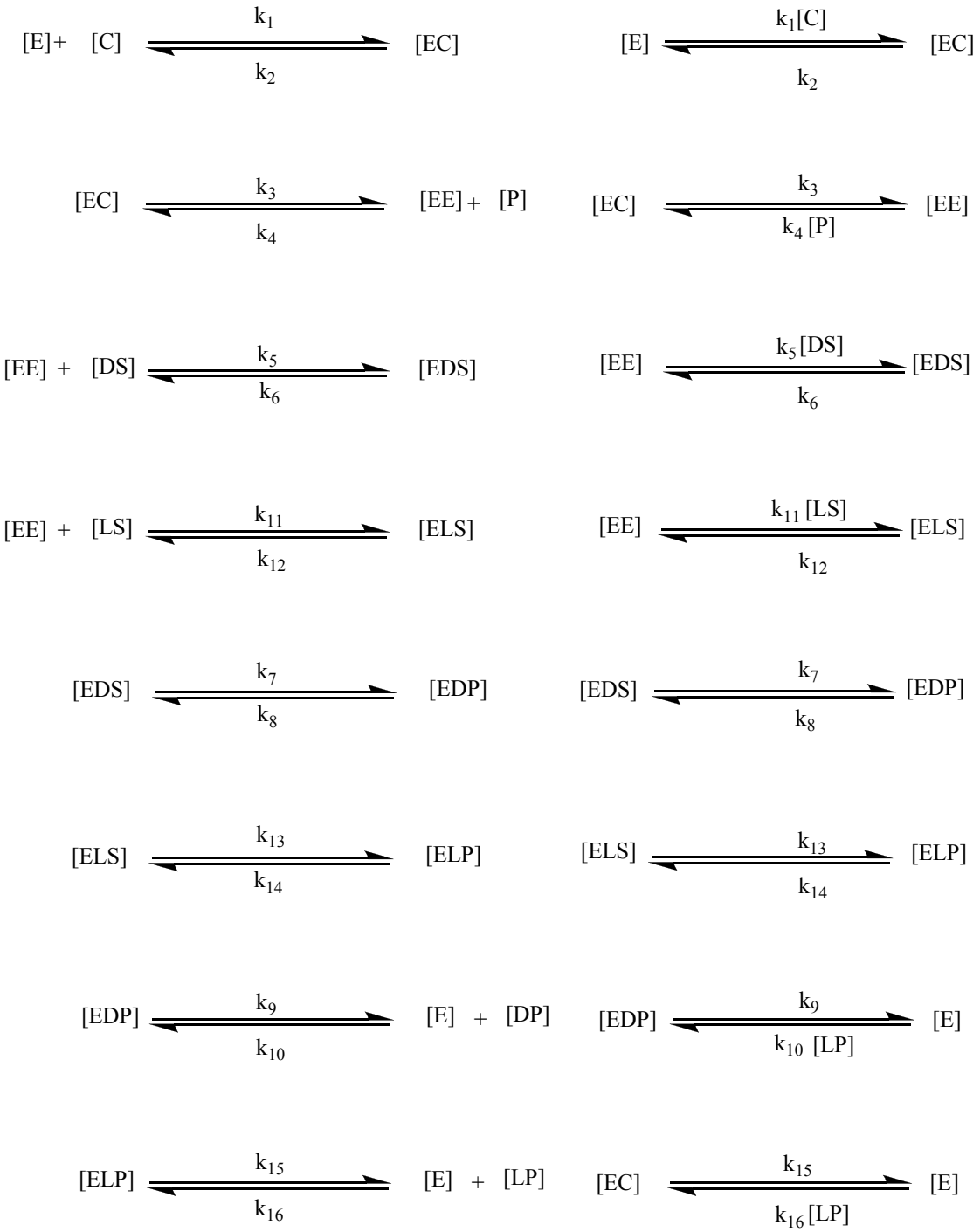


Fig . 3.2. Each reaction path included in the proposed mechanism

Equating the relative k values, it is possible to determine the appropriate kinetic mechanism by reducing the very complex non-Michaelis Menten kinetics. The rate of formation (υ_i) of each intermediate is expressed according to the Table 3.2. follows;

Table 3. 2.: the rate of formation (υ_i) of each intermediate in the general mechanistic model

$$\upsilon_1 = \frac{d[E]}{dt} = -\mathrm{k_1[C][E] + k_2[EC] + k_9[EDP] - k_{10}[E][DP] + k_{15}[ELP] - k_{16}[E][LP]} = 0 \quad (3.26)$$

$$\upsilon_2 = \frac{d[EC]}{dt} = k_1[E][C] - k_2[EC] - k_3[EC] - k_4[EE][P] = 0 \quad (3.27)$$

$$\upsilon_3 = \frac{d[EE]}{dt} = k_3[EC] - k_4[EE][P] - k_5[EE][DS] + k_6[EDS] - k_{11}[EE][LS] + k_{12}[ELS] = 0 \quad (3.28)$$

$$\upsilon_4 = \frac{d[EDS]}{dt} = k_5[EE][DS] - k_6[EDS] - k_7[EDS] + k_8[EDP] = 0 \quad (3.28)$$

$$\upsilon_5 = \frac{d[ELS]}{dt} = k_{11}[EE][LS] - k_{12}[ELS] - k_{13}[ELS] + k_{14}[ELP] = 0 \quad (3.29)$$

$$\upsilon_6 = \frac{d[EDP]}{dt} = k_7[EDS] - k_8[EDP] - k_9[EDP] + k_{10}[E][DP] = 0 \quad (3.30)$$

$$\upsilon_7 = \frac{d[ELP]}{dt} = k_{13}[ELS] - k_{14}[ELP] - k_{15}[ELP] + k_{16}[E][LP] = 0 \quad (3.31)$$

$$[E]_{tot} = [E] + [EC] + [EE] + [EDS] + [ELS] + [EDP] + [ELP] \quad (3.32)$$

$$\frac{d[C]}{dt} = 0$$

And the rates of formation of DP and LP are;

$$\upsilon_{DP} = -\frac{d[DS]}{dt} = \frac{d[DP]}{dt} = k_9[EDP] - k_{10}[E][DP] \quad (3.33)$$

$$\upsilon_{LP} = -\frac{d[LS]}{dt} = \frac{d[LP]}{dt} = k_{15}[ELP] - k_{16}[E][LP] \quad (3.34)$$

If it is required to solve these equations by the determinant model, the relative matrix form[604] of these equations is represented on Fig. 3.3.,

$$\frac{\mathrm{d}}{\mathrm{dt}}\begin{bmatrix}[E]\\[EC]\\[EE]\\[EDS]\\[ELS]\\[EDP]\\[ELP]\end{bmatrix} = \begin{bmatrix} -(\mathrm{k_1[C] + k_{10}[DP] + k_{16}[LP]}) & \mathrm{k_2} & 0 & 0 & 0 & \mathrm{k_9} & \mathrm{k_{15}} \\ \mathrm{k_1[C]} & -(\mathrm{k_2 + k_3}) & -\mathrm{k_4[P]} & 0 & 0 & 0 & 0 \\ 0 & \mathrm{k_3} & -(\mathrm{k_4[P] + k_5[DS] + k_{11}[LS]}) & \mathrm{k_6} & \mathrm{k_{12}} & 0 & 0 \\ 0 & 0 & \mathrm{k_5[DS]} & -(\mathrm{k_6 + k_7}) & 0 & \mathrm{k_8} & 0 \\ 0 & 0 & \mathrm{k_{11}[LS]} & 0 & -(\mathrm{k_{12} + k_{13}}) & 0 & \mathrm{k_{14}} \\ \mathrm{k_{10}[DP]} & 0 & 0 & \mathrm{k_7} & 0 & -(\mathrm{k_8 + k_9}) & 0 \\ \mathrm{k_{16}[LP]} & 0 & 0 & 0 & \mathrm{k_{13}} & 0 & -(\mathrm{k_{14} + k_{15}}) \end{bmatrix} \cdot \begin{bmatrix}[E]\\[EC]\\[EE]\\[EDS]\\[ELS]\\[EDP]\\[ELP]\end{bmatrix} = \begin{bmatrix}0\\0\\0\\0\\0\\0\\0\end{bmatrix}$$

Fig. 3.3.: Martix form of rate of each intermadiates.

The net result is that the corresponding network of equations contains so many parameters that meaningful numerical solutions of these differential equations can be very difficult to obtain. Therefore, the number of variables and the number of equations should be equal for the model to be solved.

By steady-state approach to calculate the intermediates, the each rate (velocity) of the intermediates is equated to zero. There are 16 parameters of k_1 to k_{16}, and 13 variables which are: [E], [EE], [EC], [EDS], [EDP], [DP], [DS], [LS], [LP], [ELP], [ELP], [P] and [C]. These variables have 8 equations (eqns. 3.26 to 3.32) given on Table 3.2.

Since [P] and [C] are dependent on [LS] and [DS],

$$[C] = [C_0] - [DP] - [LS] \tag{3.35}$$

$$[P] = [DP] + [LP] \tag{3.36}$$

The equations (3.35 & 3.36) reduce the number of variables by 2 and 13 – 2 = 11 variables left. At any time, the total enzyme concentration $[E]_{tot}$, total [LS] and total [DS] remain constant over time course of the reaction.

$$[DS_o] = [DS] + [EDS] + [EDP] \tag{3.37}$$

$$[LS_o] = [LS] + [ELS] + [ELP] \tag{3.38}$$

As a result, three more variables are reduced and 11 – 3 = 8 variables left.

It is assumed that all enzyme bound complexes are at steady states, which means all derivatives involving enzyme species are set to zero. By this way, the six more variables [EE], [EC], [EDS], [EDP], [ELP] and [ELP] are reduced to result in two variables (8-6=2 variables left). The rest two variables are the rate of formations of DP and LP defined in equations (eqns. 3.33 and 3.34). Accordingly, the model equation consequently becomes soluble. A detailed derivation of the rate expression is given on Appendix 3, and it is verified by Maple software (Appendix 4). The final non - Michaelis Menten form of rate expression was derived by the pseudo-steady-state approximation of rate of each enzyme bound intermediates given in equations (Table 3.2). These kinetic equations were solved with use of MAPLE software.

The rate controlling step has been considered where the production of DP and LP takes place. After defining the intermediates and total E in terms of measurable quantities and substituting into rate equations ;

$$\frac{d[DP]}{dt} = k_9[EDP] - k_{10}[E][DP] \quad (3.33)$$

$$\frac{d[LP]}{dt} = k_{15}[ELP] - k_{16}[E][LP] \quad (3.34)$$

The general rate expression of mechanistic model for the enantioselective racemic reactions have been derived as;

$$\frac{d[DP]}{dt} = \frac{E_o(\Delta_1[DS]-\Delta_2[DP])(\Theta_4+\Theta_5[DS]+\Theta_6[LS])}{(\Omega_1+\Omega_2[LP]+\Omega_3[DP])(\Theta_4+\Theta_5[DS]+\Theta_6[LS])+(\Omega_4+\Omega_5[DS]+\Omega_6[LS])(\Theta_1+\Theta_2[DP]+\Theta_3[LP])} \quad (3.39)$$

$$\frac{d[LP]}{dt} = \frac{E_o(\Delta_3[LS]-\Delta_4[LP])(\Theta_4+\Theta_5[DS]+\Theta_6[LS])}{(\Omega_1+\Omega_2[LP]+\Omega_3[DP])(\Theta_4+\Theta_5[DS]+\Theta_6[LS])+(\Omega_4+\Omega_5[DS]+\Omega_6[LS])(\Theta_1+\Theta_2[DP]+\Theta_3[LP])} \quad (3.40)$$

Where the constants Δ_i, Ω_i, Θ_i are combinations of rate contants k given in Appendix 3 and 4.

Since the both racemates are effective in the bulk of reactions, the rate expression of DS and LS cannot be considered separately. In order to make data fitting and to evaluate the kinetic parameters, these two equations must be solved simultaneously, as a system of differential equations.

Mathematical modeling of biological systems provides better understanding of the biological complexity. Such models of biochemical reaction networks have large number of variables and parameters involving nonlinear differential equations with a number of parameters whose values are not known generally. Analysis of these networks includes both analytical and numerical work[604]. Evaluation of the kinetic parameters is a central point in enzyme research, but the graphical methods, commonly suggested to determine these parameters, have some

limitations because of the massive algebraic expressions that may become challenging due to the inherent nonlinearities and unknown parameters in the model. Hence the constants can be evaluated using data sets of substrate concentration as a function of time[605]. Computer algebra systems (CASs), which are also capable of performing calculations with symbols and computer codes for numerical computations, are excellent tools in such analysis[606].

3.3. Enantioselectivity based on Mechanistic model

After postulating a general rate expression for each enantiomers, it could be possible to work on a general enantiomeric ratio, E value, and suggest a general equation based on mechanistic model. Since selectivity is the ratio of both rates of racemic components (eqn 3.39 and 3.40), taking this ratio it is shown that;

$$E = \frac{(1+K_{LS})}{(1+K_{DS})} \frac{\ln((1+K_{DS})\frac{DS}{DS_o}-K_{DS})}{\ln((1+K_{LS})\frac{LS}{LS_0}-K_{LS})} \qquad (3.41)$$

where the detailed derivation of above equation and details of the constants $K_{eq,DS}$ and $K_{eq,LS}$ are given on Appendix V.

Later this equation (3.41) has been studied for the possible cases.

a) If any reverse reaction of the D-racemate is inhibited ($K_{eq,DS}$=0), and L-racemate stays reversible, then E value becomes as;

$$E = \frac{(1+K_{eq,LS})\ln(\frac{DS}{DS_o})}{\ln((1+K_{eq,LS}))\frac{LS}{LS_0}-K_{eq,LS})} \qquad (3.42)$$

b) If any reverse reaction of the L-racemate is inhibited ($K_{eq,LS}$=0), and D-racemate stays reversible, then E value becomes as;

$$E = \frac{\ln((1+K_{DS})\frac{DS}{DS_o}-K_{DS})}{(1+K_{DS})\ln(\frac{LS}{LS_0})} \qquad (3.43)$$

c) If both enantiomers are irreversible (this is the usual case, since enzyme react in the same way with both enantiomers,

$$\frac{d[DS]}{d[LS]} = \frac{k_5}{k_{11}}\frac{DS}{LS} \qquad \frac{d[DS]}{d[LS]} = E\frac{DS}{LS} \qquad (3.44)$$

The case c (discussed in section 3.1.1 and 3.1.2.) and equation 3.43, turns to be in the form of irreversible reactions. The result is the same as E value suggested by Chen& Sih[564]. Comapring the equation 3.43 with the Chen & Sih definition,

$$E = \frac{k_5}{k_{11}} \tag{3.45}$$

Enantioselectivity ratio becomes the ratio of rate constant of active enzyme (EE) for DS and LS components. These rate constants are the ones where the racemic substrates compete to react with the active enzyme.

Chapter IV. Materials und Methods

4.1. Experimental Setup for reactions in *n*-Hexane (ref: [303])

4.1.1. The transesterification of IPG with Vinyl acetate in *n*-Hexane

In this study, the *Burkholderia cepacia* lipase (BCL) catalyzed reaction of isopropylidene glycerol (IPG) with vinyl acetate, as acyl donor[153,607], has been examined in *n*-Hexane. The chemicals used in this work were listed at Appendix VI. This specific reaction was considered as a case study for the general mechanistic model of transesterification reactions, constructed in the previous section. The experiments at different temperatures have been taken place with 10 mmol racemic (IPG) (Fluka, Buchs, Switzerland) and 30 mmol vinyl acetate (Merck, Darmstadt, Germany) as excess component in a 20 ml volume of a batch bioreactor. As soon as the total volume was completed to 10 ml by adding *n*-Hexane (Fluka, Buchs, Switzerland), 50 mg of lipase (Amano, Nagoya,Japan) was consequently suspended in this conventional medium to start the reaction. Three different set of data were taken for the curve fitting and to check the reliability of results calculated according to the model developed.

i. Experiments at different temperatures
ii. Experiments at different concentrations of excess compound
iii. Experiment at different enzyme concentrations

4.1.2. Reaction unit for *n* - Hexane Experiments

The reaction was carried out in a 20 ml bioreactor, which is kept constant at desired temperature with use of a water bath. The reaction medium was homogeneously agitated with a magnetic stirrer. The whole set-up was constructed in an isothermal incubator (model BE 50,Memmert, Schwabach, Germany) in order to eliminate any temperature fluctuation within the system.

4.1.3. Sample taking

At the beginning of reaction, 300 µl samples were taken with a sensitive pipette (Eppendorf) in each half an hour intervals. As the reaction tends to reach the steady state after 12 hours, the samples were taken in one hour intervals. The pipette tips were changed after each sample to eliminate any experimental error due to any possible dilution error, as a result of the previous

samples. The samples were immediately centrifuged, and the settled enzyme was removed from reaction medium to stop the reaction. Consequently, the enzyme free medium is diluted 10 times more with acetone, and analyzed with the gas chromatography (GC-14A, Shimadzu, Tokyo, Japan), whose column consists of FS-Hydrodex® ß-3P with a length of 25 m and an inside diameter of 0.25 mm (Macherey-Nagel, Düren, Germany), according to the procedure given in Appendix VIII.

4.2. Supercritical CO_2 set-up (ref: [310])

A setup for the experiments under supercritical carbon dioxide conditions, within which many experiments have been carried out, was securely constructed in Technical Chemistry Institute (TCI) by many workers , *e.g.* Bornscheuer, Tservistas, Schwabe, Hartmann[279, 308, 309, 310], respectively. A schematic High Pressure Reactor (HPR) constructed in TCI, and the one used by Tservistas is shown in Fig. 4.1.

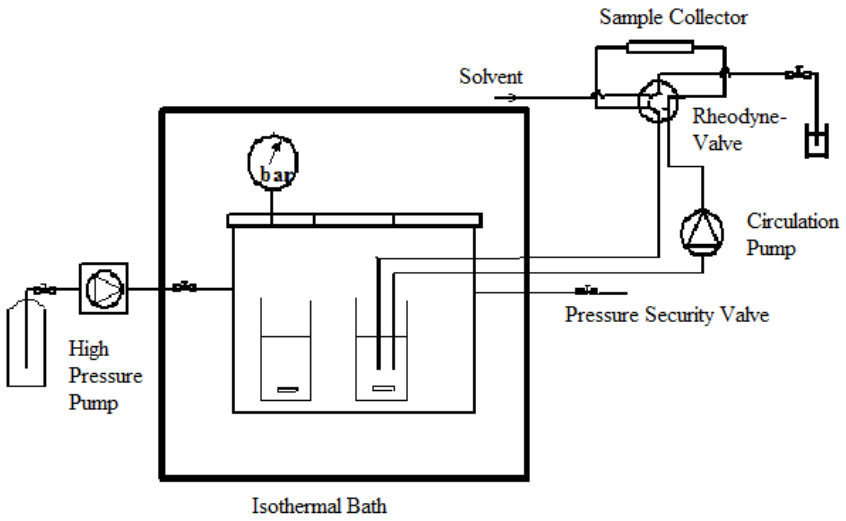


Fig. 4.1.: Experimental setup of HPR with external sample collection unit[310].

4.2.1. The construction of scCO_2 reaction unit

The each component used in constructiong a supercritical unit was listed on Appendix VII. During the construction of an experimental setup for the supercritical CO_2 experiments, a cylindrical stainless steel vessel, with 1 cm wall thickness and an inner volume of 60 ml, was constructed in the mechanical workshop of TCI. The top of the vessel was fitted with a screw cap and sealed with O-rings of Viton 500 (Otto Gehrkens GmbH, Pinneberg, Germany). A

sapphire glass window (Spindler & Hoyer, Göttingen, Germany) was integrated in the reactor cap for controlling the dissolution of reagent, visually. The sampling system was constructed from stainless steel HPLC-tubing (1/16“ OD, 10 mm ID, Knauer, Berlin, Germany), a 4-port-injektion valve with 200 µl sample-loop (Knauer, Berlin, Germany), a needle valve (Milli Mite – Hoke, Bad Vilbel, Germany), a two-way-needle-valve (SSI), two manometers (Heusinger & Salmon, Bielefeld, Germany) and a circulation pump (Verder, Haan, Germany).

The HPR had at least two steel capillary pipe connections. One of these pipes provides the inlet of $scCO_2$ and the other is connected to a needle valve to release the content of the vessel to clean the reactor after finishing the experiment.

Fig. 4.2. High pressure reactor for $scCO_2$

The different volume of the reactors was constructed in workshop of TCI, such as 19.8 ml, 60 ml of reactors. A high pressure pump (mini-pump duplex, NSI33R, Milton-Roy, Obertshausen, Germany) with cryostatic head (-12 °C) was used to compress CO_2 into the system. The pumped gas was passed through a long capillary pipe lying in a water bad, before it reaches the reaction unit. This pre-heating was done to stabilize the pressure by controlling any temperature fluctuations within the system.

The pressure was monitored by manometers (Hensinger & Salmon, Germany) and needle valves (ERC, Altegolfsheim, Germany) were used to open and close connections. The whole apparatus was set in a isothermal incubator (Memmert, Schwabach, Germany) to keep the whole system at the desired constant temperature in[608].

For the security point of view, a piece of rupture disc (bursting disc) was constructed to reactor. This disc resists up to 20 MPa and bursts immediately as soon as the system exceeds the uncontrollable maximum pressure. Although the (HPR)s run under high pressure, the setup was constructed in such a flexible way that it is quite easy to dismantle, clean and recharge for a new experiment.

4.3. Reaction in $scCO_2$

For the transesterification reaction in $scCO_2$ (grade 4.5, Linde AG), 20 mmol *rac*-isopropylideneglycerol (IPG), (Fluka, Buchs, Switzerland), was put in one side of chamber in reactor shown on Fig 3.2. 60 mmol of the excess component vinyl acetate (Merck, Darmstadt, Germany) and 100 mg lipase from *Burkholderia cepacia* (Amano, Nagoya,Japan) were put into the other chamber of the reactor (Fig. 4.3). The apparatus was then pressurized up to 10 MPa and the magnetic stirrer and the circulation pump was turned on. As the system becomes supercritical, the reagents dissolve in this homogeneous single supercritical phase. As a result, the reaction starts as soon as the components reach to enzyme distributed in the reactor.

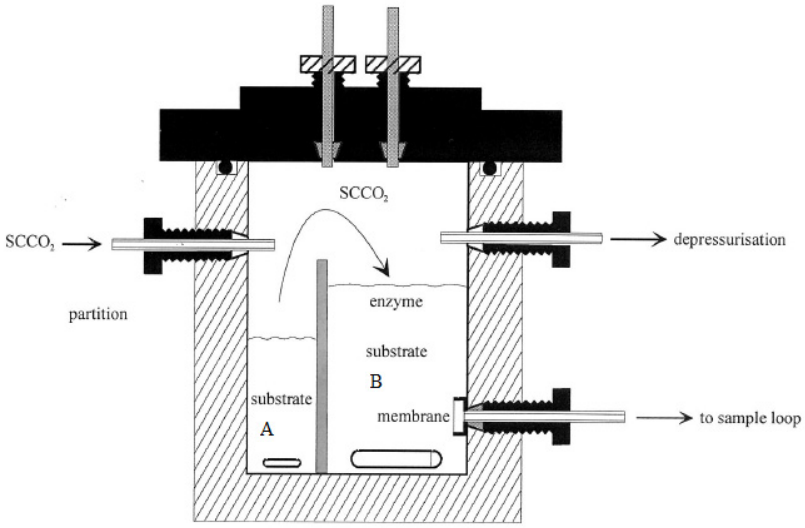


Fig. 4.3. Inside the reactor for $scCO_2$ system

The exit of the capillary pipe, connected to sample loop, was filtered with a membrane. This membrane protects any enzyme loss from the reaction volume, as well as prevents the capillary pipes from being plugged with enzyme. 100 µl of samples were taken through a sample loop, which is controlled with a needle valve. This sample was expanded into a small cup (Eppendorf, Hamburg, Germany) and rinsed with 1.5 ml acetone. The samples were analyzed by gas-chromatography (GC-14A, Shimadzu, Tokyo, Japan) using a chiral capillary-column (Hydrodex-ß3P, Macherey-Nagel, Düren, Germany) according to the gas chromatography procedure given on Appendix VIII. By these experiments, it is aimed to observe how the concentration varies with respect to time elapsed during the reaction course. These results were used during the numerical calculations to understand how the general model works for a specific reaction.

4.4. Calculation Methods

4.4.1. Goodness of fit (R^2)

Nonlinear regression changes the values of the variables to minimize the sum of squares (SS), which is the sum of the square of the vertical distances of the points from the curve.

The goodness of a fit can be quantified by the R^2 value, which is a dimensionless fraction between 0.0 and 1.0. Higher R^2 values indicate that the model fits the data better. When R^2=1.0, it means that all points lie exactly on the curve. R^2 is computed from the sum of the squares of the distances of the points from the best-fit curve determined by nonlinear regression. This sum of squares value is called SS_{reg}, which is in the units of the Y-axis squared. The results are normalized to the sum of the square of the distances of the points from a horizontal line through the mean of all Y values, called SS_{tot}.

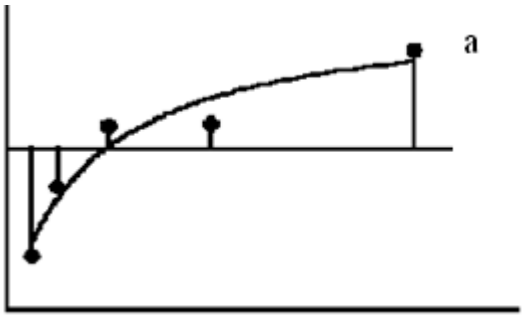


Fig 4.4. SS_{tot} , the sum of the square of the distances of the points from a horizontal line through the mean of all Y values,

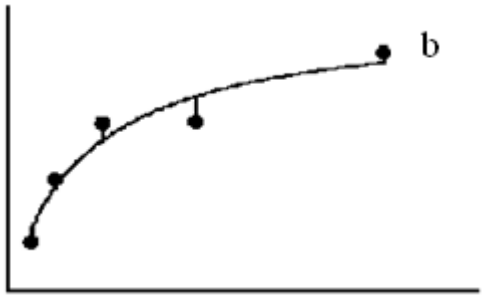


Fig 4.5. SS_{reg}, the sum of the square of the distances of the points from the fitted line by regression

R^2 is calculated using the equation $R^2 = 1 - \frac{SS_{reg}}{SS_{tot}}$ (4.1)

If SS_{reg} is larger than SS_{tot}, R^2 will be negative and the best fit curve fits the data worse than a horizontal line at the mean Y value. Thus R^2 is not actually the square of R, but only a denoion that the fraction may not be negative, since the squared values cannot have a negative value.

4.4.2. Bootstrap Method

The bootstrap method was first proposed by Efron in 1979 to study properties of various nonlinear statistics. It is a computer based procedure for estimating the accuracy of a statistical estimate derived from a set of experimental data. The method involves generation of a large number of independent resamples or bootstrap samples, each drawn from the original sample with replacement.

There are parametric and non-parametric bootstrap methods. A parametric model is fitted to the data, and samples of random numbers are drawn from this fitted model. Usually the sample drawn has the same sample size as the original data. Then the quantity, or estimate, of interest is calculated from these data.

In their book "An introduction to the bootstrap" Efron &Tibshirani described this method mathematically as follows;

Suppose that experimental data points for *n* independent variables $(x_1, x_2, \ldots., x_n)$ are observed for convenience denoted by vector **x**= $(x_1, x_2, \ldots., x_n)$ from which a statistic of interest s(**x**) can be computed. A bootstrap sample x* = $(x_1^*, x_2^*, \ldots, x_n^*)$ is obtained randomly sampling n times with replacement, from the original data point $(x_1, x_2, \ldots., x_n)$. The bootstrap algorithm begins by generating B amount of independent bootstrap samples $(\boldsymbol{x}_1^*, \boldsymbol{x}_2^*, \ldots, \boldsymbol{x}_B^*)$, each of size *n*. Corresponding to each bootstrap sample, there is a bootstrap replication of s, namely (x_b^*), with its value evaluated at (x_b^*). The bootstrap estimate of standard deviation (se) of the bootstrap replications,

The means (Φ) and standard deviation (SD) of parameters are calculated by

$$\bar{\Phi} = \frac{1}{B}\sum_{i=1}^{B} \Phi_i, \text{ where } \Phi_i = s(x_i^*) \tag{4.2}$$

$$SD(\Phi) = \sqrt{\frac{1}{B-1}\sum_{i=1}^{B}(\Phi_i - \bar{\Phi})^2} \tag{4.3}$$

As B goes to ∞, the bootstrap standard deviation approaches to population standard error value. Therefore the large number of bootstrap sampling, B, results in more trustable estimations. In this work, B=1000 has been considered during the calculations.

The bootstrap generates the values for the desired statistic. This is usually immediately followed by a histogram, which is simply the computed value of the statistic versus the subsample number. The bootstrap histogram shows the location and variation of the sampling distribution of the statistic.

Chapter V. Case study: Transesterification of isopropylidene glycerol with *Burkholderia cepacia* lipase

In this chapter, the general mechanistic model developed in Chapter 3 for the enantioselective reactions will be applied for a specific reaction; the transesterification reaction between vinyl acetate, as acyl donor, and an industrially important synthon component racemic isopropylideneglycerol (IPG) in *n*-hexane[303], and also the reaction in supercritical CO2 were considered[310].

5.1. Significance of IPG

There is an increasing demand for optically pure enantiomers in the chemical industry[609]. The chiral compounds are obviously important due to the demands of the pharmaceutical industry. More drugs are marketed as single enantiomer instead of a racemic mixture, and this process is named as "chiral switching"[610,611]. Organic acids and amino acids represent a large portion of this market, but for these useful organic compounds, only one enantiomer is known to be normally biologically active. Many researchers have attempted to separate one of optically active compounds [569, 612]. The methods (crystallization, solvent extraction, etc)[613] to separate one of the enantiomer from racemic mixture is generally time-consuming and prone to interconversion of enantiomers [614].

Chiral 1,2-*O*-isopropylideneglycerols (D)-(1) and (L)-1 and the corresponding aldehydes (L)-(2) and (D)-(2) , (IPG also called solketal), is an important chiral building synthon for the synthesis of many optically active compounds[615], such as glycerophospholipids[616,617], *β*-adrenoceptor antagonists[618], PAF (Platelet Aggregating Factor)[619,620], aryloxypropanolamines, prostaglandins [621], and leucotrienes used in the treatment of epilepsy and hypertension[622-624].

The chemical synthesis of IPG costs much for industrial applications since it requires chiral starting materials, multistep reactions, and expensive separation procedures[625]. Despite the simplicity of the reactions, the costs of production are high, so research has focused on developing biotechnological methods which allow the production of enantiomerically enriched chiral synthons with high yields and low costs[626]. Biological methods to obtain enantiomerically pure (s)-1,2-0- isopropylideneglycerol have thus received increasing attention[627,628].

The use of commercial lipases was shown to be moderately enantioselective for the esterification of IPG esters[629-631]. Different methods have been reported to enhance the enantioselectivity of lipase catalyzed reactions[257].

In present work, the transesterification of isopropylidene-glycerol (D,L – IPG) with vinyl acetate as an acyl donor[632-634] under *B.Cepacia* lipase catalysis in *n*-hexane[280,300,310] has been examined (Fig.5.1). In order to apply the general model to this specific reaction, the molecular basis for lipase catalyzed enantioselective transesterification can guide the rational improvement and tailoring of catalyst performance. By combining approaches from chemistry and biology, much information is revealed about the most important parameters controlling lipase enantioselectivity for organic synthesis[148].

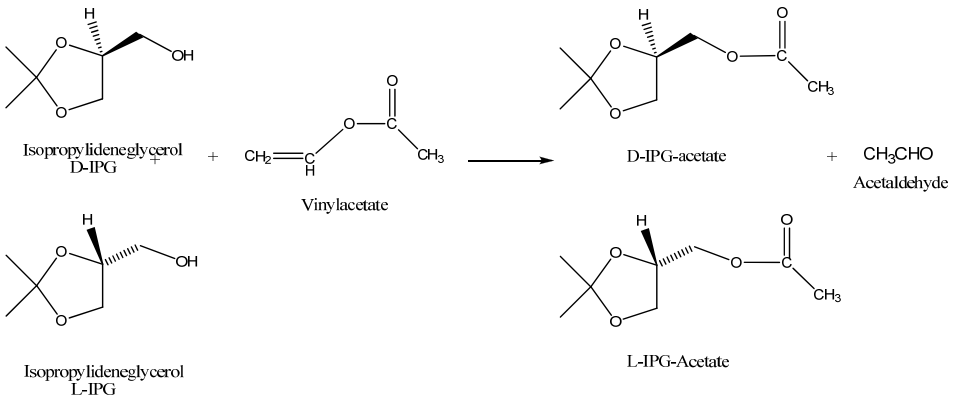


Fig.5.1. Reaction of isopropylideneglycerol with vinylacetate as acyl donor

5.2. Active site of *Burkholderia cepacia* lipase

The molecular-mechanics based modeling of substrate pathways toward reaction sites remains a difficult and time consuming task[635]. The methods used to investigate the molecular factors of enantioselectivity generally combine kinetic results and molecular modelling[636]. Enantioselectivity is mainly governed by the existence of appropriate fitting between the enzyme and each enantiomer, this being responsible for the preferred kinetic transformation of one of them[637].

Most lipases consist of a mobile element at the surface, a lid, which covers the active site [638]. The lid is opening at a hydrophobic interface, making the active site accessible for substrates and enhancing the activity of the lipase[87]. Because the exterior of the lid is hydrophilic and its interior is hydrophobic, the hydrophobic surface of lipases increases upon lid opening [639].

All lipases have a similar architecture, the α/β hydrolase fold[640-643]. Viewing the catalytic triad Asp264–His286–Ser87, the binding site of BCL has been found to consist of three subsites that are responsible for the hydrolysis reaction of the substrates by the enzyme[642, 644, 645].

i) The large hydrophobic pocket includes the catalytic triad formed by the residues Ser87, His286, and Asp264, where the acyl chain binds. This pocket is flanked by residues Val266 and Val267 on the left, Leu167 on the right, Phe119 at the top, and Pro113 in the middle, shown as green space fill above the catalytic triad on fig A1. In size 7X6.6×4.4 A°,

ii) The medium sized pocket is the oxyanion hole formed by Gln88 (yellow space fill on Fig A1) and Leu17 (red spac efill on Fig A1). This pocket stabilizes the negative charge present on one of the oxygens of the tetrahedral intermediates by hydrogen bonding. The nucleophile is placed here and is adjacent to the catalytic His286 and Leu287. In size 1.8x1.8×1.5A

iii) The alternate hydrophobic pocket to the right of the medium pocket, which can also bind parts of the nucleophile. This alternate hydrophobic pocket lies below the catalytic triad in a narrow region between Ile290, Leu287, Thr18, and Tyr29. the alternate hydrophobic pocket (dark blue space fill on Fig A1). it is estimated in size 2A° wide and 1.9A°

BCL is a globular enzyme with approximate dimensions of 30Åx40Åx50Å and its structure may be divided into one large and two smaller domains[646]. The active site of BCL is located at the bottom of a narrow 17 °A deep pocket[647,648].

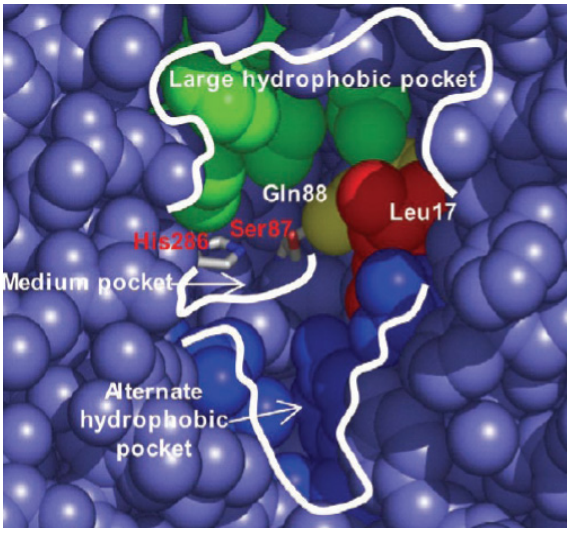


Fig. 5.2. BCL active-site structure from X-ray crystallography [ref: 645]

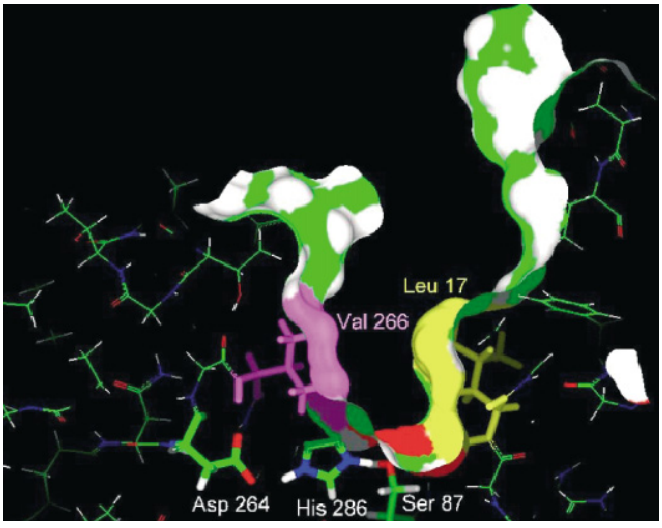


Fig 5.3. Perpendicular cross section of BCL cavity (at level Ser87) showing the funnel shape of the access path (Ser87 at the bottom and His286, Asp264 at the left).[ref: 648]

5.3. Molecular Modeling of Transition State Analogues of IPG bound to BCL

Transesterification is the result of a two-step mechanism with an acylation leading to the formation of an acyl-enzyme intermediate, which is then followed by a deacylation step[636].

In this work, the King–Altman scheme for the model was proposed and drawn as given on Fig. 5.4[32]. In the model, the first chemical step is that the acyl donor attacks to the hydroxyl group of serine in the active site, forming a tetrahedral intermediate. Collapse of this tetrahedral intermediate releases the alcohol[649]. In this specific reaction considered for the verification of the model, the acyl donor is the vinyl acetate (denoted as C on the scheme), which covalently bonds to the free enzyme (E). The excess component vinyl acetate (C) is a potential and practical acetylating reagent for the optical resolution of enantiomers in the racemate by lipase catalyzed transesterification in organic solvent. It irreversibly reacts with the active serine (Ser) residue in lipase to give the acyl-enzyme intermediate (corresponds to EC on the scheme, Fig 5.4)[650]. Acyl –enzyme complex is an irreversible step in the mechanism[651], and these complexes are crucial intermediates in all lipase catalyzed reactions[652]. As vinyl acetate (C) attacks to –OH group of serine, the H atom binds to carbon atom of –C=O part of the acetate and double bound cleaves towards the O atom. As a result of this reaction, vinyl alcohol formed as a by-product undergoes keto–enol tautomerization yielding the corresponding carbonyl compound (acetaldehyde, denoted as P on the scheme)[653-655]. This irreversibility is considered to accelerate the reaction rate. These steps correspond to $E \rightarrow EC \rightarrow EE$ steps on the scheme.

An acyl-enzyme intermediate followed by the release of alcohol forms an enzyme–ester complex (EE). This intermediate itself is formed by a hydrogen transfer from the serine, and it is the central complex upon isomerization forming the acyl-enzyme intermediate, after the release of the by-product, which is aldehyde (P)in this specific reaction. The transition state involved in formation of first tetrahedral intermediate (EE) defines the selectivity of BCL toward alcohols[649]. Consequently, enantioselectivity can occur during the steps involving the nucleophilic attack of an alcohol. The nature of the binding interactions between an ester's acyl and alcohol moieties and the active site binding pocket of a lipase critically affects enzyme performance [656,657].

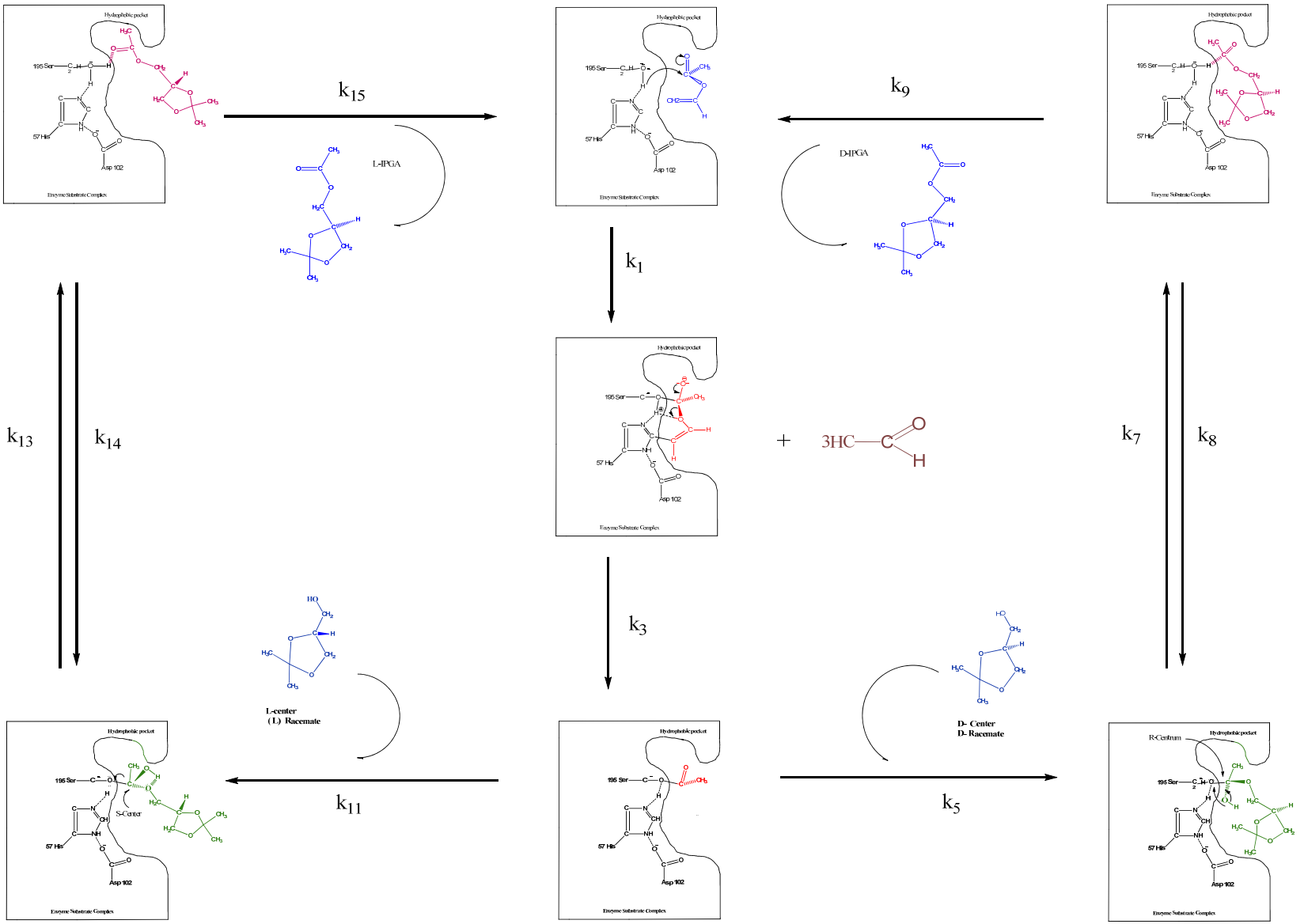


Fig 5.4. The enantioselective IPG transesterication with *B. cepacia* lipase

The second substrate, (D,L)-IPG reacts to the acyl-enzyme intermediate and forms other complexes (EDS or ELS) according to the selected form of the racemate. These intermediates further undergo isomerization to form the ester–enzyme complexes (EDP and ELP), which consequently brake into the final products, (D,L) - IPG-acetate (DP and LP) and the free enzyme. Final decomposition of the enzyme-substrate complex with the release of the transesterified product is considered as the rate controlling step and the calculations in the model were based on this step.

Eventually, the overall goal of a kinetic study is to discriminate the model and to identify the parameters for interpretation of modeling results. Thus, the obtained parameters can then be used for comparison with findings from a molecular modeling point of view[590]. As the above mechanism is drawn in King –Altman scheme, it is demonstrated as follows;

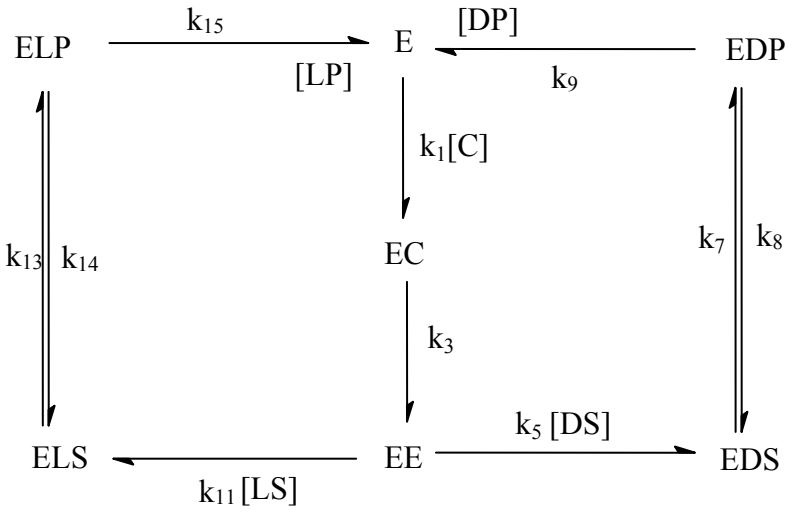


Fig. 5.5. King – Altman scheme of the enantioselective IPG transesterication with BCL.

The solution of the rate expression for individual reactions (on Fig 5.6.) includes a combination of both symbolic (solution of simultaneous equations, substitution of variables, determination of higher derivatives) and numerical (solution of differential equations, finding roots of a polynomials, fitting experimental data) computation methods[606].

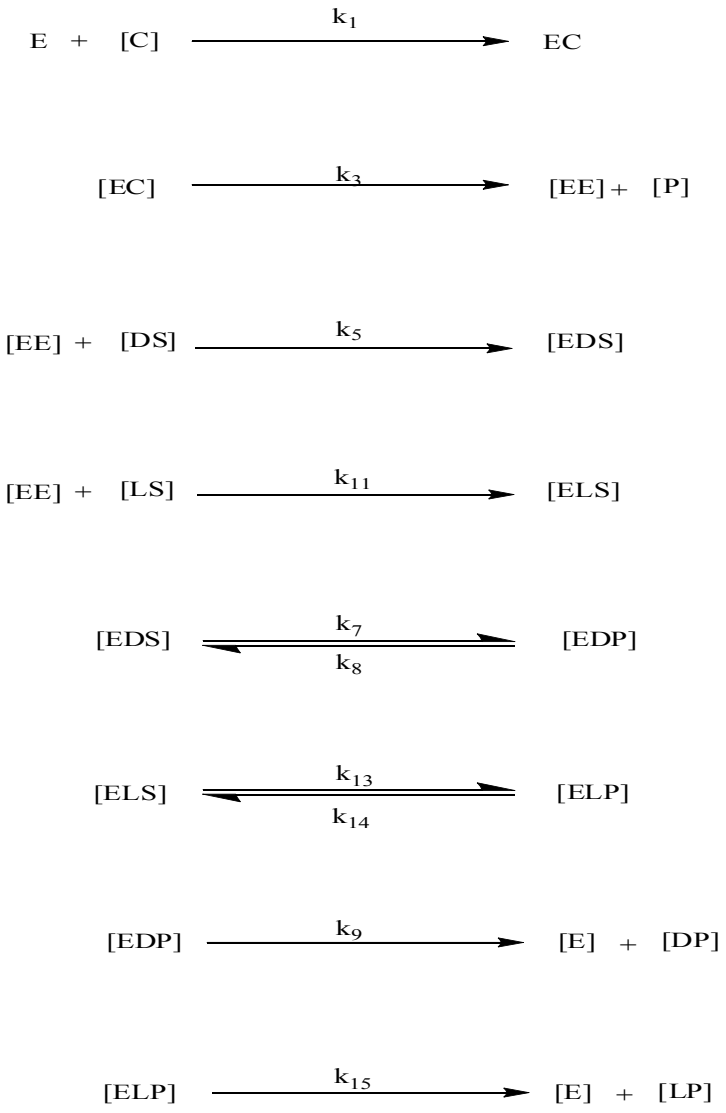


Fig.5.6. The reaction steps of each intermediate

The mathematical model for the kinetics of the each reaction and the computation with this system in MAPLE's syntax [Waterloo, Ont., Canada] was used and the set of general reversible rate expressions was written for the above case by consideration of related irreversible reaction. The results can be defined as follows:

```
v1:=k1*E-k2*EC:          (k2=0)        v2:=k3*EC-k4*EE:          (k4=0)
v3:=k5*EE*DS-k6*EDS:     (k6=0)        v4:=k7*EDS-k8*EDP:
v5:=k9*EDP-k10*E*DP:     (k10=0)       v6:=k11*LS*EE-k12*ELS:    (k12=0)
v7:=k13*ELS-k14*ELP:                   v8:=k15*ELP-k16*E*LP:     (k16=0)

E:=E[0]-(E+EC+EE+EDS+EDP+ELP+ELS):
```

These sets were computed with MAPLE syntax,[658-660], whose detailed enzyme kinetics explanation, in the applied mathematics point of view, is given by Yildirim *et.al.*[661-663].

In modifying the general model for this specific reaction mechanism of the transesterification of IPG, it can be easily seen the number of 16 rate constant parameters reduces to 10 parameters, because some intermediate steps are irreversible, which means that relative rate constants are equal to zero ($k_2 = k_4 = k_6 = k_{10} = k_{12} = k_{16} = 0$). The basic assumption done during this derivation is that the excess components is relatively very much in amount compared to the reactants, such that it remains almost constant along the course of the reaction ($\frac{dC}{dt} = 0$). In other words, it could be said that the derivation is performed under pseudo-steady state assumptions. In order to determine the rate of reaction for each enantiomer, these k values are substituted in the rate expressions derived for the general mechanism given by the equations, eqn.(3.39) and (3.40) on the maple worksheet, and the specific rate expression for D,L- IPG with vinylacetate in *Burkholderia cepacia* lipase (BCL) were derived. The derivation and the open form of the consntants in the equation as a result of Maple worksheet was given in Appendix IX and the general rate expressions simply reduce into the following forms for this specific reaction,

$$\nu_{DS} = -\frac{d[DS]}{dt} = \frac{k_1 k_5 C_o E_o [DS]}{(K_{M1} C_o + k_{11})[LS] + (K_{M2} C_o + k_5)[DS] + k_1 C_0} \tag{5.1}$$

$$\nu_{LS} = -\frac{d[LS]}{dt} = \frac{k_1 k_{11} C_o E_o [LS]}{(K_{M1} C_o + k_{11})[LS] + (K_{M2} C_o + k_5)[DS] + k_1 C_0} \tag{5.2}$$

5.4. Estimation of parameters

In determining the constants in the above derived rate expressions, the number of 10 k parameters reduces to 5 parameters, while the effective rate constants K_{M1},and K_{M2}, are the combinations of other constants. In the simulations, these two effective constants could be evaluated in addition to k_1, k_5, k_{11}. Thus, the number of parameters was reduced to 5, by which simulation of data becomes more reliable.

The rate constants k_1, k_5, k_{11} and effective kinetic constants (K_{M1}, K_{M2}) for the proposed model were obtained from a global estimation by non linear regression of all experimental

data. On the whole, data from 22 different experiments at five temperature values [35°C, 40 °C, 45 °C, 50 °C, 55 °C] were used in the calculations, comprising approximately 350 experimental data points taken into account to fit the mechanistic model in kinetics point of view.

Since each rate is a function of both of the racemates (DS, LS), the changes in the concentration of each enantiomer has an effect on the rate of other one. This means that both rates should have been solved simultaneously. As a result of this fact, the experimental data for the both reactions should be undoubtly used at the same time and this brings a benefit of increasing the number of data for the estimation of parameters. It is clear that temperature is an important parameter in kinetic calculations, since k values are function of this parameter related by Arrhenius equation[664]:

$$k = Ae^{-E_a/RT} \tag{5.3}$$

Hence, the data from the experiments at the same temperatures could be considered as a one set of data for fitting[665]. The Matlab algorithm has been written considering this opportunity. Another opportunity of the Matlab code is that it gives rise to possibility by which the reliability of the code could be tested. In verifying the coherency of the code, it is possible to select certain experiments in fitting and simulate the other experiments with the estimated values obtained by the simulation.

In running the Matlab code, the simulation was started by taking the initial conditions of the total enzyme E_o (25, 50, 75, 100 mg), the excess component vinyl acetate C_o(10–30 mM) and each enantiomers as D-IPG_o, L-IPG_o,(10 – 180 mM for *rac*- IPG). The limiting cases were adjusted to be as lower range of 10^{-12} and upper range of 10^8. The kinetic parameters were determined by fitting the model to the experimental data, through minimization of the sum of the squared residues[666]. A software system of non-linear least square regression in MATLAB consisting of the software packages (curve fitting toolbox) [The MathWorks, Inc., Michigan, USA] was used for the parameter estimation., This toolbox works to find the comparison between the measured data with predicted ones by the proposed kinetic equation of the suggested mechanistic model[667].

$$F = \sum_i([Q]_{Simulated_i} - [Q]_{Theoretical_i})^2 \tag{5.4}$$

At the minimum point of the objective function F due to equation (5.4), the small residual errors are obtained in each point when using estimated values for selected kinetic parameters. For each simulated pair of concentration R against time, one of the residual errors is randomly selected, and then added into the simulated data in turn. By this way, a set of new values for Q concentrations are obtained. Then, these values are used as though they were simulated data in a second round of minimization to calculate new estimates for selected parameters. After repeating this many times, the best fit is detected. The experimental data and the theoretical fitting model are compared if they are in a good agreement by terms of R^2 values given at the description of each figure. Convergence of the estimation procedure is tested for the experimental data which was not used in estimation[663].

In the following section, the experiments will be fitted with the model according to this parameter estimation method.

5.5. Simulation of Experimental Data with the proposed model

For the simulation of the experimental data, the rate expression derived from the suggested model has been used. The experiments were categorized according to the temperature, because the rate constants are function of the temperature.

5.5.1. The simulation of the rates at 35 °C

In these experiments, the transesterification of *rac*- IPG (10 mmol) with of vinyl acetate (30 mMol) in different amount of enzymes (50, 75, 100 mg) at 35 °C has been investigated. In each experiment, the difference was only in the amount of the enzyme. The experimental data points and the simulated curves obtained with the model developed are shown in Fig. 5.7. In order to verify the written Matlab code, first only two experiments at 50 and 75 mg of lipase at 35 °C (Fig 5.7.) were simulated.

The curves 1 and 2 on the Fig.5.7 are the results of the experiments for the 25 and 50 mg of lipase, and curve 3 is for 75 mg of lipase. First, only the curves 1 and 2 were simulated together from the all data of both experiments. The curve 3 was plotted due to the estimanted parameters of the curves 1and 2. The result seems to be fit quite well.

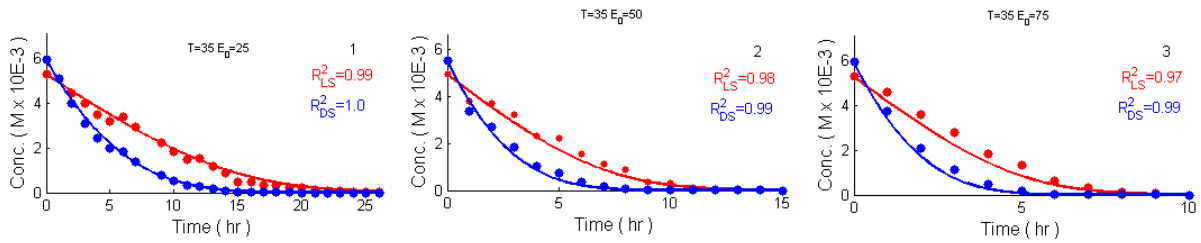


Fig 5.7. The verification of validity of the model by the simulation.

After this verification, the Matlab has run 100 times for the experiments at 35 °C for the amount of 25, 50 and 50 mg of enzyme (curves 1, 2, 3 respectively on Fig 5.8) and to fit the data set simulatenously. The 100 mg of lipase data was fitted separately on Fig 5.9, because of the reason that there might be experimental error on the data, which distribute scatteringly. The R^2 values (as described in section 4.4.1.) were given for each curve to satistfy that the model fits quite well.

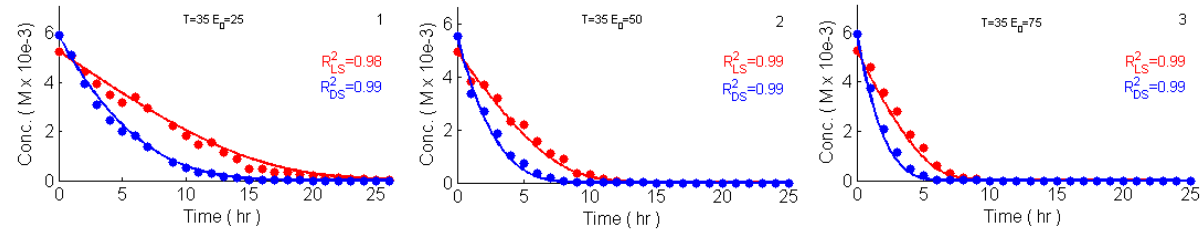


Fig. 5.8. The data fit of the experiments simulated by the mechanistic model proposed

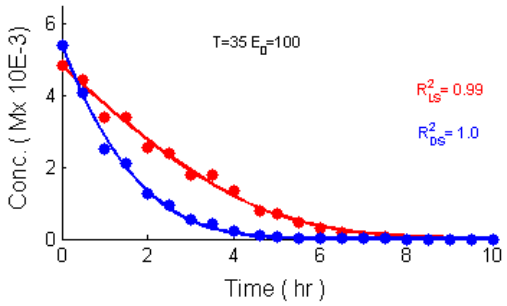


Fig.5.9. The simulation of the data for 100 mg lipase at 35 °C

5.5.2. The simulation of the rates at 50 °C

The lipase supplying company informs that *B. Cepacia* lipase has an optimum temperature at 50 °C (Fluka Buchs, Switzerland). Therefore, the most of the experiments were carried out at this temperature.

The Matlab code's algorithm was written in such a way that it is possible to simulate the data either from one experiment only or from a set of the experiments simulatenously. In the experiments, the temperature of the reacton, the amount of enzyme, initial concentration of *rac*-IPG and the amount of acylating agent were changed. Since the reaction rate constant k is a function of temperature, the whole data obtained from all experiments at 50 °C with 50 mg of lipase can be simulated at the same time (curves 1-4 at Fig 5.10), and it has been observed that the data fit well at this temperature. The curves at other temperatures were also drawn according to the estimated parameters of experiments at 50 °C. As expected, the curves 5 to 7 at Fig 5.10 do not fit reasonably, because the reactons were at 35, 40, 45, 55 °C. However, an unexpected result was obtained on the curve 8, on which L-IPG fits relatively well although the reaction temperature was at 55 °C.

The model can used for the detailed works for the perdiction of reaction conditions. Either of initial concentration, amount of excess component or enzyme quantity may be preferably changed and studied due to the results pointed out from the model simulations.

The effective K_{M1}, and K_{M2} values were calculated using the data 50 °C at which more than 10 different experiments were carried out. Some of the data have been left to verify the model. After simulation of the data fit, the reliability of the results was tested by generating the data points with the estimated parameters and plotting with the experiments which was not used for this check up.

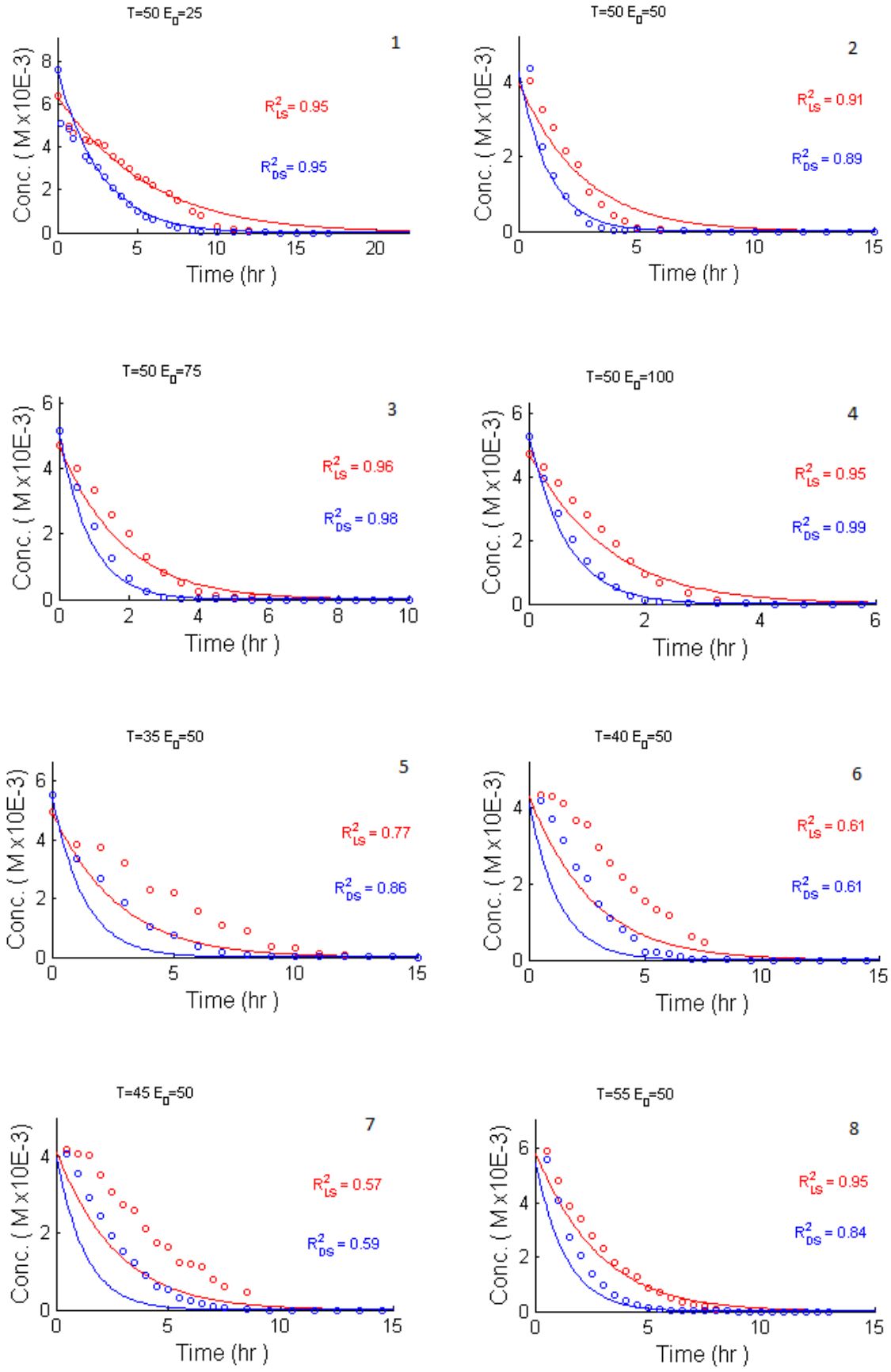


Fig. 5.10. The simulation of data at 50 °C and data fitting with estimated paramentes for the experiments at different temperatures.

In the derivation of the mechanistic model, the basic assumption was that the acyl donor component is in excess amount compared to the racemates, which means its concentration remains almost constant. Another fitting was perfomed for the validity of the model to verify the effect of this liminting condition by using the data obtained from the experiment where 15, 20 mmol of vinylacatate were used with an increasing amount of the enzyme at 50 °C, which are plotted in the curve 1 and 2 on Fig 5.11, respectively.

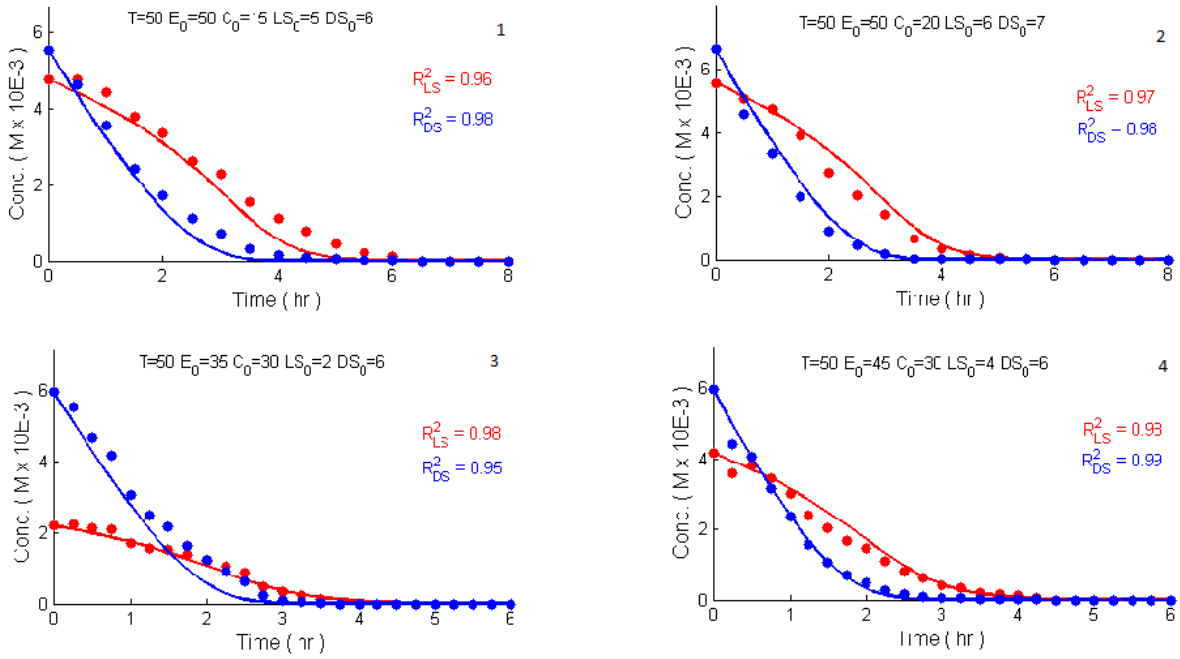


Fig 5.11. The simulation for the less amount of acylating agent

However, it is seen that the data for the experiments, with 35 mg of lipase and 2/6 mmol of L-IPG to D-IPG, and 45 mg of lipase with 4/6 ratio of racemates, have the same rate of reaction. On curve 1 and 2, the rate of L-IPG is relatively slow since the production of acylated active enzyme (EE in model) is not so much, and D-IPG was used up selectively more. As the amount of D-IPG reduces then a sudden decrease in L-IPG reduction resulted in a sigmoid shape on the figure.

The curves 3 and 4 are plotted with the use of the estimated parameters from the curves 1 and 2. The result shows that the same rate can ben obtained by adjusting the reaction conditions, like amount of enzyme, acylating agent or racemates ratio.

5.5.3. The simulation of the rates at 40, 45, 55 °C

In this section, the experiments done at different temperatures were considered. The same amount of vinyl acetate (30 mmol) and D,L-IPG (10 mmol) were used with 50 mg of lipase. The results of simulations on 40 °C and 45 °C are given on Fig. 5. 12

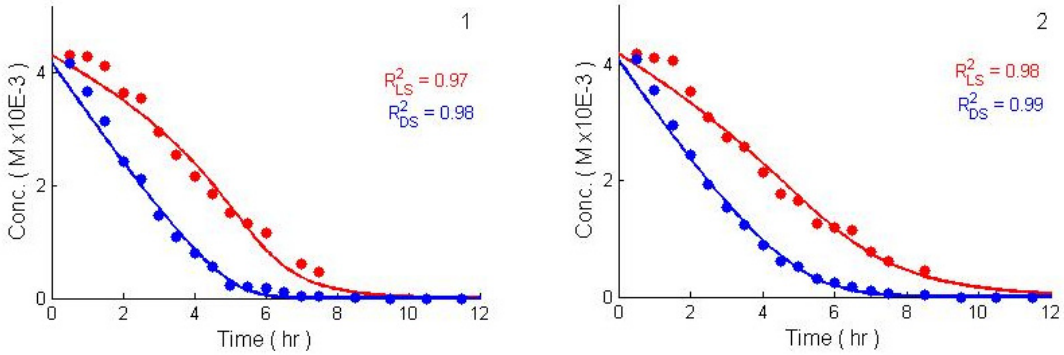


Fig. 5.12. The simulation of the data set at (1) 40 °C , (2) 45 °C

When the data from the experiment at 55 °C with 50 mg enzyme was simulated and it was interstingly detected that these estimated parameters can also fit the data of the experiment at 50 °C, with 75 mg of lipase (Fig. 5.13). This shows that the same reaction rate can be obtained by adjusting the enzyme concentration and reaction temperatures.

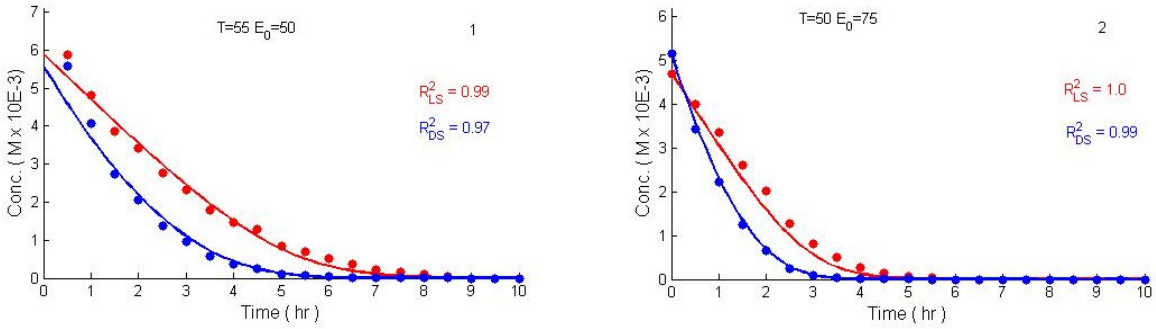


Fig. 5.13. The simulation of the data set at (1) 55 °C, (2) of the data at 45 °C with estimated parameter from (1)

5.5.4. Estimated rate constants

The reaction rate parameters have been evaluated by fitting the data points. In this fitting process, how to trust the data was inquired, and this question was solved with the goodness of the best fit, which is verified by the use of R^2 method (section 4.4.1).

The error analysis of the data has been performed with bootstrap method (section 4.4.2.), in which the big number of bootstrap sampling is preferred to obtain the reasonable results. Therefore the bootstrap sampling of thousand (B = 1000) has been considered during the calculations. The reaction rate constants have been determined due to the bootstrap histograms. A sample rate constant determination for the experiment at 35 °C and 25 mg lipase with the use of bootstrap histogram was given on Appendix X.

The estimated kinetic constants at all temperatures used in the experiments have been given on the following table (Table 5.1), and the enantiomeric ratio has been calculated according to these parameters.

Table 5.1. Estimated rate constants and enantiomeric ratio

Temp. °C	k_1(1/h)	k_5(1/h)	k_{11}(1/h)	K_{M1}(mmol/L)	K_{M2}(mmol/L)	$E=k_5/k_1$
35	5.113 ± 6.7x10^{-3}	0.0274 ± 8x10^{-4}	0.011 ±15x10^{-4}	2.932 ±0.005x10^{-3}	0.502 ± 27.8 x10^{-4}	2,49
40	4.273 ± 12.5x10^{-3}	0.0222 ± 48 x10^{-4}	0.0112 ± 27x10^{-4}	2.452 ± 4x10^{-3}	0.565 ± 15 x10^{-4}	2,01
45	3.425 ± 15 x10^{-3}	0.0249 ± 71x10^{-4}	0.0118 ± 35x10^{-4}	2.489 ± 6.2x10^{-3}	0.584 ± 7.3x10^{-4}	2,11
50	3.0299 ± 42.7x10^{-3}	0.0743 ± 8.7x10^{-4}	0.0318 ±42x10^{-4}	2.485 ±37.2x10^{-3}	0.595 ±10.6x10^{-4}	2,34
55	2.850 ± 10.2x10^{-3}	0.0484 ± 24x10^{-4}	0.0258 ±13.4 x10^{-4}	2.099 ± 8x10^{-3}	0.560 ± 27x10^{-4}	1.87

5.6. Single reactions of each racemates; Only L- IPG and D – IPG

In enantioselective reactions, the substrate inhibition by both enantiomers on the active site of the enzyme becomes significant. In order to understand the behavior of the enzyme towards the single enantiomer, the kinetic investigations involving ping-pong bi-bi models have been reported for cases, where only one enantiomer is converted[668]. To analyze this situation, the experiments were performed with D – IPG in the absence of L- IPG, and with L- IPG in the absence of D – IPG[303].

In this experiment, 10 mmol of single D and single L – IPG is mixed with 30 mmol of vinyl acetate in 50 mg of *B. Cepacia* lipase, and the total reaction volume was completed to 10 ml of *n*- hexane in 20 ml of bioreactor. The reaction was carried out at temperature of 50 °C.

As a result of these experiments, it is observed that the lipase reacts with each enantiomer almost with the same rate (Fig 5.14).

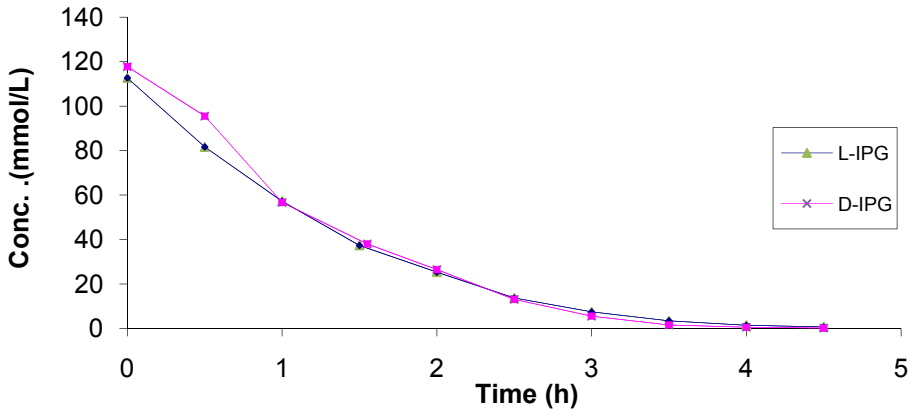


Fig.5.14. The overlapping of the D- L-IPG reactions in the absence of each other[303].

In this work, the rate expression for the single enantiomeric reactions has also been winnowed out after specifying the conditions of single enantiomeric reaction according to the general mechanistic model applied for a case study; IPG transesterification reaction.

For single D – IPG reaction, $k_{11} = 0$, hence the left side of the scheme (Fig 5.15) vanishes, and the same is true for the right side of scheme for the single L- IPG, when $k_5 = 0$. Consequently, the following scheme is obtained as independent of each other.

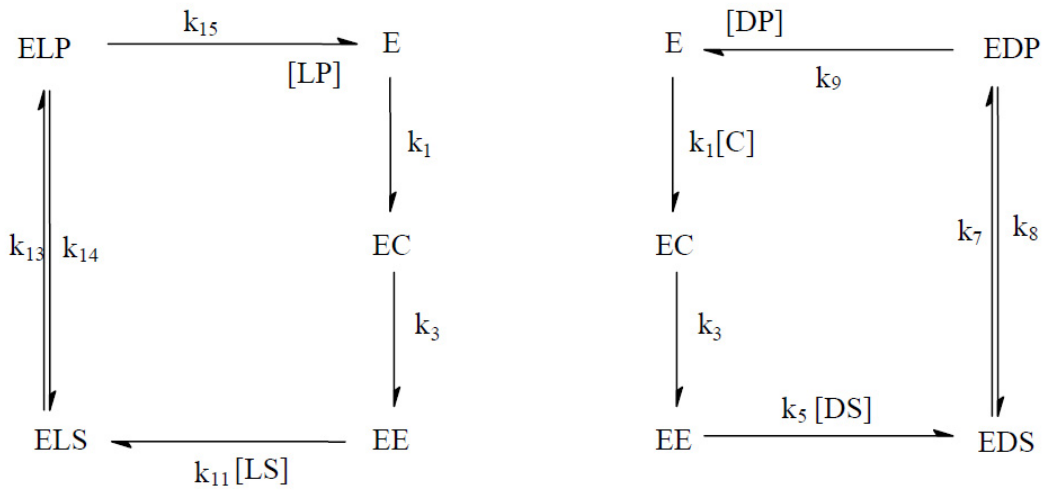


Fig. 5.15. The King –Altman scheme for single enantiomer a) for L- IPG, b) D- IPG

For determining the rate expressions of each enantiomers, this special case was solved with the Maple software, where k_5=0, and k_{11}= 0. The rates obtained by Maple software were later analytically tackled with for further mathematical simplifications, and it was found that the result simply turned to be an exact Michaelis - Menten kinetics. The details of derivation for the Michaelis – Menten form and V_{max}, K_m constants for each enantiomer were given on Appendix XI.

$$V_{DS} = \frac{d[DP]}{dt} = -\frac{d[DS]}{dt} = \frac{V_{max,DS}[DS]}{K_{m,DS}+[DS]} \tag{5.5}$$

$$V_{LS} = \frac{d[LP]}{dt} = -\frac{d[LS]}{dt} = \frac{V_{max,LS}[LS]}{K_{m,LS}+[LS]} \tag{5.6}$$

Actually, this integrated equation is useful to obtain more accurate kinetic parameters[669]. Therefore, the integrated form of the above equations were derived and given as ;

$$[DS] - [DS]_o + K_m \ln\left(\frac{DS}{DS_o}\right) = V_{max}t \tag{5.7}$$

$$[LS] - [LS]_o + K_m \ln\left(\frac{LS}{LS_o}\right) = V_{max}t \tag{5.8}$$

However, the integrated form of Michaelis – Menten equation is an implicit function, in which the concentration terms cannot be collected on one side of the reaction. This result brings difficulties to calculate the V_{max} and K_m terms by direct simulation of the experimental data. Therefore further linearization is done as by use of conversion term, as follows;

The turnover ratio is defined as $U = \frac{[S]-[S_o]}{[S_o]}$ (5.9)

$[S] = [S_o](1-U)$ and is obtained (5.10)

$$\frac{t}{U} = \frac{K_m}{v_{max}}\left[\frac{1}{U}\ln\left(\frac{1}{1-U}\right)\right] + \frac{[S_o]}{v_{max}} \quad (5.11)$$

for any species. Vmax and Km terms can now be calculated if equation (5.11) is plotted for $\frac{t}{U}$ vs $\left[\frac{1}{U}\ln\left(\frac{1}{1-U}\right)\right]$ terms.

In the program code written in Matlab, this situation has also been considered, and the constants were solved with nonlinear least square regression. The data points and the simulated curve were given on (Fig.5.16).

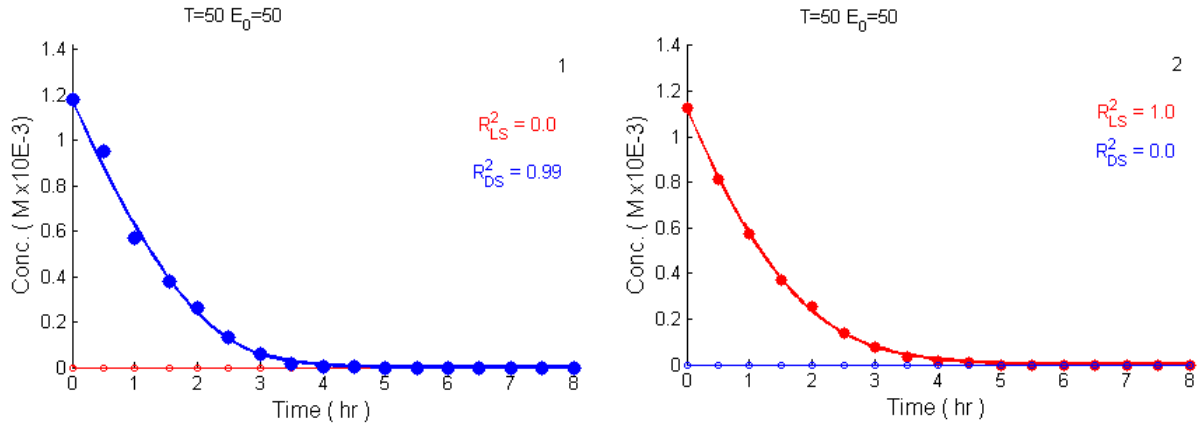


Fig.5.16. The simulation of single racemates (10 mmol D and L form of IPG with 30 mmol vinylacetate in 50 mg of enzyme in 10 ml solution)

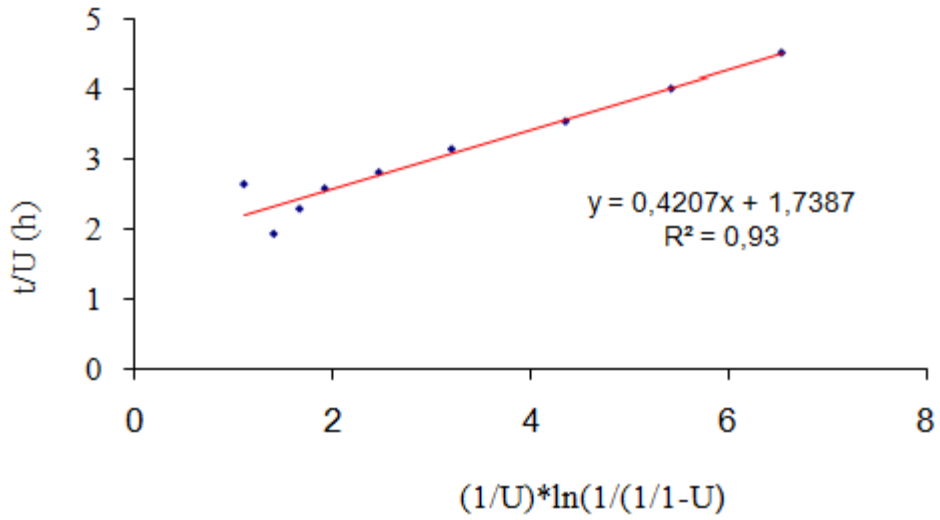


Fig.5.17. Integrated Michaelis – Menten equation of D-IPG with 50 mg lipase at 50 °C.

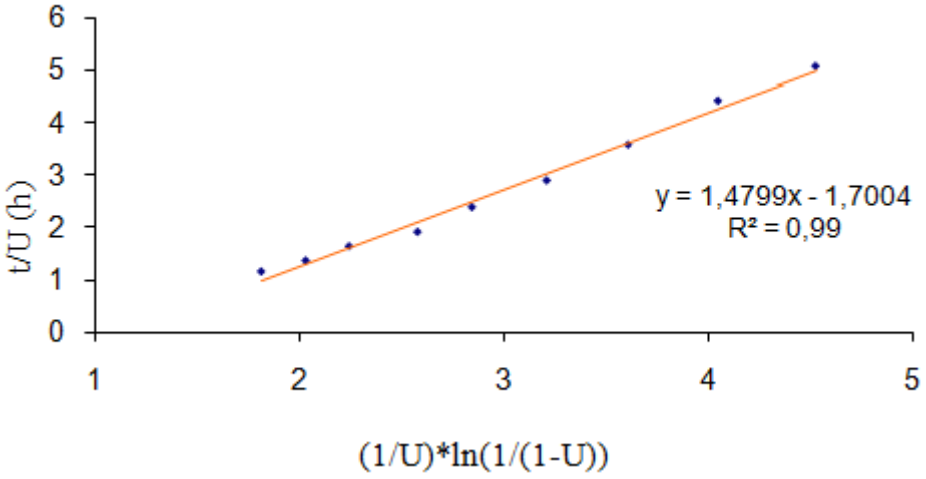


Fig. 5.18. Integrated Michaelis – Menten equation of L-IPG with 50 mg lipase at 50 °C.

Tab. 5.2. The values of v_{max} and K_m of D and L – IPG at 50 °C.

	D - IPG	L - IPG
v_{max} (mol / L. h)	0.677	0.66
K_m (mol /L)	0.285	0.98

5.7. Reaction kinetics in scCO$_2$ medium [310]

In previous reaction was done in the conventional medium organic medium, n-hexane, which is explained in detail in chapter 2. Recently, the environmentally benign mediums are usually preferred, among which scCO2 is mostly preferrd due its physicochemical properties. In scCO2 , the mass transfer limitations are negligible, thus a faster rate is expected.

An experiment in scCO2 at 10 MPa and 40 °C, with 60 mmol of vinyl acetate and 20 mmol of D;L IPG with 101,62 mg of lipase made in Technical Chemistry Institute (TCI) by Tservistas [310]. The reaction taking place in this medium has been simulated with the model developed in this work. A very good ftting with the data was obtained (Fig.5.19).

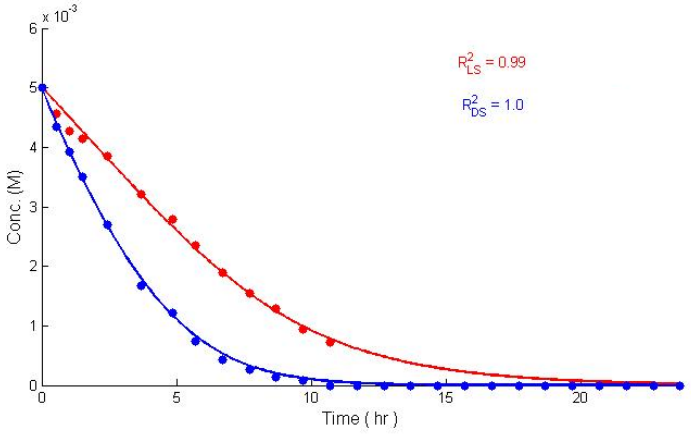


Fig. 5.19. The simulation of the data taken in super critical CO_2.

where the values of the estimation parameters are given on the table 5.3, and the enantiomeric ratio was calculated as 2.3 for the reaction in supercritical conditions.

Table 5.3. Estimated rate constant in sc CO_2

Parameterandard	Value	Standard Error
k_1(1/h)	8.5117	±1.0815
k_5(1/h)	0.0181	±0.0013
k_{11}(1/h)	0.0079	±0.00052
K_{M1}(mmol/L)	2.2221	±0.52930
K_{M2}(mmol/L)	0.4604	±0.1802

5.8. Enantioselectivity by mechanistic model

As previously derived that, enantioselective ratio E, (E-value) has become the ratio of the rate constant at the activated enzyme intermediate, where it selects either of the racemates. Experimentally it is observed that the enantioselectivity ratio E is around 2, which has been evaluated by the model as $E = \frac{k_5}{k_{11}} = 2.27$. This is the ratio of rate constants where EE reacts with any one of the enantiomers.

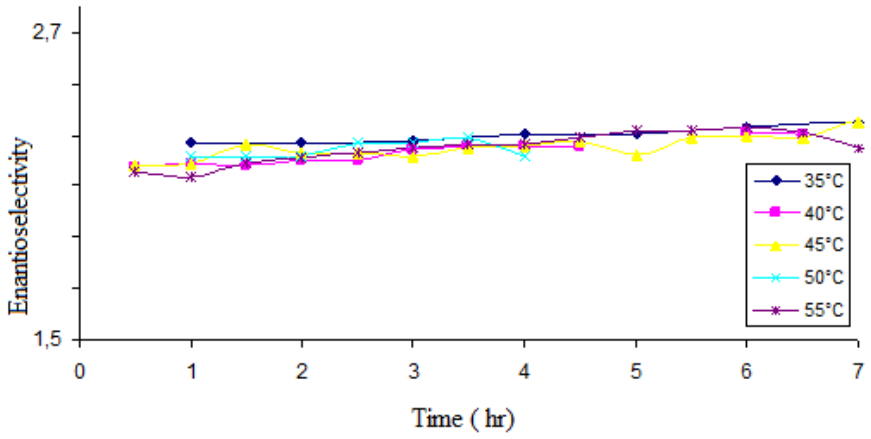


Fig.5.20. The effect of temperature on the enantioselectivity [303]

In above Fig.5.18, the eantioselectitivity dependent on temperature is given. It is obvious that there is no big change between E- values at 35, 40, 45, 50, 55 °C temperature.

It is been observed in the experiments that the enantioselectivity descends very slowly almost remaining in the range of 2.2, although the amount of enzyme increases. This is what can be explained by the ratio of k_5/ k_{11} is 2.47. As the amount of enzyme increases, the more enzymes can possibly react with L-IPG thus a slight tendency for decrease could be this result.

The experimental and the estimated E values by the model (the ratio of $\frac{k_5}{k_{11}}$) was given on the Table (5.3). The experimental enantioselectivity ratio has been calculated by the equation;

$$E = \frac{[LS]-[DS]}{[LS]+[DS]} \tag{5.12}$$

Table 5.4.: Comparision of enantioselectivity between experimental data and model

Enzyme (mg)	Temperature (°C)	Experimental E	Estimated E
25	35	2.269	2.41
50	35	2.24	2.49
50	40	2.27	2.01
50	45	2.25	2.11
50	50	2.24	2.34
50	55	2.249	1.87
75	35	2.24	2.43
100	35	2.21	2.5
Mean		**2.25**	**2.27**

The experimental results and the the ones evaluated by the estimated parameters seems to be suitable in te range of 2. It is found that the E value became in this range even in all iteration values.

Chapter 6. Discussion and Conclusion

6.1. Discussion

Either in scientific research area or in industry, the enantioselectivity is an important field studied over the years. The aim is to increase the enantiomeric ratio (E- value) of the enzyme to produce the desired product.

In this work, a general mechanistic model for the enantioselective reactions was proposed and its kinetic equations were derived with pseudo-steady state assumption. It was successfully pondered that this model can be used for any other type of the enzyme catalysed racemic reactions.

A general rate expression was derived with the use of Maple software package and this model has been used in this work. In the first part of the chapter 3, the concept of enantioselectivity was overviewed and a general enantioselectivity for overall bi-bi reactions was derived. In fact this is equation is an Abel's type equation, whose solution might be further studied. Fortunately, this handicap was eliminated by the mechanistic model. The enantioselectivity based on mechanistic model has resulted in a general equation which was tested if it becomes the well known equation for the irreversible reactions given in literature. It is demonstrated that is converts the same equation for the irreversible one if the rate constants are zero.

The use of this equation may give information if all rates are reversible. In this case it could be possible to calculate the enantioselectivity if any path of the reaction is inhibited.

After derivation of the model from mechanism of the reaction, the experimental data was fitted with least square toolbox of Matlab software package. The model was based on the pseudo-steady state assumption, since the acyl donor was in excess amount and assumed to be constant during the course of the reaction. It has been also shown that the model fits well for the less amount of excess reagent. The model could be in future modified by considering the excess component as variable.

In fact, Matlab coding can be modified for the real case fitting, which means all differential equations of the each intermediates are solved simultaneously. In such a case, it is possible to find out the concentration variation of each intermediate along the reaction.

The model was simulated with different experimental data and it has been shown that it works quite well in fitting these data. The goodness of the curve fit has been statistically verified with R^2 analysis (Section 4.4.1). The error analysis has been done with bootstrap method (Section 4.4.2).

The enantiomeric selectivity is an intrinsic property of the enzyme. The factors affecting this property have been studied in the literature as given in chapter 2 in detail. The type of solvent, solute, enzyme structure and the reaction temperature may be effective on the enantioselectivity.

One of the interesting results derived is that E value has become the ratio ($\frac{k_5}{k_{11}}$) of the rate constants of acylated active enzyme, EE, towards the enantiomers. This is physically logical but derivations have also proved this reality.

For the specific reaction of D,L – IPG considered as a case study in this work, the experiments have been performed in the temperature range of 35 – 55 °C, and the enzyme amount from 25 -100 mg. During the calculations, it is detected that the enantiomeric ratio has shown a robust character, and has not been drastically changed, staying almost around 2.

The enantiomeric ratios calculated from experiment done by Yildirim in his master work[303] and from model were compared and almost the same results were obtained.

Parameter	Experimental E-value	E-value by Model
Temperature	2,26	2,11
Enzyme consentration	2,24	2,27

In fact except the rate constants k_1, k_5, k_{11}, the other k constants for related intermediates cannot be estimated with this model, since some of them are in the combination as effective constants, k_{M1}, k_{M2}. In order to determine each k constant, the inhibition for each step in

general mechanism can be studied. Since intermediates are in very small time intervals (*e.g.* nano or femto second), very sophisticated and sensitive spectrophotometric analysis is required. In order to eliminate any volume change effect in the reactor, an online measuring system is strongly suggested.

As well as experiments done in conventional mediums, the effect tof medium on the rate constants can be studied with the use of the model, by this way the effect of mass transfer limitations on the rate can be determined.

The influence of acyl donor and temperature were investigated in details for many reactions in the literature. These two factors are very important to be analyzed and the general model may allow the scientists to work further by changing these parameters in the model.

The general mechanistic model can be later investigated for the different mechanisms given in the literature for any specific reactions. As well as the model considers the effect of one enantiomer on the other, the elimination of this effect has also been analyzed for single racemates. During the derivation of the rate expression for single enantiomer, it was not expected that the reaction becomes a Michaelis – Menten type, since the rate of enantioselective transesterification of IPG was derived as a non- Michaelis – Menten reaction type.

The $_{Vmax,\ DS}$, $V_{max,\ LS}$, and $K_{m,DS}$, $K_{m,LS}$ of single IPG at 50 °C were given at Table 5.2 in this work. In future these constants can be calculated for the reactions at different temperatures to get more information about the kinetics of single D or L - IPG reaction.

In fact this mechanistic model is for a general enantioselective reaction kinetics, and can be applied any type of racemic reaction. The systems of differential equations have been simulated with the experimental data from in vitro measurement, *i.e,* not in living cell. Thus, in future work, this study can hopefully be applied to large living systems or human lipase metabolism from the systems biology point of view.

6.2. Conclusion:

The mechanistic model developed in this work may be further applied for the online measuring systems. The program algorithm written for general enantioselective reactions may be used for any type of enzymatic reaction which can be defined by equating the k values to zero for the irreversible paths, according to mechanism proposed due to organic chemistry.

A close system is strongly suggested for the kinetic analysis. The benefit of the close systems is that any disturbance into reaction media from the environment is eliminated, thus more trustable data could be collected. Software can be developed for simultaneous analysis of the parameters within system. Thus, the dynamics of the reaction course depending on the mechanistic model can be performed simultaneously during the experiments carried out.

X - Appendixes

Appendix I. Nomenclature of Lipase (EC 3.1.1.3)

Accepted name: triacylglycerol lipase

Reaction: triacylglycerol + H2O = diacylglycerol + a carboxylate

Other name(s): lipase; triglyceride lipase; tributyrase; butyrinase; glycerol ester hydrolase; tributyrinase; Tween hydrolase; steapsin; triacetinase; tributyrin esterase; Tweenase; amno N-AP; Takedo 1969-4-9; Meito MY 30; Tweenesterase; GA 56; capalase L; triglyceride hydrolase; triolein hydrolase; tween-hydrolyzing esterase; amano CE; cacordase; triglyceridase; triacylglycerol ester hydrolase; amano P; amano AP; PPL; glycerol-ester hydrolase; GEH; meito Sangyo OF lipase; hepatic lipase; lipazin; post-heparin plasma protamine-resistant lipase; salt-resistant post-heparin lipase; heparin releasable hepatic lipase; amano CES; amano B; tributyrase; triglyceride lipase; liver lipase; hepatic monoacylglycerol acyltransferase

Systematic name: triacylglycerol acylhydrolase

Comments: The pancreatic enzyme acts only on an ester-water interface; the outer ester links are preferentially hydrolysed.

References:

1. Korn, E.D. and Quigley, T.W. Lipoprotein lipase of chicken adipose tissue.
J. Biol. Chem. Vol.226(2), pp: 833-839, (1957) [PDF]

2. Lynn, W.S. and Perryman, N.C. Properties and purification of adipose tissue lipase.
J. Biol. Chem.Vol. 235(7), pp: 1912-1916, (1960) [PDF]

3. Sarda, L. and Desnuelle, P. Action de la lipase pancréatique sur les esters en emulsion
Biochim. Biophys. Acta 30 (1958) 513-521. [PDF]

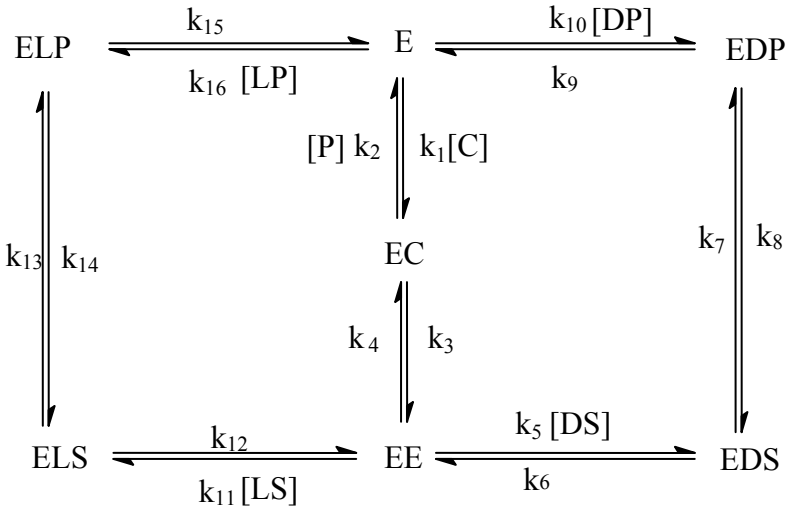
4. Singer, T.P. and Hofstee, B.H.J. Studies on wheat germ lipase. I. Methods of estimation, purification and general properties of the enzyme. *Arch. Biochem.* 18 (1948) 229-243.

5. Singer, T.P. and Hofstee, B.H.J. Studies on wheat germ lipase. II. Kinetics.
Arch. Biochem. 18 (1948) 245-259.

Appendix II. Maple Sheet for Two component enantioselectivity

```
> restart;
>
>
> # Derivation of Two Component Enantiomeric Ratio
>
>
>#LS+C+E<==========>LP+P+E
>#DS+C+E<==========>DP+P+E
>
>
> DP := DSo - DS; LP := LSo - LS; C := Co - DP - LP; P := DP
      + LP;
k2 := K·k1;
> k4 := K·k3;
>
> # rate1 = diff ( LS(t), t)
>
> rate1 := expand (k1·LS·C·E - k2·LP·P·E);
> rate11 := collect( ??, [k1, LS, DS, LS·LS ]);
>
> #rate2 = diff ( DS(t), t)
>
> rate2 := expand (k3·DS·C·E - k4·DP·P·E);
> rate22 := collect( ??, [k3, DS, LS, DS·DS] );
>
>
> k3 := k1/E;
> Enan1 := simplify( rate11/rate22 );
> enan2 := collect( ??, [ LS, DS, LS·LS, DS·DS ]);
>
> enan3 = subs( 1 - K = α1, enan2 );
>
> α1 := 1 - K;
> α2 := Co + 2 K LSo - DSo - LSo + K DSo;
> α4 := -K LSo DSo - K LSo^2;
> α3 := K LSo;
>
> α5 := Co + 2·K·DSo - DSo - LSo + K·LSo;
>
> α7 := -K DSo^2 - K LSo DSo;
> α6 := K·DSo;
>
```

APPENDIX III.: DERIVATION OF THE MODEL UNDER STEADY STATE ASSUMPTION ON ENZYME BOUND COMPLEXES



Equation Chapter (Next) Section 1

$$\frac{d[EC]}{dt}=k_1C[E]+k_4[EE]-(k_2P+k_3)[EC] \tag{1.1}$$

$$\frac{d[EE]}{dt}=k_3[EC]+k_6[EDS]+k_{12}[ELS]-(k_5[DS]+k_{11}[LS]+k_4)[EE] \tag{1.2}$$

$$\frac{d[ELS]}{dt}=k_{11}[LS][EE]+k_{14}[ELP]-(k_{13}+k_{12})[ELS] \tag{1.3}$$

$$\frac{d[ELP]}{dt}=k_{13}[ELS]+k_{16}[LP][E]-(k_{15}+k_{14})[ELP] \tag{1.4}$$

$$\frac{d[EDS]}{dt}=k_5[DS][EE]+k_8[EDP]-(k_7+k_6)[EDS] \tag{1.5}$$

$$\frac{d[EDP]}{dt}=k_7[EDS]+k_{10}[DP][E]-(k_8+k_9)[EDP] \tag{1.6}$$

$$\frac{d[DP]}{dt}=k_9[EDP]-k_{10}[E][DP] \tag{1.7}$$

$$\frac{d[LP]}{dt}=k_{15}[ELP]-k_{16}[E][LP] \tag{1.8}$$

If enzyme complexes are all at steady state, then all the time derivatives vanish from Eq**Fehler! Verweisquelle konnte nicht gefunden werden.** through Eq**Fehler! Verweisquelle konnte nicht gefunden werden.**. That is to say

$$\frac{d[EC]}{dt}=\frac{d[EE]}{dt}=\frac{d[ELS]}{dt}=\frac{d[ELP]}{dt}=\frac{d[EDS]}{dt}=\frac{d[EDP]}{dt}=0 \tag{1.9}$$

We assume total concentration of DS and LS remain unchanged during the reaction period, so we can write

$$DS_0=[DS]+[EDS]+[EDP]+[DP] \tag{1.10}$$

$$LS_0=[LS]+[ELS]+[ELP]+[LP] \tag{1.11}$$

Since enzyme concentration is too small compare to initial LS and DS concentrations by assumption, we can ignore contributions of the enzyme bound complexes in the conservation of total LS and DS concentrations. So we get

$$DS_0 \approx [DS]+[DP] \tag{1.12}$$

$$LS_0 \approx [LS]+[LP] \tag{1.13}$$

We also assume the enzyme is stable throughout the reaction and its total concentration remains constant that gives us,

$$E_0=[E]+[EC]+[EE]+[EDS]+[ELS]+[ELP]+[EDP] \tag{1.14}$$

Solve [ELP] Eqn for [ELP] to get

$$[ELP]=\alpha_0[ELS]+\alpha_1[E][LP] \tag{1.15}$$

where,

$$\alpha_0=k_{13}/(k_{14}+k_{15})$$
$$\alpha_1=k_{16}/(k_{14}+k_{15}) \tag{1.16}$$

Solve [EDP] Eqn for [EDP] to get

$$[EDP]=\alpha_2[EDS]+\alpha_3[E][DP] \tag{1.17}$$

$$\alpha_2 = k_7 / (k_8 + k_9)$$

$$\alpha_3 = k_{10} / (k_8 + k_9) \quad (1.18)$$

Put [ELP] into [ELS] then solve it for [ELS] to get

$$[ELS] = \alpha_4[LS][EE] + \alpha_5[E][LP] \quad (1.19)$$

Here,

$$\alpha_4 = \frac{k_{11}(k_{14} + k_{15})}{k_{12}(k_{14} + k_{15}) + k_{13}k_{15}}$$

$$\alpha_5 = \frac{k_{14}k_{16}}{k_{12}(k_{14} + k_{15}) + k_{13}k_{15}} \quad (1.20)$$

Put [EDP] into EDS eqn and then solve it for [EDS] to get

$$[EDS] = \alpha_6[DS][EE] + \alpha_7[E][DP] \quad (1.21)$$

$$\alpha_6 = \frac{k_5(k_8 + k_9)}{k_6(k_8 + k_9) + k_7k_9}$$

$$\alpha_7 = \frac{k_8k_{10}}{k_6(k_8 + k_9) + k_7k_9} \quad (1.22)$$

Solve [EC] Eqn to get [EC] as:

$$[EC] = \alpha_8[E] + \alpha_9[EE] \quad (1.23)$$

$$\alpha_8 = \frac{k_{1C}}{k_{2P} + k_3}$$

$$\alpha_9 = \frac{k_4}{k_{2P} + k_3} \quad (1.24)$$

Put [EC] in [EE] eqn and solve it for [EE] to get

$$[EE] = g_0[E] \quad (1.25)$$

$$g_0 = \frac{k_3\alpha_8 + k_6\alpha_7[DP] + k_{12}\alpha_5[LP]}{k_4\text{-}k_3\alpha_9 + (k_5 - k_6\alpha_6)[DS] + (k_{11} - k_{12}\alpha_4)[LS]} \quad (1.26)$$

$$g_0 = \frac{\Theta_1 + \Theta_2[DP] + \Theta_3[LP]}{\Theta_4 + \Theta_5[DS] + \Theta_6[LS]} \quad (1.27)$$

Here, Θ_i 's are defined as

$$\Theta_1 = k_3\alpha_8, \Theta_2 = k_6\alpha_7, \Theta_3 = k_{12}\alpha_5, \Theta_4 = k_4 - k_3\alpha_9, \Theta_5 = k_5 - k_6\alpha_6, \Theta_6 = k_{11} - k_{12}\alpha_4 \quad (1.28)$$

Put [EE] into [EC] equation above and solve it for [EC]

$$[EC] = (\alpha_8 + \alpha_9 g_0)[E] \quad (1.29)$$

Put [EE] into [EDS] Eqn and solve it for [EDS] to get

$$[EDS] = (\alpha_6[DS]g_0 + \alpha_7[DP])[E] \quad (1.30)$$

Put [EE] into [ELS] Eqn and solve it for [ELS] to get

$$[ELS] = (\alpha_4 g_0[LS] + \alpha_5[LP])[E] \quad (1.31)$$

Put [ELS] into [ELP] eqn and solve it for [ELP] to get

$$[ELP] = (\alpha_0\alpha_4 g_0[LS] + (\alpha_0\alpha_5 + \alpha_1)[LP])[E] \quad (1.32)$$

Put [EE] into [EDS] to get

$$[EDS] = \alpha_6[DS]g_0[E] + \alpha_7[E][DP] \quad (1.33)$$

Then substitute [EDS] into EDP to get

$$[EDP] = (\alpha_2\alpha_6 g_0[DS] + (\alpha_2\alpha_7 + \alpha_3)[DP])[E] \quad (1.34)$$

Let's E_0 is the total enzyme concentration when time t=0. Since we assume that the total enzyme concentration remains constant throughout the reactions, we can write

$$E_0 = [E] + [ELP] + [EDP] + [ELS] + [EDS] + [EE] + [EC] \quad (1.35)$$

After substituting the expressions we derived for each enzyme bound complexes in this equation we get

$$E_0 = \begin{pmatrix} 1 + \alpha_0\alpha_4 g_0[LS] + (\alpha_1 + \alpha_0\alpha_5)[LP] + \alpha_2\alpha_6 g_0[DS] + (\alpha_3 + \alpha_2\alpha_7)[DP] \\ + \alpha_4 g_0[LS] + \alpha_5[LP] + \alpha_6 g_0[DS] + \alpha_7[DP] + g_0 + \alpha_8 + \alpha_9 g_0 \end{pmatrix}[E] \quad (1.36)$$

After some rearrangements made, solution of this equation for [E] becomes

$$E = \frac{E_0}{\begin{pmatrix} 1 + \alpha_0\alpha_4 g_0[LS] + (\alpha_1 + \alpha_0\alpha_5)[LP] + \alpha_2\alpha_6 g_0[DS] + (\alpha_3 + \alpha_2\alpha_7)[DP] \\ + \alpha_4 g_0[LS] + \alpha_5[LP] + \alpha_6 g_0[DS] + \alpha_7[DP] + g_0 + \alpha_8 + \alpha_9 g_0 \end{pmatrix}} \quad (1.37)$$

$$E = \frac{E_0}{\begin{pmatrix} 1 + \alpha_8 + (\alpha_1 + \alpha_5(\alpha_0 + 1))[LP] + (\alpha_3 + (1+\alpha_2)\alpha_7)[DP] \\ + (1 + \alpha_9 + \alpha_4(1+\alpha_0)[LS] + \alpha_6(1+\alpha_2)[DS])g_0 \end{pmatrix}} \quad (1.38)$$

$$[E] = \frac{E_0}{\Omega_1 + \Omega_2[LP] + \Omega_3[DP] + (\Omega_4 + \Omega_5[DS] + \Omega_6[LS])g_0} \quad (1.39)$$

where,

$$\Omega_1 = 1+\alpha_8,\ \Omega_2 = \alpha_1+(1+\alpha_0)\alpha_5,\ \Omega_3 = \alpha_3+(1+\alpha_2)\alpha_7,\ \Omega_4 = 1 + \alpha_9,\ \Omega_5 = \alpha_6(1+\alpha_2),\ \Omega_6 = \alpha_4(1+\alpha_0) \quad (1.40)$$

After putting g0 given by Eq**Fehler! Verweisquelle konnte nicht gefunden werden.** into we get [E] as

$$[E] = \frac{E_0}{\Omega_1 + \Omega_2[LP] + \Omega_3[DP] + (\Omega_4 + \Omega_5[DS] + \Omega_6[LS])\dfrac{\Theta_1 + \Theta_2[DP] + \Theta_3[LP]}{\Theta_4 + \Theta_5[DS] + \Theta_6[LS]}} \quad (1.41)$$

After some rearrangement made, then E becomes

$$[E] = \frac{E_0\left(\Theta_4 + \Theta_5[DS] + \Theta_6[LS]\right)}{\left(\begin{array}{l}\left(\Omega_1 + \Omega_2[LP] + \Omega_3[DP]\right)\left(\Theta_4 + \Theta_5[DS] + \Theta_6[LS]\right) \\ \\ +\left(\Omega_4 + \Omega_5[DS] + \Omega_6[LS]\right)\left(\Theta_1 + \Theta_2[DP] + \Theta_3[LP]\right)\end{array}\right)} \tag{1.42}$$

The rate of [DP] Production is given by

$$\frac{d[DP]}{dt} = k_9[EDP] - k_{10}[E][DP] \tag{1.43}$$

Putting [EDP] and [E] expressions into this equation gives us

$$\frac{d[DP]}{dt} = \left(\Delta_1[DS] - \Delta_2[DP]\right)\frac{E_0\left(\Theta_4 + \Theta_5[DS] + \Theta_6[LS]\right)}{\left(\begin{array}{l}\left(\Omega_1 + \Omega_2[LP] + \Omega_3[DP]\right)\left(\Theta_4 + \Theta_5[DS] + \Theta_6[LS]\right) \\ \\ +\left(\Omega_4 + \Omega_5[DS] + \Omega_6[LS]\right)\left(\Theta_1 + \Theta_2[DP] + \Theta_3[LP]\right)\end{array}\right)} \tag{1.44}$$

Here Δ_1 and Δ_2 are defined as

$$\begin{aligned}\Delta_1 &= k_9\alpha_2\alpha_6 \\ \Delta_2 &= k_{10} - k_9\left(\alpha_2\alpha_7 + \alpha_3\right) > 0\end{aligned} \tag{1.45}$$

The rate of [LP] Production is given by

$$\frac{d[LP]}{dt} = k_{15}[ELP] - k_{16}[E][LP] \tag{1.46}$$

Putting [ELP] and [E] expressions into this equation gives us

$$\frac{d[LP]}{dt} = \left(\Delta_3[LS] - \Delta_4[LP]\right)\frac{E_0\left(\Theta_4 + \Theta_5[DS] + \Theta_6[LS]\right)}{\left(\begin{array}{l}\left(\Omega_1 + \Omega_2[LP] + \Omega_3[DP]\right)\left(\Theta_4 + \Theta_5[DS] + \Theta_6[LS]\right) \\ \\ +\left(\Omega_4 + \Omega_5[DS] + \Omega_6[LS]\right)\left(\Theta_1 + \Theta_2[DP] + \Theta_3[LP]\right)\end{array}\right)} \tag{1.47}$$

Here Δ_3 and Δ_4 are defined as

$$\begin{aligned}\Delta_3 &= k_{15}\alpha_0\alpha_4 \\ \Delta_4 &= k_{16} - k_{15}\left(\alpha_0\alpha_5 + \alpha_1\right) > 0\end{aligned} \tag{1.48}$$

Appendix IV.: Maple worksheet of derivation given on Appendix 3

```
#################################################################
```

DERIVATION OF THE MODEL UNDER STEADY STATE ASSUMPTION ON ENZYME BOUND COMPLEXES

```
#################################################################
```

```
> restart;with(Groebner):
```

Rates for individual reactions

```
v1:=k[1]*E-k[2]*EC:

v2:=k[3]*EC-k[4]*EE:

v3:=k[5]*EE*DS-k[6]*EDS:

v4:=k[7]*EDS-k[8]*EDP:

v5:=k[9]*EDP-k[10]*E*DP:

v6:=k[11]*LS*EE-k[12]*ELS:

v7:=k[13]*ELS-k[14]*ELP:

v8:=k[15]*ELP-k[16]*E*LP:
```

Mathematical Model

```
 dELP:=v7-v8;

 dEDP:=v4-v5;

> dEC:=v1-v2;

> dEEE:=v2-v3-v6;

dELS:=v6-v7;

dEDS:=v3-v4;
```

```
dDP:=v5;

dLP:=v8;
```

$$dELP := k_{13}\,ELS - k_{14}\,ELP - k_{15}\,ELP + k_{16}\,E\,LP$$

$$dEDP := k_{7}\,EDS - k_{8}\,EDP - k_{9}\,EDP + k_{10}\,E\,DP$$

$$dEC := k_{1}\,E - k_{2}\,EC - k_{3}\,EC + k_{4}\,EE$$

$$dEEE := k_{3}\,EC - k_{4}\,EE - k_{5}\,EE\,DS + k_{6}\,EDS - k_{11}\,LS\,EE + k_{12}\,ELS$$

$$dELS := k_{11}\,LS\,EE - k_{12}\,ELS - k_{13}\,ELS + k_{14}\,ELP$$

$$dEDS := k_{5}\,EE\,DS - k_{6}\,EDS - k_{7}\,EDS + k_{8}\,EDP$$

$$dDP := k_{9}\,EDP - k_{10}\,E\,DP$$

$$dLP := k_{15}\,ELP - k_{16}\,E\,LP$$

```
>
> ELP1:=collect(solve(dELP,ELP),[ELS,LP],`recursive`);

> ELP:=alpha[0]*ELS+alpha[1]*E*LP;

A0:=coeff(ELP1,ELS);

A1:=coeff(coeff(ELP1,LP),E);
```

$$ELP1 := \frac{k_{13}\,ELS}{k_{14} + k_{15}} + \frac{k_{16}\,E\,LP}{k_{14} + k_{15}}$$

$$ELP := \alpha_{0}\,ELS + \alpha_{1}\,E\,LP$$

$$A0 := \frac{k_{13}}{k_{14} + k_{15}}$$

$$A1 := \frac{k_{16}}{k_{14} + k_{15}}$$

```
> EDP1:=collect(solve(dEDP,EDP),[EDS,DP],`recursive`);;

> EDP:=alpha[2]*EDS+alpha[3]*E*DP;
```

$$EDP1 := \frac{k_{7}\,EDS}{k_{8} + k_{9}} + \frac{k_{10}\,E\,DP}{k_{8} + k_{9}}$$

$$EDP := \alpha_{2}\,EDS + \alpha_{3}\,E\,DP$$

```
> A2:=coeff(EDP1,EDS);
A3:=coeff(coeff(EDP1,DP),E);
```

$$A2 := \frac{k_7}{k_8 + k_9}$$

$$A3 := \frac{k_{10}}{k_8 + k_9}$$

```
> ELS1:=collect(solve(dELS,ELS),[EE,LS,LP],`recursive`);

> ELS:=beta[0]*LS*EE+beta[1]*E*LP;
```

$$ELS1 := \frac{k_{11}\, LS\, EE}{k_{12} + k_{13} - k_{14}\,\alpha_0} + \frac{k_{14}\,\alpha_1\, E\, LP}{k_{12} + k_{13} - k_{14}\,\alpha_0}$$

$$ELS := \beta_0\, LS\, EE + \beta_1\, E\, LP$$

```
> B0:=simplify(subs(alpha[0]=A0,coeff(coeff(ELS1,LS),EE)));

B1:=simplify(subs(alpha[0]=A0,alpha[1]=A1,coeff(coeff(ELS1,LP)
,E)));
```

$$B0 := \frac{k_{11}\left(k_{14} + k_{15}\right)}{k_{12}\,k_{14} + k_{12}\,k_{15} + k_{13}\,k_{15}}$$

$$B1 := \frac{k_{16}\,k_{14}}{k_{12}\,k_{14} + k_{12}\,k_{15} + k_{13}\,k_{15}}$$

```
>

EDS1:=collect(subs(a2=A2,a3=A3,solve(dEDS,EDS)),[EE,DS,DP],`re
cursive`);

> EDS:=beta[2]*DS*EE+beta[3]*E*DP;

B2:=simplify(coeff(coeff(EDS1,EE),DS));

B3:=simplify(coeff(coeff(EDS1,DP),E));
```

$$EDS1 := \frac{k_5\, DS\, EE}{k_6 + k_7 - k_8\,\alpha_2} + \frac{k_8\,\alpha_3\, E\, DP}{k_6 + k_7 - k_8\,\alpha_2}$$

$$EDS := \beta_2\, DS\, EE + \beta_3\, E\, DP$$

$$B2 := \frac{k_5}{k_6 + k_7 - k_8\,\alpha_2}$$

$$B3 := \frac{k_8\,\alpha_3}{k_6 + k_7 - k_8\,\alpha_2}$$

```
> EC1:=collect(solve(dEC,EC),[E,EE],`recursive`);
```

```
> EC:=alpha[8]*E+alpha[9]*EE;

A8:=coeff(EC1,E);

A9:=coeff(EC1,EE);

>
```

$$EC1 := \frac{k_1 E}{k_2 + k_3} + \frac{k_4 EE}{k_2 + k_3}$$

$$EC := \alpha_8 E + \alpha_9 EE$$

$$A8 := \frac{k_1}{k_2 + k_3}$$

$$A9 := \frac{k_4}{k_2 + k_3}$$

```
> EE1:=collect(solve(dEEE,EE),[E,EE],`recursive`);
```

$$EE1 := \frac{E\left(k_3 \alpha_8 + k_{12} \beta_1 LP + k_6 \beta_3 DP\right)}{-k_3 \alpha_9 + k_4 - k_6 \beta_2 DS + k_{11} LS + k_5 DS - k_{12} \beta_0 LS}$$

```
> g00:=coeff(EE1,E);
```

$$g00 := \frac{k_3 \alpha_8 + k_{12} \beta_1 LP + k_6 \beta_3 DP}{-k_3 \alpha_9 + k_4 - k_6 \beta_2 DS + k_{11} LS + k_5 DS - k_{12} \beta_0 LS}$$

```
>
G0:=(Theta1+Theta2*DP+Theta3*LP)/(Theta4+Theta5*DS+Theta6*LS);
```

$$G0 := \frac{\Theta 1 + \Theta 2\, DP + \Theta 3\, LP}{\Theta 4 + \Theta 5\, DS + \Theta 6\, LS}$$

```
> Theta1:=coeff(coeff(numer(g00),LP,0),DP,0);
Theta2:=coeff(numer(g00),DP);
Theta3:=coeff(numer(g00),LP);
Theta4:=coeff(coeff(denom(g00),DS,0),LS,0);
Theta5:=coeff(denom(g00),DS);
Theta6:=coeff(denom(g00),LS);
```

$$\Theta 1 := k_3 \alpha_8$$

$$\Theta 2 := k_6 \beta_3$$

$$\Theta 3 := k_{12} \beta_1$$

$$\Theta 4 := -k_3 \alpha_9 + k_4$$

$$\Theta 5 := -k_6 \beta_2 + k_5$$

$$\Theta 6 := k_{11} - k_{12} \beta_0$$

```
> EEx:=g0*E;
```

$$EEx := g0\ E$$

```
> EE2:=collect(subs(EE=EEx,EC),E);
```

$$EE2 := \left(\alpha_8 + \alpha_9\ g0\right) E$$

```
> ELS2:=collect(subs(EE=EEx,ELS),E);
```

$$ELS2 := \left(\beta_0\ LS\ g0 + \beta_1\ LP\right) E$$

```
> ELP2:=collect(subs(EE=EEx,ELP),E);
```

$$ELP2 := \left(\alpha_0\ \left(\beta_0\ LS\ g0 + \beta_1\ LP\right) + \alpha_1\ LP\right) E$$

```
> EDP2:=collect(subs(EE=EEx,EDP),[E,DP],`recursive`);
```

$$EDP2 := \left(\left(\alpha_2\ \beta_3 + \alpha_3\right) DP + \alpha_2\ \beta_2\ DS\ g0\right) E$$

```
> E1:=solve(subs(EE=EEx,E[0]=(E+EC+EE+EDS+EDP+ELP+ELS)),E);
```

$$E1 := E_0 \Big/ \left(1 + \alpha_8 + \alpha_9\ g0 + g0 + \beta_2\ DS\ g0 + \beta_3\ DP + \alpha_2\ \beta_2\ DS\ g0 + \alpha_2\ \beta_3\ DP + \alpha_3\ DP + \alpha_0\ \beta_0\ LS\ g0 + \alpha_0\ \beta_1\ LP + \alpha_1\ LP + \beta_0\ LS\ g0 + \beta_1\ LP\right)$$

```
>
E2:=E[0]/(Omega1+Omega2*LP+Omega3*DP+(Omega4*DS+Omega5+Omega6*
LS)*g0);
```

$$E2 := \frac{E_0}{\Omega 1 + \Omega 2\ LP + \Omega 3\ DP + \left(\Omega 4\ DS + \Omega 5 + \Omega 6\ LS\right) g0}$$

```
> Omega1:=coeff(coeff(coeff(denom(E1),LP,0),g0,0),DP,0);
Omega2:=collect(coeff(denom(E1),LP),beta[1]);
Omega3:=collect(coeff(denom(E1),DP),beta[3]);
Omega4:=collect(coeff(coeff(coeff(denom(E1),g0,1),DS,0),LS,0),
beta[3]);
Omega5:=collect(coeff(coeff(denom(E1),g0,1),DS),beta[2]);
Omega6:=collect(coeff(coeff(denom(E1),g0,1),LS),beta[0]);
```

$$\Omega 1 := 1 + \alpha_8$$

$$\Omega 2 := \left(\alpha_0 + 1\right) \beta_1 + \alpha_1$$

$$\Omega 3 := \left(1 + \alpha_2\right) \beta_3 + \alpha_3$$

$$\Omega 4 := \alpha_9 + 1$$

$$\Omega 5 := \left(1 + \alpha_2\right) \beta_2$$

$$\Omega 6 := \left(\alpha_0 + 1\right) \beta_0$$

Rate equations

DP production rate:

```
v1:=collect(collect(subs(EE=EEx,dDP),[DP]),E);
```

$$v1 := \left(\left(k_9 \left(\alpha_2 \beta_3 + \alpha_3\right) - k_{10}\right) DP + k_9 \alpha_2 \beta_2 DS\, g0\right) E$$

```
> Delta[1]:=coeff(coeff(coeff(v1,DS),E),g0);
> Delta[2]:=coeff(coeff(v1,DP),E);
Delta[11]:=simplify((subs(alpha[2]=A2,(subs(beta[2]=B2,Delta[1
]))));
Delta[22]:=simplify((subs(alpha[2]=A2,subs(alpha[3]=A3,(subs(b
eta[3]=B3,Delta[2])))));
```

$$\Delta_1 := k_9 \alpha_2 \beta_2$$

$$\Delta_2 := k_9 \left(\alpha_2 \beta_3 + \alpha_3\right) - k_{10}$$

$$\Delta_{11} := \frac{k_5 k_7 k_9}{k_6 k_8 + k_6 k_9 + k_7 k_9}$$

$$\Delta_{22} := -\frac{k_8 k_6 k_{10}}{k_6 k_8 + k_6 k_9 + k_7 k_9}$$

LP production rate:

```
v2:=collect(collect(subs(EE=EEx,dLP),[LP]),E);
```

$$v2 := \left(\left(k_{15} \left(\alpha_0 \beta_1 + \alpha_1\right) - k_{16}\right) LP + k_{15} \alpha_0 \beta_0 LS\, g0\right) E$$

```
> Delta[3]:=coeff(coeff(coeff(v2,LS),E),g0);
Delta3:=simplify((subs(alpha[0]=A0,(subs(beta[0]=B0,Delta[3]))
)));
```

$$\Delta_3 := k_{15} \alpha_0 \beta_0$$

$$\Delta 3 := \frac{k_{15} k_{13} k_{11}}{k_{12} k_{14} + k_{12} k_{15} + k_{13} k_{15}}$$

```
> Delta[4]:=coeff(coeff(v2,LP),E);
Delta4:=simplify((subs(alpha[1]=A1,alpha[0]=A0,(subs(beta[1]=B
1,Delta[4])))));
```

$$\Delta_4 := k_{15} \left(\alpha_0 \beta_1 + \alpha_1\right) - k_{16}$$

$$\Delta 4 := -\frac{k_{14} k_{12} k_{16}}{k_{12} k_{14} + k_{12} k_{15} + k_{13} k_{15}}$$

>

Appendix V. Derivation of Enantioselectivity based on Mechanistic Model

The rate expressions found in model were;

$$\frac{d[DP]}{dt} = \frac{E_o(\Delta_1[DS]-\Delta_2[DP])(\Theta_4+\Theta_5[DS]+\Theta_6[LS])}{(\Omega_1+\Omega_2[LP]+\Omega_3[DP])(\Theta_4+\Theta_5[DS]+\Theta_6[LS])+(\Omega_4+\Omega_5[DS]+\Omega_6[LS])(\Theta_1+\Theta_2[DP]+\Theta_3[LP])} \quad \text{(App.1)}$$

$$\frac{d[LP]}{dt} = \frac{E_o(\Delta_3[LS]-\Delta_4[LP])(\Theta_4+\Theta_5[DS]+\Theta_6[LS])}{(\Omega_1+\Omega_2[LP]+\Omega_3[DP])(\Theta_4+\Theta_5[DS]+\Theta_6[LS])+(\Omega_4+\Omega_5[DS]+\Omega_6[LS])(\Theta_1+\Theta_2[DP]+\Theta_3[LP])} \quad \text{(App.2)}$$

$$\frac{d[DP]}{dt} = -\frac{d[DS]}{dt}$$
$$\frac{d[LP]}{dt} = -\frac{d[LS]}{dt}$$

By the definition of enantioselectivity, it is the ratio of both enantioselective rate,

$$\frac{d[DS]}{d[LS]} = \frac{(\Delta_1[DS]-\Delta_2[[DS]_o-[DS]])}{(\Delta_3[LS]-\Delta_4[[LS]_o]-[LS])} \quad \text{(App 3)}$$

$$\frac{d[DS]}{d[LS]} = \frac{(\Delta_1+\Delta_2)[DS]-\Delta_2[DS]_o}{(\Delta_3+\Delta_4)[LS]-\Delta_{42}[LS]_o} \quad \text{(App.4)}$$

Taking the numerator and denominator by $\Delta_1$and Δ_3, the it is obtained as,

$$\frac{d[DS]}{d[LS]} = \frac{\Delta_1\left(1+\frac{\Delta_2}{\Delta_1}\right)[DS]-\left(\frac{\Delta_2}{\Delta_1}\right)[DS]_o}{\Delta_3\left(1+\frac{\Delta_4}{\Delta_3}\right)[LS]-\left(\frac{\Delta_4}{\Delta_3}\right)[LS]_o} \quad \text{(App.5)}$$

Substituting Δ_1, Δ_2, $\Delta_3$and Δ_4 from Appendix 4 in Maple worksheet it simplifies to be the multiplication of equilibrium constants of each racemate.

$$\frac{\Delta_2}{\Delta_1} = K_{eq,DS} = \frac{k_6 k_8 k_{10}}{k_5 k_7 k_9} \quad \text{(App.6)}$$

$$\frac{\Delta_4}{\Delta_3} = K_{eq,LS} = \frac{k_{12} k_{14} k_{16}}{k_{11} k_{13} k_{15}} \quad \text{(App.7)}$$

If enantiomeric ratio is defined to be the ratio of rate constants; $\frac{\Delta_1}{\Delta_3}$

$$E = \frac{\Delta_1}{\Delta_3} = \frac{k_5 k_7 k_9 (k_{12} k_{14} + k_{12} k_{15} + k_{13} k_{15})}{k_{11} k_{13} k_{15} (k_6 k_8 + k_6 k_9 + k_7 k_9)} \quad \text{(App.8)}$$

Substituting equations (App,6,7,8) into eqn(App.5);

$$\frac{d[DS]}{d[LS]} = E \frac{(1+K_{eq,DS})[DS] - K_{eq,DS}[DS]_o}{(1+K_{eq,LS})[LS] - K_{eq,ls}[LS]_o} \quad \text{(App.9)}$$

This equation can now become explicit and arranging for DS; LS, it becomes as;

$$\frac{d[DS]}{(1+K_{eq,DS})[DS] - K_{eq,DS}[DS]_o} = E \frac{d[LS]}{(1+K_{eq,LS})[LS] - K_{eq,ls}[LS]_o} \quad \text{(App.10)}$$

Integration of (App.10) is in the form of

$$\int_{x_o}^{x} \frac{1}{(a*x-b)} dx = \frac{1}{a} ln \frac{(ax-b)}{ax_o-b)}$$

$$E = \frac{(1+K_{LS})}{(1+K_{DS})} \frac{\ln((1+K_{DS})\frac{DS}{DS_0} - K_{DS})}{\ln((1+K_{LS})\frac{LS}{LS_0} - K_{LS})} \quad \text{(App.11)}$$

a) If D-racemate is irrversible, L-racemate reversible

Since none of forward rate constant could not be zore, the situations where any of the reversible reaction has been considered. When anyone of the reversible reaction on DS loop, k6=k8, k10 = 0, then $K_{eq,DS}$ becomes zero and Enantioselectivity turns to be

If right loop on scheme becomes irreversible, any of the rate is irreversible (e.g. inhibited)

$$E = \frac{(1+K_{eq,LS})\ln(\frac{DS}{DS_0})}{\ln((1+K_{eq,LS})\frac{LS}{LS_0}-K_{eq,LS})} \quad \text{(App.12)}$$

b) If D-racemate reversible, L-rac irreversible

If any of reversible rate constant on LS loop is zero, k12=k14=k16 = zero

$$E = \frac{\ln((1+K_{DS})\frac{DS}{DS_0}-K_{DS})}{(1+K_{DS})\ln(\frac{LS}{LS_0})} \quad \text{(App.13)}$$

c) If enzyme irreversibly reacts with enantiomers

d)

For an irreversible enantioselective reaction,

For case study rate expression :

$$\frac{d[DS]}{d[LS]} = \frac{k_5}{k_{11}}\frac{DS}{LS} \qquad \frac{d[DS]}{d[LS]} = E\frac{DS}{LS} \quad \text{(App.14)}$$

This is same as Chen and Sih definition , then

$$E = \frac{k_5}{k_{11}} \quad \text{(App.15)}$$

For the specific case, where both enantiomers are irreversible k6 and k12 are zero the general

Appendix VI.: List of Chemicals

Racemic IPG ($C_6H_{12}O_3$) , with synonyms
1,2-isopropylidene glycerol,
Solketal = 2,2-Dimethyl-4-hydroxymethyl-1,3-dioxolane (TFA),
acetone gylcerol ,
glycerol dimethylketal ,

Chemicals

Aceton n-Hexan D-L-- Isopropylidene glycerol	Fluka, Buchs, Switzerland
Carbondioxide (CO_2)	Linde
Isopropylidene glycerol, Solketal Enantiomers	Aldrich
Vinylacetate	Merck, Darmstadt, Germany
Lipase	Amano, Nagoya,Japan

Appendix VII. Apparatus used in Experiments

Sampling Appratus : Vortexer Heidopf

Zentrifuge Eppendorf

Components of High Pressure Reactor Unit for $scCO_2$ system

High pressure pump (mini-pump duplex)	NSI 33R ,Milton-Roy
Circulation pump	PKP P250V 225 BTG, Buddeber
Thermostate,	RM 6 (-15 C° +100 C°) mgw, Lauda
Capillary pipes Knauer	stainless steel, 1/16 AD 10 mm ID,
Ferrule	stainless steel, Knauer
O- Ring ,	Viton 500 Balster , Otto Gehrkens GmbH
Manometer	Hensing&Salmon
Needle valve	Mill Mite, 1/16 Hoke
Needle valve	Two Way, SSI
Needle valve	ERC, Altegolfsheim, Germany
6-Port/ 3-Channel-Injection valve	Knauer
7-Port- distrubutor	Knauer
Probenschlaufe 100 µl	Knauer
Sapphire glass window	Spindler & Hoyer

Appendix VIII. Gas Chromatography

In this work, the gas chromatography CC-14A (Shimadzu) with a terminal C-R4AX CHROMATOPAC from the same company has been used.

The column of FS-Hydrodex® ß-3P (Heptakis(2,6-di-Omethyl-3-O-pentyl)-ß-cyclodextrin) with 25 m length inner diameter of 0,25 mm (Macherey-Nagel) was utilized for the analysis of chiral components.

7.1. *Adjustment oft he Chromatography*

Carrier Gas H_2	1,2	bar
Synthetic airt	0,65	bar
Make-up Gas	0,5	bar
Injector temperatur	180	°C
Detector temperatur	250	°C
Injection volume	1	µl

7.2 *Retention time*

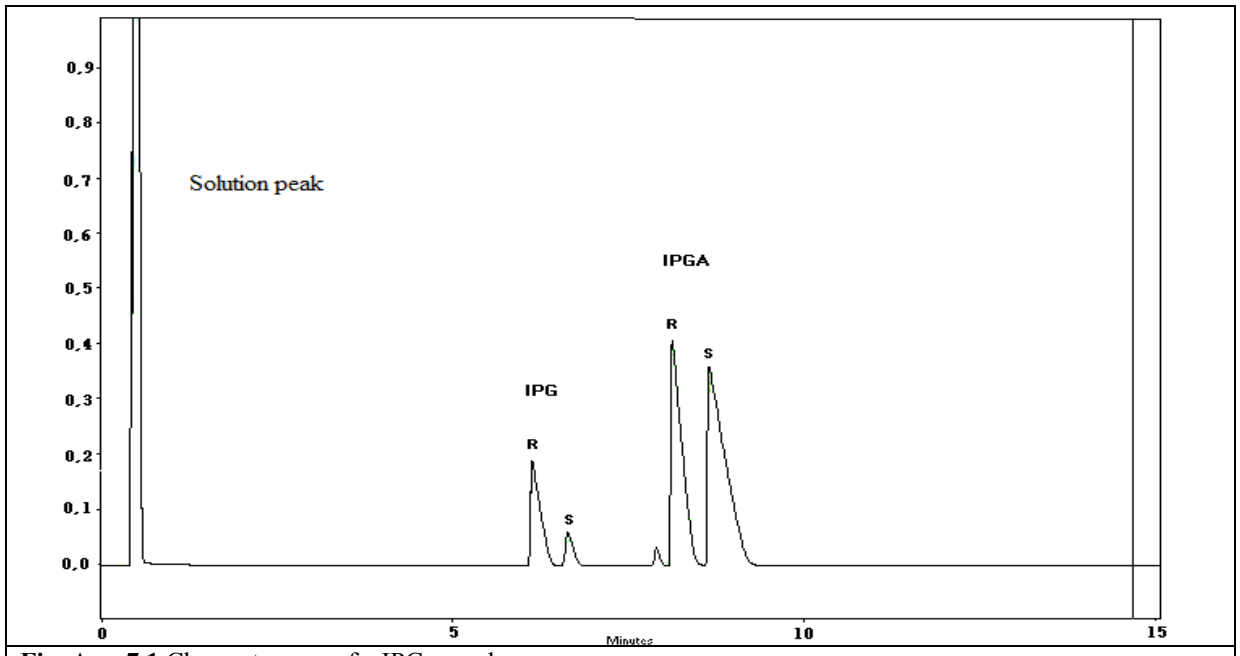


Fig .App 7.1 Chromatogram of a IPG sample

(D)-IPG	6, 13	min
(L)-IPG	6, 45	min
(D)-IPGA	8, 24	min
(L)-IPGA	8, 83	min

Appendix IX. Derivation of rate expressions for lipase catalyzed IPG reaction.

```
> restart;with(Groebner):                    ## (1) ##
k[2]:=0:k[4]:=0:k[6]:=0:k[12]:=0:k[16]:=0:k[10]:=0:
```

Rates for individual reactions

```
v1:=k[1]*C[0]*E-k[2]*EC:
v2:=k[3]*EC-k[4]*EE:
v3:=k[5]*EE*DS-k[6]*EDS:
v4:=k[7]*EDS-k[8]*EDP:
v5:=k[9]*EDP-k[10]*E*DP:
v6:=k[11]*LS*EE-k[12]*ELS:
v7:=k[13]*ELS-k[14]*ELP:
v8:=k[15]*ELP-k[16]*E*LP:
eqE:=E[0]-(E+EC+EE+EDS+EDP+ELP+ELS):
```

Mathematical Model

```
dELP:=v7-v8;
dEDP:=v4-v5;
> dEC:=v1-v2;
> dEEE:=v2-v3-v6;
dELS:=v6-v7;
dEDS:=v3-v4;
dDP:=v5;
dLP:=v8;
```

$$dELP := k_{13}\,ELS - k_{14}\,ELP - k_{15}\,ELP$$
$$dEDP := k_{7}\,EDS - k_{8}\,EDP - k_{9}\,EDP$$
$$dEC := k_{1}\,C_{0}\,E - k_{3}\,EC$$
$$dEEE := k_{3}\,EC - k_{5}\,EE\,DS - k_{11}\,LS\,EE$$
$$dELS := k_{11}\,LS\,EE - k_{13}\,ELS + k_{14}\,ELP$$
$$dEDS := k_{5}\,EE\,DS - k_{7}\,EDS + k_{8}\,EDP$$
$$dDP := k_{9}\,EDP$$
$$dLP := k_{15}\,ELP$$

```
> E:=solve(E0-(E+EC+EE+EDS+EDP+ELP+ELS),E);
```

$$E := E0\,(-k_3\,\alpha_9 + k_5\,DS + k_{11}\,LS) \Big/ (k_3\,\alpha_8\,C_0 + \alpha_7\,DP\,k_{11}\,LS + \alpha_5\,LP\,k_5\,DS + \alpha_5\,LP\,k_{11}\,LS + \alpha_8\,C_0\,k_5\,DS + \alpha_8\,C_0\,k_{11}\,LS - \alpha_3\,DP\,k_3\,\alpha_9 + \alpha_3\,DP\,k_5\,DS + \alpha_3\,DP\,k_{11}\,LS - \alpha_1\,LP\,k_3\,\alpha_9 + \alpha_1\,LP\,k_5\,DS + \alpha_1\,LP\,k_{11}\,LS - \alpha_2\,\alpha_7\,DP\,k_3\,\alpha_9 + \alpha_2\,\alpha_7\,DP\,k_5\,DS + \alpha_2\,\alpha_7\,DP\,k_{11}\,LS - \alpha_0\,\alpha_5\,LP\,k_3\,\alpha_9 + \alpha_0\,\alpha_5\,LP\,k_5\,DS + \alpha_0\,\alpha_5\,LP\,k_{11}\,LS + k_{11}\,LS + k_5\,DS - k_3\,\alpha_9 + \alpha_6\,DS\,k_3\,\alpha_8\,C_0 + \alpha_7\,DP\,k_5\,DS - \alpha_7\,DP\,k_3\,\alpha_9 + \alpha_4\,LS\,k_3\,\alpha_8\,C_0 - \alpha_5\,LP\,k_3\,\alpha_9 + \alpha_2\,\alpha_6\,DS\,k_3\,\alpha_8\,C_0 + \alpha_0\,\alpha_4\,LS\,k_3\,\alpha_8\,C_0)$$

>

```
dLPfull:=subs(alpha[0]=A0,alpha[1]=A1,alpha[2]=A2,alpha[3]=A3,alpha[4]=A4,a
lpha[5]=A5,alpha[6]=A6,alpha[7]=A7,alpha[8]=A8,alpha[9]=A9,dLP);
```

$$dLPfull := k_{11}\,LS\,k_1\,C_0\,E0 \Big/ \left(k_1\,C_0 + \frac{k_1\,C_0\,k_5\,DS}{k_3} + \frac{k_1\,C_0\,k_{11}\,LS}{k_3} + k_{11}\,LS + k_5\,DS + \frac{k_{11}\,LS\,k_1\,C_0}{k_{15}} + \frac{k_5\,DS\,k_1\,C_0}{k_9} + \frac{k_5\,(k_8+k_9)\,DS\,k_1\,C_0}{k_7\,k_9} + \frac{k_{11}\,(k_{14}+k_{15})\,LS\,k_1\,C_0}{k_{13}\,k_{15}}\right)$$

>

```
LPn:=subs(alpha[0]=A0,alpha[1]=A1,alpha[2]=A2,alpha[3]=A3,alpha[4]=A4,alpha
[5]=A5,alpha[6]=A6,alpha[7]=A7,alpha[8]=A8,alpha[9]=A9,numer(dLP));
```

$$LPn := E0\,k_{11}\,LS\,k_1\,C_0$$

>

```
LPd:=collect(simplify(subs(alpha[0]=A0,alpha[1]=A1,alpha[2]=A2,alpha[3]=A3,
alpha[4]=A4,alpha[5]=A5,alpha[6]=A6,alpha[7]=A7,alpha[8]=A8,alpha[9]=A9,den
om(dLP))),[LS,DS,C[0]],'recursive');
```

$$LPd := \left(\frac{(k_1\,k_{11}\,k_3\,k_9\,k_7\,k_{13} + k_1\,k_{11}\,k_{15}\,k_9\,k_7\,k_{13} + k_{11}\,k_1\,k_3\,k_9\,k_7\,k_{14} + k_{11}\,k_1\,k_3\,k_9\,k_7\,k_{15})\,C_0}{k_3\,k_{15}\,k_9\,k_7\,k_{13}} + k_{11}\right) LS + \left(\frac{(k_1\,k_5\,k_{15}\,k_9\,k_7\,k_{13} + k_1\,k_5\,k_3\,k_{15}\,k_7\,k_{13} + k_5\,k_1\,k_3\,k_{15}\,k_{13}\,k_8 + k_5\,k_1\,k_3\,k_{15}\,k_{13}\,k_9)\,C_0}{k_3\,k_{15}\,k_9\,k_7\,k_{13}} + k_5\right) DS + k_1\,C_0$$

>

> `dLP/dt=k1*k11*E0*C0*LS/(k1*C0+(Km1*C0+k11)*LS+(Km2*C0+k5)*DS`;

$$dLP/dt = k1*k11*E0*C0*LS/(k1*C0+(Km1*C0+k11)*LS+(Km2*C0+k5)*DS$$

>

>

```
dDPfull:=subs(alpha[0]=A0,alpha[1]=A1,alpha[2]=A2,alpha[3]=A3,alpha[4]=A4,a
lpha[5]=A5,alpha[6]=A6,alpha[7]=A7,alpha[8]=A8,alpha[9]=A9,dDP);
```

$$dDPfull := k_5\,DS\,k_1\,C_0\,E0 \Big/ \left(k_1\,C_0 + \frac{k_1\,C_0\,k_5\,DS}{k_3} + \frac{k_1\,C_0\,k_{11}\,LS}{k_3} + k_{11}\,LS + k_5\,DS + \frac{k_{11}\,LS\,k_1\,C_0}{k_{15}} + \frac{k_5\,DS\,k_1\,C_0}{k_9} + \frac{k_5\,(k_8+k_9)\,DS\,k_1\,C_0}{k_7\,k_9} + \frac{k_{11}\,(k_{14}+k_{15})\,LS\,k_1\,C_0}{k_{13}\,k_{15}}\right)$$

>

```
DPn:=subs(alpha[0]=A0,alpha[1]=A1,alpha[2]=A2,alpha[3]=A3,alpha[4]=A4,alpha
[5]=A5,alpha[6]=A6,alpha[7]=A7,alpha[8]=A8,alpha[9]=A9,numer(dDP));
```

$$DPn := E0\, k_5\, DS\, k_1\, C_0$$

>

```
DPd:=collect(simplify(subs(alpha[0]=A0,alpha[1]=A1,alpha[2]=A2,alpha[3]=A3,
alpha[4]=A4,alpha[5]=A5,alpha[6]=A6,alpha[7]=A7,alpha[8]=A8,alpha[9]=A9,den
om(dDP))),[LS,DS,C[0]],'recursive');
```

$$DPd := \left(\frac{(k_1 k_{11} k_3 k_9 k_7 k_{13} + k_1 k_{11} k_{15} k_9 k_7 k_{13} + k_{11} k_1 k_3 k_9 k_7 k_{14} + k_{11} k_1 k_3 k_9 k_7 k_{15})\, C_0}{k_3 k_{15} k_9 k_7 k_{13}} + k_{11} \right) LS + \left(\frac{(k_1 k_5 k_{15} k_9 k_7 k_{13} + k_1 k_5 k_3 k_{15} k_7 k_{13} + k_5 k_1 k_3 k_{15} k_{13} k_8 + k_5 k_1 k_3 k_{15} k_{13} k_9)\, C_0}{k_3 k_{15} k_9 k_7 k_{13}} + k_5 \right) DS + k_1\, C_0$$

>

$$v_{DS} = -\frac{d[DS]}{dt} = \frac{k_1 k_5 C_o E_o [DS]}{(K_{M1} C_o + k_{11})[LS] + (K_{M2} C_o + k_5)[DS] + k_1 C_0}$$

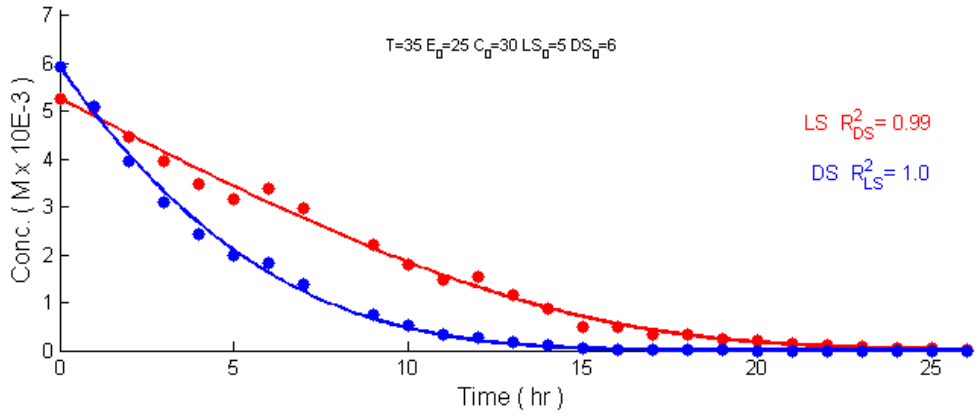
$$v_{LS} = -\frac{d[LS]}{dt} = \frac{k_1 k_{11} C_o E_o [LS]}{(K_{M1} C_o + k_{11})[LS] + (K_{M2} C_o + k_5)[DS] + k_1 C_0}$$

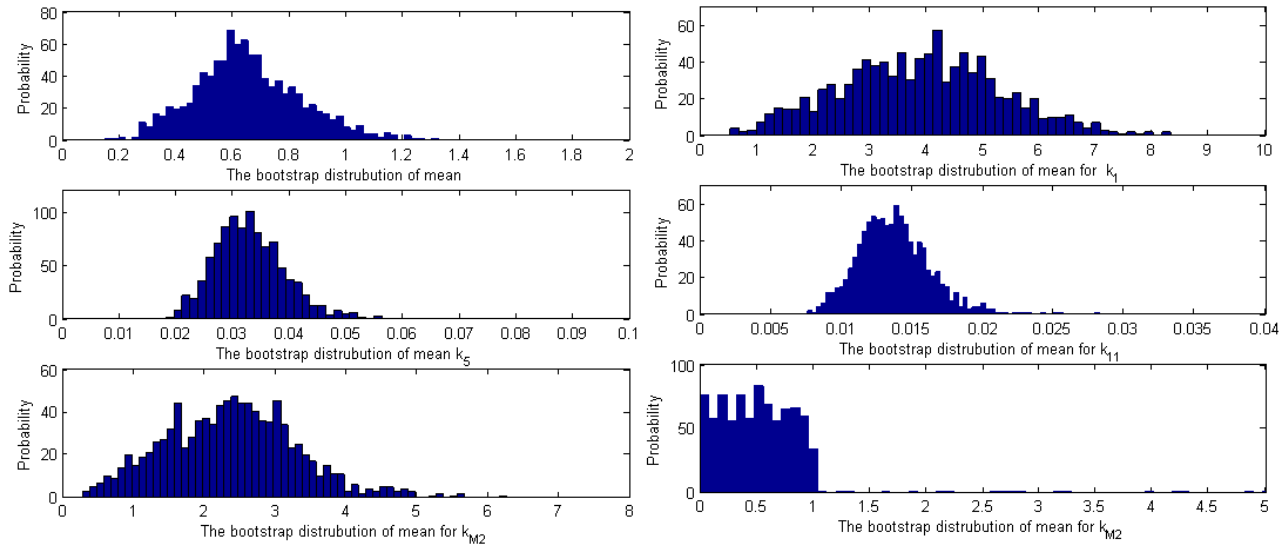
$$K_{M1} = \left(\frac{k_1 k_3 k_7 k_9 k_{11} k_{13} + k_1 k_7 k_9 k_{11} k_{13} k_{15} + k_1 k_3 k_7 k_9 k_{11} k_{14} + k_1 k_3 k_7 k_9 k_{11} k_{15}}{k_3 k_7 k_9 k_{13} k_{15}} \right) C_o + k_{11}$$

$$K_{M2} = \left(\frac{k_1 k_5 k_7 k_9 k_{13} k_{15} + k_1 k_3 k_5 k_7 k_{13} k_{15} + k_1 k_3 k_5 k_8 k_{13} k_{15} + k_1 k_3 k_5 k_9 k_{13} k_{15}}{k_3 k_7 k_9 k_{13} k_{15}} \right) C_o + k_5$$

Appendix X. Bootstrap histogram for estimations of thereaction rate constants

A sample histogram for the determination of the parameters was given for the experiment carried out at 35 °C and 25 mg of lipase in 30 mM vinly acetate.





	Bootstap mean	Standard error
mean	0.7726	±0.1193
k_1(1/h	3.8939	±0.0447
k_5(1/h	0.0331	±0.0002
k_{11}(1/h)	0.0169	±0.0032
K_{M1}(mmol/L)	2.3835	±0.0296
K_{M2}(mmol/L)	0.2553	±0.0138

Appendix XI. Derivation of the rates for the non-enantiomeric single reactions.

In this derivation, the results obtained in Appendix IX. Have been rearranged.

For single DS and LS forms,

a) If only DS exits, where LS = 0,

LS terms in the denominator disappears and rate becomes

$$\nu_{DS} = -\frac{d[DS]}{dt} = \frac{k_1 k_5 C_o E_o [DS]}{(K_{M2} C_o + k_5)[DS] + k_1 C_0}$$

Dividing numerator and denominator by $(K_{M2} C_o + k_5)$, it is obtained that;

$$\nu_{DS} = -\frac{d[DS]}{dt} = \frac{\frac{k_1 k_5 C_o E_o}{(K_{M2} C_o + k_5)}[DS]}{[DS] + \frac{k_1 C_0}{(K_{M2} C_o + k_5)}}$$

This is the Michaelis –Menten form where V_{max} and K_M are defined as;

$$V_{max,DS} = \frac{k_1 k_5 C_o E_o}{(K_{M2} C_o + k_5)}$$

$$K_{M,DS} = \frac{k_1 C_0}{(K_{M2} C_o + k_5)}$$

Thus the rate of the single DS can be written as,

$$\nu_{DS} = -\frac{d[DS]}{dt} = \frac{V_{max}[DS]}{K_M + [DS]}$$

b) If only LS exits, where DS =0

By the same prodecure explained above, then the rate of the LS is;

$$\nu_{LS} = -\frac{d[LS]}{dt} = \frac{V_{max,LS}[LS]}{K_{M,LS} + [LS]}$$

$$V_{max,LS} = \frac{k_1 k_{11} C_o E_o}{(K_{M1} C_o + k_{11})}$$

$$K_{M,LS} = \frac{k_1 C_0}{(K_{M1} C_o + k_{11})}$$

XI- References

[1] "Biotechnology At Work" and "Biotechnology in Perspective," Washington, D.C.: Biotechnology Industry Organization, 1989, 1990 [PDF]

[2] Diamond, Jared, "Guns, germs, and steel – The Fates of Human Societies", W.W. Norton & Company Inc., N.Y, (1997); ISBN : 0-393 – 03891 – 2

[3] Wallman, Sonia, A Short History of Biotechnology,NHCTC. 1997 [PDF]

[4] What is Biotechnology ? , Pamela Peters, from Biotechnology: A Guide To Genetic Engineering., Wm. C. Brown Publishers, Inc., 1993. [PDF]

[5] http://www.ncbiotech.org/biotech101/timeline.cfm http://www.bio.org/er/timeline.asp http://www.accessexcellence.org/AB/BC/1977- http://www.biotechinstitute.org/what_is/timeline.html [PDF]

[6] Madden, Dean , Food Biotechnology: An Introduction, International Life Sciences Institute and ILSI Europe, ISBN 0-944398-62-6 , 1995, Belgium , I LSI Europe Concise Monograph Series [PDF]

[7] Fari, M.G., Bud, R., Kralovanszky, P.U, History of Term Biotechnology: K.Ereky & his Contriubtion Agricultical Biotechnology Center, Szent-György, Hungary [PDF]

[8] Bhoojedhur, S., ***et.al.*** Biotechnology Report of Mauritius Research Council, University of Mauritius, September,2001, [PDF]

[9] Dahms, A. Stephen, " Biotechnology: What It Is, What It Is Not, and the Challenges in Reaching a National or Global Consensus ", *Biochem. Mol. Bio. Education,* Vol. 32, No. 4, pp. 271–278, 2004 [PDF]

[10] Lennox P, Thiele J H, Kennedy M, Underwood S, Culliford J, Walker B, Christensen M, Jamieson I & Battye J, Biotechnology for New Zealand engineers: A review. *Biotechnology for NZ Engineers*. IPENZ 2002-5,(2003) [PDF]

[11] Karen Laney-Cummings, ***et.al.***, A Survey of the Use of Biotechnology in U.S. Industry U.S. Department of Commerce Technology Administration, Bureau of Industry and Security, Oct. 2003 [PDF]

[12] Da Silva, E.J., Baydoun, E., Badran, A. , "Biotechnology and the developing world" *EJB Electronic Journal of Biotechnology* ISSN: 0717-3458 Vol.5 No.1, Iss. April 15, 2002. [PDF]

[13] Krall, A., Biotechnology in Hessen, Survey 2002/03, Wiesbaden, October 2003 Hessisches Ministrium für Wirtschaft, Verkehr und Landesentwicklung by M-Result GmbH Mainz [PDF]

[14] Oakley, P., Green Biotechnology, August 2005, BASF, [PDF]

[15] Bellmann, K., Johannes Gutenberg Universität, Mainz, White Biotech in Germany- Chances and Obstacles, Presentation in Tokyo, 20th May 2005, [PDF]

[16] Williamson, M.P., "Systems biology: will it work?, " Biochemical Society Transactions, vol.33(3), pp: 503 – 516 , (2005) [PDF]

[17] Fuyuno, I., "Systems Biology Research in Japan", Reports by S&I Section in the British Embassy Tokyo [PDF]

[18] Hiroaki Kitano, "Computational systems biology". Nature, vol. 420, pp: 206 – 210, (2002) [PDF]

[19] Hucka, A. Finney, Sauro, H. M. , "The systems biology markup language (SBML): a medium for representation and exchange of biochemical network models " , Bioinformatics, vol.19(4), pp: 524 – 531, (2003) , [PDF]

[20] Stein, M., Gabdoulline, R.R., Wade, R.C., „Integrating Structural and Kinetic Enzymatic Information in Systems Biology, pp: 129 – 131 , NIC Series, Vol. 34, ISBN-10: 3-9810843-0-6,(2006), [PDF]

[21] Suttie, John W., Introduction to Biochemistry, 2nd ed., , Holt-Saunders Int. Ed., 1982, ISBN: 0-03-048706-4

[22] Hoffmann-Ostenhof, O., "100 Years ago: The origin of the term Enzyme ", Trends Biochem. Sci. 3(3), pp: 186 – 187,(1978), [PDF]

[23] Mrs. Fullhame, "An Assay to combustion with a view to a new art of dying and painting ", London, (1794)

[24] Maria-Regina Kula, P:" Enzyme Catalysis in Organic Synthesis " , Karlheinz Draw and Herbert Waldmann, ISBN 3-527-30434-7, 2nd ed.Wiley-VCH Verlag GmbH, Weinheim, (2002)

[25] Bairoch A., The ENZYME database in 2000, Nucleic Acids Res., vol. 28(1), pp: 304-305, (2000).[PDF]

[26] Lubert Stryer John L. Tymoczko Jeremy Mark Berg , Biochemistry , ISBN: 0716730510, W. H. Freeman Company - February 2002

[27] Maugh, T.H. , "Need a Catalyst? Design an Enzyme", Science, New Series, Vol. 223(4633),, pp. 269-271., (1984), [PDF]

[28] Whitesides, G. M., Wong, C.H. , "Enzymes as Catalysts in Synthetic Organic Chemistry ", Angewandte Chem. Int. Ed., Vol. 24(8), pp: 617-638, (1985), [PDF]

[29] Cornish-Bowden, Athel, Two centuries of catalysis, *J.Biosci*., 23(2), pp: 87-92, (1998), [PDF]

[30] Laidler, Keith J. , "A Brief History of Enzyme Kinetics „ Reprinted from *New Beer in an Old Bottle: Eduard Buchner and the Growth of Biochemical Knowledge,* pp. 127–133, ed. A. Cornish-Bowden, Universitat de València, Valencia, Spain, (1997), [PDF]

[31] Monod, J., Wyman, J., Changeux, J.P., " On the Nature of Allosteric Transitions: A Plausible Model", *J. Mol. Biol.*vol. 12, pp 88-118, (1965), [PDF]

[32] King, EL and Altman, C., " A Schematic Method of Deriving the Rate Laws for Enzyme-Catalyzed Reactions" Phys. Chem." Vol. 60, pp: 1375-1378, (1956), [PDF]

[33] Boudart, M., " From the century of the rate equation to the century of the rate constants: a revolution in catalytic kinetics and assisted catalyst design", *Catalysis Letters* , vol.65, pp: 1–3, (2000), [PDF]

[34] Richmond, C.," Two and a quarter centuries of digestive juices", Trends Biochem. Sci. 11(12), pp:528 – 530,(1986), [PDF]

[35] Tipton, K., Boyce, S., " History of the enzyme nomenclature system", Bioinformatics Vol. 16(1), pp: 34-40, (2000), [PDF]

[36] Robert K. Murray, ***et.al.***, " Harper's Illustrated Biochemistry", 26th ed., ISBN 0-07-138901-6, The McGraw-Hill Comp. (2003).

[37] Holme, D.J., Peck, H., p: 257, "Analytical Biochemistry", 3rd ed.,Pearson Ed. Lt., ISBN: 0582 29438 – X

[38] Johannes, T., Simurdiak, M.R., Zhao, H., pp: 101 – 110, "Bioctalysis", Encyclopedia of Chemical Processing, Taylor & Francis, (2006), [PDF]

[39] Sharma,R., Christi,Y.,Banerjee, "Production,purification, characterization and applications of Lipases", *Biotechnology Advances*, 19, pp: 672-662 , (2001), [PDF]

[40] Understanding Enzyme Structure and Function in Terms of the Shifting Specificity Model *J. Biochem. & Mol. Biol.*, Vol. 37(4), pp. 394-401, (2004), [PDF]

[41] David Metzler, Biochemistry, Biochemistry: The Chemical Reactions of Living Cells, Harcourt/ Academic Press, ISBN : 0-12-492540-3.]

[42] Stein, R.L., "**A Process Theory of Enzyme Catalytic Power- The Interplay of Science and Metaphysics",** *Foundations of Chemistry*, vol 8, pp: 3–29, (2006), [PDF]

[43] Robert E. Kohler, Jr., "The Enzyme Theory and the Origin of Biochemistry", Isis, Vol. 64, No. 2. (Jun., 1973), pp. 181-196. [PDF]

[44] Fischer E. (1894). "Einfluss der Configuration auf die Wirkung der Enzyme". , Ber. Dt. Chem. Ges., vol. 27, pp: 2985–2993, (1894), [PDF]

[45] Lichtenthaler, F.W., " 100 Years Schlüssel-Schloss-Prinzip: What Made Emil Fischer Use this Analogy? ", Angewandte Chem. Int.Ed.(Eng.), Vol.33(23-24) , pp:2364 – 2374, (2003), [PDF]

[46] Koshland, D.E. Jr., "Molecular geometry in enzyme action**",** *J. Cellular and Comparative Physiology,* Vol.47(1), pp: 217-234, (1956), [PDF]

[47] Koshland, D. E. Jr., "Enzyme flexibility and enzyme action**",** *J Cell Comp Physiol.* Vol.54, pp:245-58, (1959), [PDF]

[48] Thoma, J.A., Koshland, D. E. Jr., "Competitive Inhibition by Substrate during Enzyme Action. Evidence for the Induced-fit Theory"., J. Am. Chem. Soc.;vol..82(13), pp: 3329-3333, (1960), [PDF]

[49] Koshland, Jr.,D. E. , "The Key-Lock Theory and the Induced Fit Theory**",** *Angew.Chem.Int.Ed.Engl,* vol. 33, pp: 2375-2378, (1994), [PDF]

[50] Jaeger, K-E., Eggert, T.„ "Enantioselective biocatalysis optimized by directed evolution", Current Opinion in Biotechnology, Vol. 15(4), pp: 305-313 , (2004), [PDF[

[51] Keith J. Laidler, The Benjamin / Cummings, " Physical Chemistry with Biological Applications ", Pub., 1978, ISBN 0-8053 – 5681 -9

[52] Oldano, C., Becchi, M. , "Natural optical activity and liquid crystals", Indian Academy of Sciences, Vol. 53(1), pp. 131 -143, (1999), [PDF]

[53] Kubinyi, H.,"Chance favors the prepared mind- from serendipity to rational drug design "
Journal of Receptor and Signal Transduction Research, vol. 19(1-4), pp::15-39, (1999), [PDF]

[54] Boersma, Y.L.,, "Evolution of Enantioselectivity Selection of Improved Hydrolase Variants ", PhD Dep. of Pharmaceutical Biology ofRijksuniversiteit Groningen, , The Netherlands, (2007), [PDF]

[55] Kostyanovsky, R.G. , "**Impossible things in stereochemistry",** *Mendeleev Commun.***,** vol.14, pp: 229 – 230,(2004), [PDF]

[56] Mason, S.,"**Biomolecular Homochirality**",*Chem. Soc. Rec.,*vol.17, pp: 347-359, (1988), [PDF]

[57] Aronson, J K , "Features of this month's Journal", Br J Clin Pharmacol. October; vol.56(4), pp: 347–348. (2003), [PDF]

[58] Bailey, J., "Chirality and the origin of life", Acta Astronautica, vol.46(10), pp:627-631, (2000), [PDF]

[59] Fuß, W.,"Does life originate from a single molecule?", Chirality, vol.21(2), pp: 299-304, (2009),[PDF]

[60] Bonner, W.A., Greenberg,J.M., Rubenstein,E.,"The Extraterrestrial Origin of the Homochirality of Biomolecules –Rebuttal to a Critique", *Origins of Life and Evolution of the Biosphere,* vol.29, pp: 215–219, (1999), [PDF]

[61] Breslow, R., Cheng, Z.L., "On the origin of terrestrial homochirality for nucleosides and amino acids"
PNAS, vol. 106(23), pp: 9144-9146, (2009), [PDF]

[62] Avalos, M., Babiano, R., Cintas, P., Jiménez, J.L. Palacios, J.C. , "***Chiral autocatalysis: where stereochemistry meets the origin of life***, *Chem. Commun.*,vol. 887–892,(2000), [PDF]

[63] Barron, L.D.,"True and false chirality and absolute asymmetric synthesis",
J. Am. Chem. Soc., vol. 108 (18), pp 5539–5542, (1986), [PDF]

[64] Barron, L.D., "Symmetry and Molecular Chirality",Chem. Soc. Rev., vol.15, pp:189-223, (1986),[PDF]

[65] Blumenthal, S.J. " **New insights into the origin of biological chirality",**
BioEssays, Vol.1(6), pp: 258-260, (1984), [PDF]

[66] Cope, M.J., "Understanding Chirality : How Molecules that are mirror Images of Each Other can Act Differently in the Body", Analytical Proceedings, Dec., vol. 30, pp: 498-500, (1993), [PDF]

[67] McConathy, J., Owens, M.J.,"Stereochemistry in Drug Action",J Clin Psychiatry, vol.5(2), pp: 70–73, (2003), [PDF]

[68] Leffler, J.E., A short course in modern organic chemistry , pp: 174,The Mc Millan Comp. 1973, N.Y.,

[69] Mori, K., "Synthetic and stereochemical aspects of pheromone chemistry",
Pure &Appl. Chem., Vol. 66(10/11), pp:1991-1998, (1994), [PDF]

[70] Park, D-S., Oh, H-W., Heo, S-Y.,, ***et.al.***, "Characterization of an Extracellular Lipase in Burkholderia sp.HY-10 Isolated from a Longicorn Beetle", The Journal of Microbiology, Vol. 45(5), pp: 409-417, (2007), [PDF]

[71] Shu, Z,, Lin, R., Jiang, H., ***et.al.***,"A rapid and efficient method for directed screening of lipase-producing *Burkholderia cepacia* complex strains with organic solvent tolerance from rhizosphere" , Journal of Bioscience and Bioengineering, Vol.107(6),pp: 658-661, (2009), [PDF]

[72] Palleroni, N.J. , "The Taxonomy of Bacteria", BioScience, Vol. 33(6), pp: 370- 377, (1983), [PDF]

[73] Miller, S.C. M., LiPuma, J.J., Parke, J.L. ,"Culture-Based and Non-Growth-Dependent Detection of the *Burkholderia cepacia* Complex in Soil Environments", Appl. Envir. Microbiol. Vol.68, pp: 3750-3758. (2002), [PDF]

[74] Moore, J.E., Williams, F.,"Cicero and *Burkholderia cepacia*: What's in a Name? ",
Emerging Infectious Diseases, vol. 9(4), pp: 506 – 507, (2003), [PDF]

[75] Lloyd, A.T., Sharp, P. M. , "Evolution of the recA Gene and the Molecular Phylogeny of Bacteria",
J Mol Evol., vol. 37, pp:399-407, (1993), [PDF]

[76] Ramette, A., LiPuma, J. J., Tiedje, J. M. ,"Species Abundance and Diversity of *Burkholderia cepacia* Complex in the Environment. ", Appl. Environ. Microbiol., vol.71, pp: 1193-1201, (2005), [PDF]

[77] Vandamme, P. "Emerging new "Pseudomonas" species in cystic fibrosis.", In 20th European Cystic Fibrosis Conference, (1995), [PDF]

[78] LiPuma, J.J.,"Update on the *Burkholderia cepacia* complex", Curr. Opin. Pulm. Med., vol. 11, pp:528—533, (2005), [PDF]

[79] Rabkin, C. S.; Jarvis, W. R.; Martone, W. J.,"Current Status of Pseudomonas cepaciaTyping Systems"
European Journal of Epidemiology, Vol. 3(4)., pp. 343-346,(1987), [PDF]

[80] Baligh, M., Delgado, M.A. , Conway, K.E., "Evaluation of *Burkholderia cepacia* Strains: Root Colonization of Catharanthus roseus and In-Vitro Inhibition of Selected Soil-borne Fungal Pathogens" Proc.Okla. Acad. Sci. 79:19-27(1999), [PDF]

[81] Palleroni, N J, Doudoroff, M .,"Some Properties and Taxonomic Sub-Divisions of the Genus *Pseudomonas*", Annual Review of Phytopathology, Vol. 10, pp: 73-100, (1972), [PDF]

[82] Burkholder, W H.,"Bacteria as Plant Pathogens", Annual Review of Microbiology, Vol. 2: pp: 389-412,(1948), [PDF]

[83] Coenye, T., Vandamme, P., Govan, J.R. W., LiPuma, J.J.,"Taxonomy and Identification of the *Burkholderia cepacia* Complex", J. of Clin. Microbiol., vol.39(10), pp: 3427-3436, (2001), [PDF]

[84] Hasan, F., Shah, A.A., Hameed, A.,"Industrial applications of microbial lipases", Enzyme and Microbial Technology, Vol. 39 (2), pp: 235-251, (2006), [PDF]

[85] Armstrong, H.E.,"Studies on Enzyme Action.—Lipase", Proceedings of the Royal Society of London. Series B, Vol. 76(513), pp. 606-608, (1905), [PDF]

[86] Armstrong, H.E., Ormerod, E.,"Studies on Enzyme Action.--Lipase: II", Proceedings of the Royal Society of London. Series B, Vol. 78(526), pp. 376-385, (1906), [PDF]

[87] Sarda, L., Desnuelle, P.,"Action de la lipase pancréatique sur les esters en emulsion", Biochim. Biophys. Acta, vol. 30(3), pp: 513 – 521, (1958), [PDF]

[88] International Union of Biochemistry and Molecular Biology, Recommendations on Biochemical & Organic Nomenclature, Symbols & Terminology etc., http://www.chem.qmul.ac.uk/iubmb/, [PDF]

[89] IUPAC-IUB Commission on Biochemical Nomenclatured, The Nomenclature of Lipids, Biochem. J., vol. 171, pp:21-35, (1978), [PDF]

[90] IUPAC-IUB Commission on Biochemical Nomenclature (CBN), The Nomenclature of Lipids, Eur. J. Biochem., vol. 79,pp: 11-21, (1977), [PDF]

[91] Jaeger K-E, Ransac S, Dijkstra BW, Colson C, Heuvel M van, Misset O., "Bacterial lipases", FEMS Microbiol Rev, Vol.15(1), pp:29 -63, (1994), [PDF]

[92] Schmid, R.D., Verger, R.,"Lipases: interfacial enzymes with attractive applications", Angew. Chem. International, 37, 1608-1633, (1998), [PDF]

[93] Villeneuve,P., Muderhwa,J. M., Graille, J., Haas, M. J.,"Customizing lipases for biocatalysis: a survey of chemical, physical and molecular biological approaches", J. Mol. Catal. B:Enzym. Vol.9(4-6), pp: 113-148, (2000), [PDF]

[94] Klibanov, A. M., "Improving enzymes by using them in organic solvents", Nature, vol.409, pp:241 - 246,(2001), [PDF]

[95] A. Schmid, J. S. Dordick, B.Hauer, A. Kiener, M. Wubbolts, B. Witholt, "Industrial biocatalysis today and tomorrow", Nature,vol.409, pp:258 - 268; (2001), [PDF]

[96] Reetz, M.T., "Lipases as practical biocatalysts", Curr. Opin. Chem. Biol., Vol.6(2), pp: 145-150, (2002), [PDF]

[97] Bornscheuer, U.T., Kazlauskas,.R.J., "Hydrolases in Organic Synthesis: Regio- and Stereoselective Biotransformations", ChemBioChem, vol.7, pp: 1279 – 1280, (2006), [PDF]

[98] Bornscheuer, U.T., Bessler, C., Srinivas, R., Krishna, S.H., "Optimizing lipases and related enzymes for efficient application", Trends in Biotechnology, Vol.20(10), pp: 433-437, (2002), [PDF]

[99] Fischer, M., Pleiss, J.,"The Lipase Engineering Database: a navigation and analysis tool for protein families", Nucleic Acids Research, Vol. 31(1), pp:319–321, (2003), [PDF]

[100] Stadler, P., Kovac, A., Haalck, L., Spener, F., Paltauf, F.,"Stereoselectivity of microbial lipases. The substitution at position sn-2 of triacylglycerol analogs influences the stereoselectivity of different microbial lipases", Eur J Biochem., vol, 227, pp: 335 – 343, (1995), [PDF]

[101] Paiva, A.L., Malcata, F.X. ,"Integration of reaction and separation with lipases: An overview", Journal of Molecular Catalysis B: Enzymatic, Vol.3 (1-4), pp: 99-109, (1997), [PDF]

[102] Houde A, Kademi A, Leblanc D.,"Lipases and their industrial applications: an overview.", Appl Biochem Biotechnol, vol.118(1–3), pp:155–70, (2004), [PDF]

[103] Zaks A., Klibanov, A.M.,"Biochemistry Enzyme-catalyzed processes in organic solvents", Proc. Natl. Acad. Sci., Vol. 82, pp. 3192-3196, (1985), [PDF]

[104] Neena N.G.,"Applications of Lipase", JAOCS, Vol. 74(6), pp: 621 – 634, (1997), [PDF]

[105] Mileto, D., Brocca,S., M. Lotti, ***et.al.***, "Characterization of the Candida rugosa lipase system and overexpression of the lipisoenzyme in a non-conventional yeast", Chemistry and Physics of Lipids, Vol.93(1-2), pp: 47-55, (1998), [PDF]

[106] Yeo S.H., Nihira T., Yamada Y., "Screening and identification of a novel lipase from Burkholderia sp. YY62 which hydrolyzes butyl esters effectively. ", J Gen Appl Microbiol; vol.44(2), pp:147–152., (1998), [PDF]

[107] Hsu, A.F., Foglia T,A., Shen, S., "Immobilization of Pseudomonas cepacialipase in a phyllosilicate sol–gel matrix effectiveness as a biocatalyst ", Biotechnol Appl Biochem., vol.;31, pp:179–83,(2000), [PDF]

[108] Jaeger, K-E., Reetz, M.T., "Microbial lipases form versatile tools for biotechnology", TIBTECH, vol.16, pp:396-403, (1998), [PDF]

[109] Roberts, S.M.,"Preparative biotransformations ", J. Chem.Soc. Perkin Trans., vol. 1, pp: 611-633, (2000), [PDF]

[110] Laumen, K., Schneider, M.P., "A highly selective ester hydrolase from Pseudomonas sp. for the enzymatic preparation of enantiomerically pure secondary alcohols; chiral auxiliaries in organic synthesis", J. Chem. Soc. Chem. Commun., iss. 9, pp: 598-600, (1988), [PDF]

[111] Nguyen, BV, Hedenström, E., "Candida rugosa lipase as an enantioselective catalyst in the esterification of methyl branched carboxylic acids: resolution of rac-3,7-dimethyl-6-octenoic acid (citronellic acid)", Tetrahedron: Asymmetry, Vol.10 (9), pp: 1821-1826 , (1999), [PDF]

[112] Berglund, P., Vallikivi, I., Fransson, L., Dannacher, H., Holmquist, M., ***et.al.***,"Switched enantiopreference of Humicola lipase for 2-phenoxyalkanoic acid ester homologs can be rationalized by different substrate binding modes", *Tetrahedron: Asymmetry*, Vol. 10 (21), pp: 4191-4202 , (1999), [PDF]

[113] Luic, M., Tomic, S., Lescic, I., Ljubovic, E., Sepac, D., Sunjic, V., ***et.al.*** "Complex of *Burkholderia cepacia* lipase with transition state analogue of 1-phenoxy-2 acetoxybutane Biocatalytic, structural and modelling study ", Eur. J. Biochem. 268, 3964-3973 (2001), [PDF]

[114] Benjamin, S., Pandey, A., "Candida rugosa lipases: Molecular biology and versatility in biotechnology", *Yeast*, vol. 14(12), pp: 1069–1087, (1998), [PDF]

[115] Pandey A., Benjamin S., Soccol C. R., Nigam P., Krieger N., Soccol,V. T., "The realm of microbial lipases in biotechnology", *Biotechnol. Appl. Biochem*., Vol.29 (2), pp: 119 – 131, (1999), [PDF]

[116] Adamczyk, M., Grote, J., Rege, S.,"Stereoselective *Pseudomonas cepacia* lipase mediated synthesis of α-hydroxyamides", *Tetrahedron: Asymmetry*, Vol. 8(15), pp. 2509-2512, (1997), [PDF]

[117] Fang, J.M.; Wong, C.H.,"Enzymes in Organic Synthesis: Alteration of Reversible Reactions to Irreversible Processes", *Synlett*, Iss.6 ,pp: 393 – 402, (1994), [PDF]

[118] Waldmann, H., Sebastian, D.," Enzymic Protecting Group Techniques", *Chem. Rev.*, Vol. 94(4), pp: 911 – 937, (1994), [PDF]

[119] Jaeger, K-E., Eggert, T.,**Lipases for biotechnology**", *Current Opinion in Biotechnol..,* Vol. 13(4), pp: 390-397, (2002), [PDF]

[120] Woodyer, R., Chen, W., Zhao, H., "Outrunning Nature: Directed Evolution of Superior Biocatalysts", J. Chem. Edu., vol 81(1), pp:126 – 133, (2004), [PDF]

[121] Turner, N.J.,"Directed evolution of enzymes for applied biocatalysis", *Trends in Biotechnology*, Vol.21(11), , pp:474-478, (2003), [PDF]

[122] Reetz, M.T., Zonta, A., Simpelkamp, J., "Efficient Heterogeneous Biocatalysts by Entrapment of Lipases in Hydrophobic Sol-Gel Materials", *Ang. Chemie Inter. Ed.in Eng.*, Vol.34 (3) , pp:301 – 303, (2003), [PDF]

[123] Marlot, C., Langrand, G. , Triantaphylides, C. I, Baratti, J.,"Ester Synthesis in Organic Solvent Catalyzed by Lipase Immobilized on Hydrophilic Supports", *Biotechnology Letters* Vol 7(9), pp: 647-650, (1985), [PDF]

[124] Ye, P, Xu, Z.K., Wang, Z.G., Wu, J., Deng, H.T., Seta, P., "Comparison of hydrolytic activities in aqueous and organic media for lipases immobilized on poly(acrylonitrile-co-maleic acid) ultrafiltration hollow fiber membrane", *Journal of Molecular Catalysis B: Enzymatic*, vol. 32, pp: 115–121, (2005), [PDF]

[125] Reetz, M.T., Zonta, A., Simpelkamp, J., Konen, W.,"*In situ* fixation of lipase-containing hydrophobic sol-gel materials on sintered glass-highly efficient heterogeneous biocatalysts", *Chem. Commun.*, pp: 1397 – 1398, (1996), [PDF]

[126] Knežević, Z. D., Šiler-Marinković, S.S., Mojović, L.V., "Immobilized Lipases as Practical Catalysts", *APTEFF*, vol.35, pp: 151- 164, (2004), [PDF]

[127] Jaeger, K.E., Schneidinger, B., Rosenau, F., Werner, M., Lang, D., *et. al.*,,"Bacterial lipases for biotechnological applications", Journal of Mol. Catal. B: Enzymatic, Vol. 3 (1-4), pp: 3-12, (1997), [PDF]

[128] Theil, F.," Lipase-supported synthesis of biologically active compounds.", Chem Rev., Vol. 95, pp: 2203 – 2227, (1995), [PDF]

[129] Cherry, J.R , Fidantsef, A.L., "Directed evolution of industrial enzymes: an update", Current Opinion in Biotechnology, Vol.14(4), pp: 438-443, (2003), [PDF]

[130] Dröge, M.J.,, "Selection of novel lipases and esterases for enantioselective biocatalysis", Dissertation, University Groningen, (2004), [PDF]

[131] Gotor, V.,"Biocatalysis Applied to the Preparation of Pharmaceuticals", Org. Proc. Res. Dev., vol. 6, pp: 420–426, (2002), [PDF]

[132] Wehtje, E., Adlercreutz, P., "Water activity and substrate concentration effects on lipase activity", Biotechnology and Bioengineering, Vol.55(5), pp: 798-806, (1997), [PDF]

[133] Jönsson, A., Wehtje, E., Adlercreutz, P., Mattiasson, B.,"Thermodynamic and kinetic aspects on water vs. organic solvent as reaction media in the enzyme-catalysed reduction of ketones", Biochim. Biophys. Acta, vol.1430(2), pp: 313 – 322, (1999), [PDF]

[134] Wescott, C.R., Klibanov,A.M.,"The solvent dependence of enzyme specificity", Biochim. Biophys. Acta, vol. 1206, pp. 1–9, (1994), [PDF]

[135] Tsai, S.W. , Chen, C.C., Yang, H.S., Ng, I-Son, Chen, T.L.,"Implication of substrate-assisted catalysis on improving lipase activity or enantioselectivity in organic solvents", Biochim. Biphys. Acta (BBA), Protein & proteomics, vol. 1764(8), pp: 1424 – 1428, (2006), [PDF]

[136] Krishna, SH, Karanth, NG., "Lipases and lipase-catalyzed esterification reactions in nonaqueous media" *Catal. Rev*., vol. 44 (4), pp: 499-591, (2002), [PDF]

[137] Zaks, A. , Russell, A. J.,"Enzymes in organic solvents: properties and applications", *Journal of Biotechnology*, vol.8(4), pp: 259-269, (1988), [PDF]

[138] Krishna, SH.,"Developments and trends in enzyme catalysis in nonconventional media", *Biotechnol. Adv.*, vol.20(3-4), pp: 239-267, (2002), [PDF]

[139] Gupta, M.N.,"Enzyme function in organic solvents", *Eur. J. Biochem*. Vol.203,pp: 25-32, (1992),[PDF]

[140] Halling, P. J., "What Can We Learn by Studying Enzymes in Non-Aqueous Media?", *Philosophical Transactions: Biological Sciences*, Vol. 359(1448), pp. 1287-1297, (2004), [PDF]

[141] Lortie, R.,"Enzyme catalyzed esterification", *Biotechnol. Advances*, vol.15(1), pp: 1-15, (1997), [PDF]

[142] Piyatheerawong, W., Iwasaki, Y., Xu, X., Yamane, T.,"Dependency of water concentration on ethanolysis of trioleoylglycerol by lipases", *J.Mol. Cat. B: Enzymatic*, vol.28(1), pp: 19-24, (2004), [PDF]

[143] Affleck, R., Xu, Z., V Suzawa, Focht, K., Clark, DS, and Dordick, JS., "Enzymatic Catalysis and Dynamics in Low-Water Environments", PNAS , vol. 89, pp: 1100-1104., (1992), [PDF]

[144] Dordick, J. S., "Enzymatic catalysis in monophasic organic solvents", *Enzyme and Microbial Technology*, Vol.11(4), pp: 194-211, (1989), [PDF]

[145] Klibanov, A.M., "Enzymatic catalysis in anhydrous organic solvents", *Trends in Biochemical Sciences*, Vol. 14 (4), pp: 141-144, (1989), [PDF]

[146] Rupley, J.A., Gratton, E., Careri, G., "Water and globular proteins" *Trends in Biochemical Sciences*, vol.8(1), pp: 18-22, (1983), [PDF]

[147] Jönsson, Å., van Breukelen, W., Wehtje, E., Adlercreutz, P., Mattiasson, B.,"The influence of water activity on the enantioselectivity in the enzyme-catalyzed reduction of 2-pentanone", *J. Mol. Catal. B: Enzym*. Vol. 5, pp. 273–276, (1998), [PDF]

[148] Berglund, P., "Controlling lipase enantioselectivity for organic synthesis", *Biomolecular Engineering*, Vol.18(1), pp: 13-22, (2001), [PDF]

[149] Edlund, H.; Berglund, P.; Jensen, M.; Hedenström, E.; Högberg, H.-E.,"Resolution of 2-Methylalkanoic Acids. Enantioselective Esterification with Long Chain Alcohols Catalysed by Candida rugosa Lipase" *Acta Chem Scand*, Vol.50, pp: 666–671, (1996), [PDF]

[150] Högberg, H.E., Edlund, H., Berglund, P., Hedenström, E. „Water Activity Influences Enantioselectlvlty in a Lipase-Catalysed Resolution by Esterificatlon in an Organic Solvent", *Tetrahedron Asymmetry*, vol.4 , pp:. 2123 – 2126, (1993), [PDF]

[151] Holmberg E, Hult K., "Transesterification with Candida Cylindracea Lipase in a Biphasic Aqueous-Organic System. Dependence of the Enantiomeric Ratio and the Reaction Rate on the Proportions of Water and Cyclohexane", *Biocatalysis*, vol.3(3), pp:243–251, (1990), [PDF]

[152] Yasufuku, Y., Ueji, S.-ichi, "High Temperature-Induced High Enantioselectivity of Lipase for Esterifications of 2-Phenoxypropionic Acids in Organic Solvent", *Bioorganic Chemistry*, Vol.25(2), pp: 88-99, (1997), [PDF]

[153] Bornscheuer, U., Herar, A., Kreye, L., ***et.al.***,"Factors affecting the lipase catalyzed transesterification reactions of 3-hydroxy esters in organic solvents.", *Tetrahedron:Asymmetry*, Vol. 4(5), pp: 1007-1016,(1993), [PDF]

[154] Gubicza, L.; Szakács-Schmidt, A., " Effects of the Reaction Conditions on the Enantio-Selective Esterification Using Candiada Cylindracea Lipase ", *Biocatalysis*, vol. 9(1-4), pp:131–143, (1994), [PDF]

[155] Orrenius, C., Norin, T., Hult,K., Carrea, G., "The *Candida antarctica* lipase B catalysed kinetic resolution of suedenol in non-aqueous media of controlled water activity", *Tetrahedron: Asymmetry*, vol. 6, pp. 3023–3030, (1995), [PDF]

[156] Reslow M, Adlercreutz P, Mattiasson B.,"Modification of the Microenvironment of Enzymes in Organic Solvents. Substitution of Water by Polar Solvents",*Biocatalysis* ,;6(4), pp:307–318, (1992), [PDF]

[157] Stokes, T.M., Oehlschlager, A.C.,"Enzyme reactions in apolar solvents: the resolution of (±)-sulcatol with porcine pancreatic lipase", *Tetrahedron Letters*, Vol. 28(19), pp: 2091-2094 (1987), [PDF]

[158] Bovara R, Carrea G, Ottolina G, Riva S.,"Water Activity does not influence the enantioselectivity of Lipase PS and Lipoprotein Lipase in Organic Solvents", *Biotechnol Lett*, vol.15(2), pp :169–174, (1993), [PDF]

[159] Nordin, O., Hedenström, E., Högberg, H.E.,"Enantioselective transesterifications of 2-methyl-1-alcohols catalysed by lipases from *Pseudomonas*", *Tetrahedron: Asymmetry*, Vol.5(5),pp: 785-788, (1994), [PDF]

[160] Wehtje, E., Costes, D., Adlercreutz, P., "Enantioselectivity if lipases: effects of water activity", *J. Mol. Catal. B. Enzym*. 3, pp. 221–230. (1997), [PDF]

[161] Antczak, T., Graczyk, J., Szczsna-Antczak, M., Bielecki, S.,"Activation of *Mucor circinelloides* lipase in organic medium", *Journal of Molecular Catalysis B: Enzymatic*, Vol.19-20, pp: 287-294, (2002), [PDF]

[162] Macrae, A.R.,"Enzyme-Catalysed Modification of Oils and Fats ", *Phil. Trans. R. Sac. Land. B* , vol.310(114), pp: 227-233, (1985), [PDF]

[163] Muralidhar, R V, Marchant, R, Nigam, P.,"Lipases in racemic resolutions", *J Chem Technol Biotechnol*., vol. 76(1), pp::3 – 8, (2001), [PDF]

[164] Shuji, A., Takashi , K.,"Synthesis of esters by immobilized-lipase-catalyzed condensation reaction of sugars and fatty acids in water-miscible organic solvent.", *Journal of Bioscience and Bioengineering*, vol.99 (2), pp.87-94, (2005), [PDF]

[165] You, L.L., Baharin, B.S.,"Effects of Enzymatic Hydrolysis on Crude Palm Olein by Lipase from *Candida Rugosa* ", *Journal of Food Lipids*, Vol.13(1), pp: 73-87,(2006), [PDF]

[166] Santaniello E, Ferraboschi P, Grisenti P, Manzocchi A., „The biocatalytic approach to the preparation of enantiomerically pure chiral building blocks.", *Chem. Rev.*, vol.92, pp:1071-1140 ,(1992), [PDF]

[167] Ulbert, O., Fráter, T., Bélafi-Bakó, K., Gubicza, L.,"Enhanced enantioselectivity of *Candida rugosa* lipase in ionic liquids as compared to organic solvents", *Journal of Molecular Catalysis B: Enzymatic*, Vol.31(1-3), pp: 39-45, (2004), [PDF]

[168] Sym, E. A.,"Action of esterase in the presence of organic solvents", *Biochem. J.*, vol. 30(4), pp: 609–617, (1936), [PDF]

[169] Esa, N.M., Yunus, W.M.Z., Ahmad, M.B., Basri, M., Razak, C.N.A., Salleh, A.B.,"Water Activity Effect on Lipase Immobilized onto Different Compositions of Poly(HEMA-MMA), *Enzyme Engineering* XIV, Vol. 864, pp: 489 – 492, (1998), [PDF]

[170] Lee, S.B., Kim, K.-J.,"Effect of water activity on enzyme hydration and enzyme reaction rate in organic solvents", *J. Ferment. Bioeng.* , vol.79, pp. 473–478, (1995), [PDF]

[171] Kanerva, L.; Klibanov, A. M.,"Hammett analysis of enzyme action in organic solvents", *J. Am. Chem. Soc.*, 111(17), 6864 - 6865, (1989), [PDF]

[172] Carrea, G. Ottolina, G., Riva, S.,"Role of solvents in the control of enzyme selectivity in organic media",*TIBTECH*, vol. 13, pp: 63–70,. (1995), [PDF]

[173] Kamat, SV, Iwaskewycz, B., Beckman,,EJ and Russell, AJ., "Biocatalytic Synthesis of Acrylates in Supercritical Fluids: Tuning Enzyme Activity by Changing Pressure", *PNAS* vol. 90, pp: 2940-2944,(1993), [PDF]

[174] Zaks, A.,Dodds, D.R.,"Application of biocatalysts and biotransformations to the synthesis of pharmaceuticals ", *Drug Discovery Today*, 2(12), pp: 513-531, (1997), [PDF]

[175] Gupta, M. N., Roy, I., "Enzymes in organic media. Forms, functions and applications", *Eur. J. Biochem.*, Vol. 271(13), pp. 2575-2583, (2004), [PDF]

[176] Mine, Y., Zhang, L., Fukunaga, K., Sugimura, Y.,"Enhancement of enzyme activity and enantioselectivity by cyclopentyl methyl ether in the transesterification catalyzed by *Pseudomonas cepacia* lipase co-lyophilized with cyclodextrins *Biotechnology Letters*, vol.27, pp: 383–388, (2005), [PDF]

[177] Mezzetti, A., Keith, C., Kazlauskas, R. J.,"Highly enantioselective kinetic resolution of primary alcohols of the type Ph-X-CH(CH3)CH2OH by *Pseudomonas cepacia* lipase. Effect of acyl chain length and solvent", *Tetrahedron: Asymmetry*, vol.14, pp: 3917-3924, (2003), [PDF]

[178] Carrea, G.,Riva, S., "Properties and synthetic applications of enzymes in organic solvents." *Angew. Chemie Int.*, vol. 39, pp: 2226–2254, (2000), [PDF]

[179] Ogino, H., Ishikawa, H.,"Enzymes Which Are Stable in the Presence of Organic Solvents", *J. Biosci. Bioeng.*,vol. 91(2),pp: 109–116, (2001), [PDF]

[180] Ru, M. T., Dordick, J. S., Reimer, J. A., Clark, D. S., "Optimizing the salt-induced activation of enzymes in organic solvents: Effects of lyophilization time and water content", *Biotechnol.Bioeng.*vol. 63(2),pp: 233–241, (1999), [PDF]

[181] Ru, M.T., Wu, K.C., Lindsay, J.P., Dordick, J.S., Reimer, J.A., Clark, D.S., "Towards more active biocatalysts in organic media: Increasing the activity of salt-activated enzymes",
Biotechnology and Bioengineering, Vol.75(299, pp: 187-196, (2001), [PDF]

[182] Das, D., Das, P.K.,"Improving the Lipase Activity Profile in Cationic Water-in-Oil Microemulsions of Hydroxylated Surfactants", *Langmuir*, vol. 19(22), pp: 9114 -9119, (2003), [PDF]

[183] Chai, J.L., Wang, S.Q., Li, G.Z., Xu,Q., Gao, Y.H., "Kinetics of the Esterification Reaction Catalyzed by Lipase in W/O Microemulsions of Alkyl Polyglucoside",
Chinese Chemical Letters Vol. 15(6), pp 699-702, (2004), [PDF]

[184] Bovara, R., Carrea, G., Ottolina, G., Riva, S., "Effects of water activity on V_{max} and K_M of lipase catalyzed transesterification in organic media", *Biotech. Lett.*, Vol. 15 No.9, pp.937442, (1993), [PDF]

[185] Castro, G. R., Knubovets,T.,"Homogeneous Biocatalysis in Organic Solvents and Water-Organic Mixtures", *Crit. Rev. Biotechnol.*,vol. 23, pp: 195–231, (2003), [PDF]

[186] Maruyama, T., Yamamura, H., Kotani, T., Kamiya, N., Goto, M., "Poly(ethylene glycol)-lipase complexes that are highly active and enantioselective in ionic liquids", *Org. Biomol.Chem.*, Vol. 2, pp: 1239 – 1244, (2004), [PDF]

[187] Adlercreutz, P., "Immobilization of Enzymes for Use in Organic Media",
Methods in Biotechnology, Vol. 22, pp: 251-256, (2006), [PDF]

[188] Buisson, P., Pierre, A.C., "Immobilization in quartz fiber felt reinforced silica aerogel improves the activity of *Candida rugosa* lipase in organic solvents", *Journal of Molecular Catalysis B: Enzymatic*, vol. 39,pp: 77–82, (2006), [PDF]

[189] Gutiérrez-Ayesta, C., Carelli, A.A., Ferreira, M.L.,"Relation between lipase structures and their catalytic ability to hydrolyse triglycerides and phospholipids", *Enzyme and Microbial Technology*, vol.41(1-2), pp: 35-43, (2007), [PDF]

[190] Schilke, K.F., Kelly, C.,"Activation of immobilized lipase in non-aqueous systems by hydrophobic poly-DL-tryptophan tethers", Biotechnology and Bioengineering, Vol. xxx, No. xxx, (2008), [PDF]

[191] Takaç, S., Bakkal, M.,"Impressive effect of immobilization conditions on the catalytic activity and enantioselectivity of Candida rugosa lipase toward S-Naproxen production",
Process Biochemistry, Vol.42(6), pp: 1021-1027, (2007), [PDF]

[192] Urban, PL, Goodall, DM, Bruce, NC., "Enzymatic microreactors in chemical analysis and kinetic studies.", Biotechnol Adv., Vol. 24(1), pp :42–57, (2006), [PDF]

[193] Yujun, W., Jian, X., Guangsheng, L., Youyuan, D., "Immobilization of lipase by ultrafiltration and cross-linking onto the polysulfone membrane surface",
Bioresource Technology, Vol. 99(7), pp: 2299-2303, (2008), [PDF]

[194] Chauhan, G.S., Kanwar, S.S., Kumar, R., Kumar, Y., Chauhan, S., „Solvent free biocatalytic synthesis of vinyl monomers by lipase immobilized on hydrogels", *Journal of Applied Polymer Science*, Vol.108(5), pp: 3200-3209,(2008), [PDF]

[195] Han, D., Rhee, J. S.,"Characteristics of lipase-catalyzed hydrolysis of olive oil in AOT-isooctane reversed micelles", *Biotechnology and Bioengineering*, vol.28(8), pp: 1250–1255, (1986), [PDF]

[196] Mojović, L., Šiler-Marinković, S., Kukić, G., Vunjak-Novaković, G., "*Rhizopus arrhizus* lipase-catalyzed interesterification of the midfraction of palm oil to a cocoa butter equivalent fat", Enzyme Microb. Technol., vol. 15(5), pp: 438–443, (1993), [PDF]

[197] Yao, C., Tang, S., He, Z., Deng, X., "Kinetics of lipase-catalyzed hydrolysis of olive oil in AOT/isooctane reversed micelles", Journal of Molecular Catalysis B: Enzymatic, Vol.35(4-6), pp: 108-112, (2005), [PDF]

[198] Cotterill, I.C., Sutherland, A.G., Roberts, S.M., Grobbauer, R., Spreitz, J., Faber, K.,"Enzymatic resolution of sterically demanding bicyclo[3.2.0]heptanes: evidence for a novel hydrolase in crude porcine pancreatic lipase and the advantages of using organic media for some of the biotransformations", *J. Chem. Soc., Perkin Trans.* 1, pp: 1365 - 1368, (1991), [PDF]

[199] Koeller, K.M., Wong, C.H., "Enzymes for chemical synthesis",
Nature, vol.409, pp: 232 – 240, (2001), [PDF]

[200] Miyazawa, T., Kurita, S., Shimaoka, M., Ueji,S., Yamada, T., "Resolution of Racemic Carboxylic Acids via the Lipase-Catalyzed Irreversible Transesterification of Vinyl Esters", *Chirality*, vol. 11, pp: 554 – 560, (1999), [PDF]

[201] Nakamura, K., Matsuda, T., Harada,T.,"Chiral synthesis of secondary alcohols using *Geotrichum candidum*", *Chirality*, vol. 14, pp. 703–708, (2002), [PDF]

[202] Schoffers, E., Golebioeski, A.,Johnson, C.R.,"Enantioselective Synthesis Through Enzymatic Asymmetrization", *Tetrahedron*, vol. 52, pp. 3769–3826, (1996), [PDF]

[203] Raston, C.L.,"Versatility of 'alternative' reaction media: solventless organic synthesis",
Chemistry in Australia, pp: 10 – 13, (2004), [PDF]

[204] Dossat, V., Combes, D., Marty, A.,"Efficient lipase catalysed production of a lubricant and surfactant formulation using a continuous solvent-free process", *Journal of Biotechnology*, Vol.97(2),pp: 117-124, (2002), [PDF]

[205] Cave G.W.V., Raston C.L., Scott J.L.,"**Recent advances in solventless organic reactions: towards benign synthesis with remarkable versatility**",*Chem. Commun*., iss.21, pp: 2159–69., (2001), [PDF]

[206] Soumanou, M.M., Bornscheuer, U.T.,"**Lipase-catalyzed alcoholysis of vegetable oils**" *Eur. J. Lipid Sci. Technol*., vol. 105,pp: 656–660, (2003), [PDF]

[207] Xiong, J., Wu, J., Xu, G., Yang, L.,"**Kinetic study of lipase catalyzed asymmetric transesterification of mandelonitrile in solvent-free system**",*Chemical Engineering Journal*, Vol.138(1-3), pp: 258-263, (2008), [PDF]

[208] Ghanem, A., Aboul-Enein, H.Y."Lipase-mediated chiral resolution of racemates in organic solvents", *Tetrahedron: Asymmetry*, Vol.15 (21), pp: 3331-3351, (2004), [PDF]

[209] Torres, S., Castro, R."Non-Aqueous Biocatalysis in Homogeneous Solvent Systems", *Food Technol. Biotechnol*. Vol.42 (4), pp: 271–277, (2004), [PDF]

[210] Foresti, M.L., Pedernera, M., Bucalá, V., Ferreira, M.L."Multiple effects of water on solvent-free enzymatic esterifications", *Enzyme and Microbial Technology*, vol.41(1-2), pp: 62-70, (2007),[PDF]

[211] Laane, C., Boeren, S., Vos, K., Veeger, C."Rules for Optimization of Biocatalysis in Organic Solvents", *Biotechnology and Bioengineering*, Vol. XXX, Pp. 81-87 (1987), [PDF]

[212] Salis, A., Svensson, I., Monduzzi, M., Solinas, V., Adlercreutz, P.,"The atypical lipase B from Candida antarctica is better adapted for organic media than the typical lipase from Thermomyces lanuginose ", *Biochimica et Biophysica Acta (BBA) - Proteins & Proteomics*, Vol.1646(1-2), pp: 145-151,(2003), [PDF]

[213] Yahya ARM, Anderson WA, Moo-Young M.,"Ester synthesis in lipase catalyzed reactions.", Enzyme Microb. Technol.,vol.23(7-8), pp:438–450, (1998), [PDF]

[214] Dzyuba, S.V., Bartsch, R.A.,"Recent Advances in Applications of Room-Temperature Ionic Liquid/Supercritical CO2 Systems", *Angew. Chem. Int. Ed.*, Vol.42(2), pp: 148-150, (2003), [PDF]

[215] Feher, E., Illeova, V., Kelemen-Horvath, I. , ***et.al.***, „Enzymatic production of isoamyl acetate in an ionic liquid-alcohol biphasic system ", *Journal of Molecular Catalysis. B,* vol.50 (1), p.28-32, (2008), [PDF]

[216] Hernández-Fernández, F.J., de los Río,s A.P., Tomás-Alonso, F., ***et.al.***, "Integrated reaction/separation processes for the kinetic resolution of rac-1-phenylethanol using supported liquid membranes based on ionic liquids", *Chemical Engineering and Processing*, Vol.46(9), pp: 818-824, (2007), [PDF]

[217] van Rantwijk, F., Sheldon, R.A., "Biocatalysis in Ionic Liquids", *Chem. Rev*., vol.107(6), pp: 2757 – 2785, (2007), [PDF]

[218] Chiappe, C., Pieraccini, D.,"Ionic liquids: solvent properties and organic reactivity", *J. Phys. Org. Chem*. Vol.18(4), pp: 275 - 297,(2005), [PDF]

[219] Dupont, J., de Souza, R.F., Suarez, P.A.Z.,"Ionic Liquid (Molten Salt) Phase Organometallic Catalysis", *Chem. Rev.*, vol. 102(10), pp: 3667-3692, (2002), [PDF]

[220] Johnson, K.E., "What's an Ionic Liquid? ", *The Electrochemical Society Interface*, pp: 38 – 41, (2007), [PDF]

[221] Kragl, U., Eckstein, M., Kaftzik, N., " Enzyme catalysis in ionic liquids", *Curr. Opin. Biotechnol*., vol.13(6), pp: 565-71. (2002), [PDF]

[222] Lee, S.H., Ha, S.H., Hiep, N.M., Chang, W.J., Koo, Y.M., " Lipase-catalyzed synthesis of glucose fatty acid ester using ionic liquids mixtures*", J. Biotechnol.*, Vol.133(4), pp: 486-489, (2008), [PDF]

[223] Singh, M., Singh, R.S., Banerjee, U.C., " Stereoselective synthesis of (R)-1-chloro-3(3,4-difluorophenoxy)-2-propanol using lipases from *Pseudomonas aeruginosa* in ionic liquid-containing system ", *J. Mol. Catal.B: Enzym*. June (2008), in press, [PDF]

[224] Wasserscheid, P., Wilhelm, K., "Ionic Liquids - New Solutions for Transition Metal Catalysis", Angew. *Chem. Int. Ed. Engl.*, vol. 39,pp: 3772, (2000), [PDF]

[225] Welton, T., "Room-Temperature Ionic Liquids. Solvents for Synthesis and Catalysis", *Chem. Rev.*, vol. 99(8), pp: 2071 - 2084, (1999), [PDF]

[226] Zhao, H., " Effect of ions and other compatible solutes on enzyme activity, and its implication for biocatalysis using ionic liquids. *", J. Mol. Catal. B: Enzym.*, vol. 37(1-6), pp: 16–25, (2005), [PDF]

[227] Adam, D., " Clean and green. . but are they mean? ", *Nature*, vol. 407, pp:938-940, (2000), [PDF]

[228] Barahona, D., Pfromm, P.H., Rezac, M.E., " Effect of Water Activity on the Lipase Catalyzed Esterification of Geraniol in Ionic Liquid [bmim]PF6 ", *Biotechnol. & Bioengr.,* vol.93(2), pp: 318 – 324, (2006), [PDF]

[229] Kim, M.J., Choi, M. Y., Lee, J.K., Ahn, Y., " Enzymatic selective acylation of glycosides in ionic liquids: significantly enhanced reactivity and regioselectivity ", *Journal of Molecular Catalysis B: Enzymatic,* Vol. 26(3-6), pp: 115-118, (2003), [PDF]

[230] Nara, S.J., Mohile, S.S., Harjani, J.R., Naik, P.U., Salunkhe, M.M., " Influence of ionic liquids on the rates and regioselectivity of lipase-mediated biotransformations on 3,4,6-tri-O-acetyl- -glucal"
Journal of Molecular Catalysis B: Enzymatic, Vol.28(1), pp: 39-43, (2004), [PDF]

[231] Davis, J.H.; Fox, P.A., "From Curiosities to Commodities: Ionic Liquids Begin the Transition", Chem.Comm., vol.11,pp: 1209-1212., (2003), [PDF]

[232] Gordon, C.M.," New developments in catalysis using ionic liquids ",
Applied Catalysis A: General, Vol.222(1-2), pp: 101-117, (2001), [PDF]

[233] Sheldon, R.A., Lau, R.M., Sorgedrager, M.J., ***et.al.***, " Biocatalysis in ionic liquids ",
Green Chem., vol. 4, pp: 147 – 151, (2002), [PDF]

[234] Sheldon, R., " Catalytic reactions in ionic liquids", *Chem. Comm.*,vol.23,pp: 2399-2407, (2001), [PDF]

[235] Rogers, R.D., Seddon, K.R., " Ionic Liquids--Solvents of the Future? ", *Science*, Vol. 302(5646), pp: 792 – 793, (2003), [PDF]

[236] Holbrey, J. D.; Seddon K. R. " The phase behaviour of 1-alkyl-3-methylimidazolium tetrafluoroborates; ionic liquids and ionic liquid crystals", *J. Chem. Soc., Dalton Trans.*, Iss.13, pp: 2133-2139, (1999), [PDF]

[237] Huddleston, J. G.; Willauer; H. D.; Swatloski, R. P.; Visser, A. E.;Rogers, R. D., " Room temperature ionic liquids as novel media for clean liquid–liquid extraction", *Chem. Commun.*, iss. 16 , pp: 1765 – 1766, (1998), [PDF]

[238] Rasalkar, M.S., Potdar, M.K., Salunkhe, M.M., " Pseudomonas cepacialipase-catalysed resolution of racemic alcohols in ionic liquid using succinic anhydride: role of triethylamine in enhancement of catalytic activity", *Journal of Molecular Catalysis B: Enzymatic*, Vol.27(4-6), pp: 267-270, (2004), [PDF]

[239] Erbeldinger, M., Mesiano, A.J., Russel, A.J., " Enzymatic catalysis of formation of Z-aspartame in ionic liquid – an alternative to enzymatic catalysis in organic solvents. ", *Biotechnol Prog.*, vol. 16(6), pp: 1129–1131, (2000), [PDF]

[240] Madeira, L.R., van-Rantwijk, F, Seddon, K.R., Sheldon, R.A., " Lipase-Catalyzed Reactions in Ionic Liquids", *Org. Lett.*, vol. 2(26), pp: 4189–4191, (2000), [PDF]

[241] Mantarosie, L., Coman, S., Parvulescu, V.I. " Comparative behavior of various lipases in benign water and ionic liquids solvents", *J. Mol. Catal. A: Chem.* , vol.279, pp: 223–229, (2008), [PDF]

[242] Sawant, D.P., Vinu, A., Justus, J., Srinivasu, P., Halligudi, S.B., " Catalytic performances of silicotungstic acid/zirconia supported SBA-15 in an esterification of benzyl alcohol with acetic acid", *J. Mol. Catal.A: Chem.*, Vol.276(1-2), pp: 150-157, (2007), [PDF]

[243] Van Rantwijk, F.V., Secundo, F., Sheldon, R.A., " Structure and activity of Candida antarctica lipase B in ionic liquids", *Green Chem.*, vol. 8(3), pp: 282 – 286, (2006), [PDF]

[244] Vidya, P., Chadha, A., " The Role Of Different Anions In Ionic Liquids On *Pseudomonas cepacia*Lipase Catalyzed Transesterification And Hydrolysis", Journal of Molecular Catalysis B: Enzymatic, vol.57(1-4), pp:145-148, (2009), [PDF]

[245] Schöfer, S. H., Kaftzik, N., Wasserscheid, P., Kragl, U.,"Enzyme catalysis in ionic liquids: lipase catalysed kinetic resolution of 1-phenylethanol with improved enantioselectivity", *Chem. Commun.*, pp: 425–426, (2001), [PDF]

[246] Zhang, D.H., Bai, S., Ren, M.Y., Sun, Y.,"Optimization of lipase-catalyzed enantioselective esterification of (±)-menthol in ionic liquid", Food Chemistry, vol. 109, pp: 72–80, (2008), [PDF]

[247] Bai, S., Ren, M., Wang, L., Sun, Y., " Regioselective acylation of pyridoxine catalyzed by immobilized lipase in ionic liquid ", Frontiers of Chemical Engineering in China, vol.2(3), (2008), [PDF]

[248] Li, N., Zong, M-H., Ma, D., "Regioselective acylation of nucleosides and their analogs catalyzed by *Pseudomonas cepacia* lipase: enzyme substrate recognition",*Tetrahedron*, vol.65(5), pp: 1063-1068, (2009), PDF (204 K),[PDF]

[249] Li, C., Wang, P., Zhao, D., Cheng, Y., Wang, L., Wang, L., Wang, Z., " Enantioselective enzymatic hydrolysis of racemic glycidyl butyrate by lipase from *Bacillus subtilis* with improved catalytic properties", *Journal of Molecular Catalysis B: Enzymatic*, vol.55(3-4), pp: 152-156, (2008), [PDF]

[250] Li, N., Ma, D., Zong, M-H., " Enhancing the activity and regioselectivity of lipases for 3′-benzoylation of floxuridine and its analogs by using ionic liquid-containing systems", *Journal of Biotechnology*, vol.133(1), pp: 103-109, (2008), [PDF]

[251] Shi, Y-G., Fang, Y., Ren, Y-P, Wu, H-P., Guan, H-L., " Effect of ionic liquid [BMIM][PF6] on asymmetric reduction of ethyl 2-oxo-4-phenylbutyrate by Saccharomyces cerevisiae ", *J. Ind. Microbiol Biotechnol* ., vol.35, pp: 1419–1424, (2008), [PDF]

[252] Adamczak, M., Bornscheuer, U.T., "Improving ascorbyl oleate synthesis catalyzed by Candida antarctica lipase B in ionic liquids and water activity control by salt hydrates", *Process Biochemistry*, vol. 44(3), pp: 257 – 261, (2009), [PDF]

[253] Gary A. Baker, William T. Hellera, " Small-angle neutron scattering studies of model protein denaturation in aqueous solutions of the ionic liquid 1-butyl-3-methylimidazolium chloride ", *Chem. Eng. J.,*vol. 147(1), pp: 6-12, (2009), [PDF]

[254] De Los Rios, A.P., Hernandez-Fernandez, F.J., Martinez, F.A. *et.al.*, " The effect of ionic liquid media on activity, selectivity and stability of Candida antarctica Lipase B in transesterification reactions. ", Biocatal Biotransform., vol.25(2), pp: 151–156, (2007), [PDF]

[255] de los Rios, A.P., Hernandez-Fernandez, F.J., Tomas-Alonso, F., et al., "Synthesis of esters in ionic liquids - The effect of vinyl esters and alcohols ", Process Biochemistry, Vol. 43(8), pp: 892 – 895, (2008), [PDF]

[256] Ha, S.H., Lee, S.H., Dang, D.T., et. Al., "Enhanced stability of Candida antarctica lipase B in ionic liquids", Korean J. Chem. Eng., vol.25(2), pp: 291-294, (2008), [PDF]

[257] Hernández-Fernández, F.J, de los Ríos, A.P, Rubio, M.l, ***et.al.***, " Enhancement of activity and selectivity in lipase-catalyzed transesterification in ionic liquids by the use of additives", Journal of Chemical Technology & Biotechnology, Vol.82(10),pp: 882-887, (2007), [PDF]

[258] Katsoura, M.H., Polydera, A.C., Tsironis, L., Tselepis,A.D., Stamatis, H., " Use of ionic liquids as media for the biocatalytic preparation of flavonoid derivatives with antioxidant potency", J. Biotechnol., vol. 123(4), pp: 491 – 503, (2006), [PDF]

[259] Shi-Hui, H., Hirakawa, T., Fukuba, T., ***et.al.***, " Synthesis of enantiomerically pure cycloalkenols via combination strategy of enzyme-catalyzed reaction and RCM reaction", Tetrahedron: Asymmetry, vol.18(20), pp: 2484-2490, (2007), [PDF]

[260] Husum, T.L. *et al.* , "Enzyme catalysed synthesis in ambient temperature ionic liquids. ", Biocatal. Biotransform., vol. 19, pp: 331–338, (2001), [PDF]

[261] Kim, K-W. et al." Biocatalysis in ionic liquids: markedly enhanced enantioselectivity of lipase. ", Org. Lett., vol. 3(10), pp: 1507–1509, (2001), [PDF]

[262] Park, S. and Kazlauskas, R.J., "Improved preparation and use of room-temperature ionic liquids in lipase-catalyzed enantio- and regioselective acylations. ", J. Org. Chem., vol. 66(25), pp: 8395–8401, (2001), [PDF]

[263] Pilissão, C., Nascimento, M.G., "Effects of organic solvents and ionic liquids on the aminolysis of (RS)-methyl mandelate catalyzed by lipases", Tetrahedron: Asymmetry, Vol.17(3), pp: 428-433, (2006), [PDF]

[264] Kwang-Wook K, Boyoung S, Min-Young C., et al., " Biocatalysis in ionic liquids: markedly enhanced enantioselectivity of lipase. ", *Org Lett.*, vol. 3, pp: 1507–1509, (2001), [PDF]

[265] Lau, R.M., Sorgedrager, M.J., Carrea, G. et al., " Dissolution of Candida antartica lipase B in ionic liquids: effects of structure and activity. ", *Green Chem*, vol. 6(9), pp: 483–487, (2004), [PDF]

[266] Lozano P, de Diego T, Carrie D., et al., " Over-stabilization of Candida antartica lipase B by ionic liquids in ester synthesis. ", Biotehnol Lett., vol. 23, pp: 1529–1533,(2001), [PDF]

[267] Sunitha, S., Kanjilal, S., Reddy, P.S., Prasad, R.B.N., " Ionic liquids as a reaction medium for lipase-catalyzed methanolysis of sunflower oil ", Journal Biotechnology Letters, vol.29(12), pp: 1881-1885, (2007), [PDF]

[268] Toral, A.R., Rios, A.P. , Hernandez, F.J. , ***et.al.*** "Cross-linked *Candida antarctica* lipase B is active in denaturing ionic liquids", Enzyme and Microbial Technology,vol. 40, pp: 1095–1099, (2007), [PDF]

[269] Arai, M., Fujita, S-i, Shirai, M., " Multiphase catalytic reactions in/under dense phase CO_2 " The Journal of Supercritical Fluids, Vo.47(3), pp: 351-356, (2009), [PDF]

[270] Damen, M.R., Brand, R.W., Bloem, S.C., ***et.al.***," Process intensification by combining ionic liquids and supercritical carbon dioxide applied to the design of Levodopa production ", Chemical Engineering and Processing: Process Intensification, vol.48(1), pp: 549-553, (2009), [PDF]

[271] Jiang, T., Han, B.," Chemical Reactions and Related Phase Behavior in Supercritical Co2 and Co2/Ionic Liquid Mixtures ", Green Chemical Reactions, ISBN 978-1-4020-8458-4, Ed. 2008, NATO Science for Peace and Security Series C: Environmental Security, [PDF]

[272] Keskin, S., Akman, U., Hortaçsu, Ö., " Soil remediation via an ionic liquid and supercritical CO_2 " Chem. Eng. Proc., vol. 47(9-10), pp: 1693–1704, (2008), [PDF]

[273] Lozano P, De Diego T, Mira C, et al., " Long term continuous chemoenzymatic dynamic kinetic resolution of rac-1-phenylethanol using ionic liquids and supercritical carbon dioxide ", Green Chem., vol.11(4), pp: 538 – 542, (2009), [PDF]

[274] Miyawaki, O., Tatsuno, M., " Lipase-catalyzed butanolysis of triolein in ionic liquid and selective extraction of product using supercritical carbon dioxide ", Journal of Bioscience and Bioengineering, Vol.105(1), pp: 61-64, (2008), [PDF]

[275] Roth, M., " Partitioning behaviour of organic compounds between ionic liquids and supercritical fluids", Journal of Chromatography A, Vol. 1216(10), pp: 1861-1880, (2009), [PDF]

[276] Xing, H., Wang, T., Dai, Y.," Continuous synthesis of d,l-α-tocopherol catalyzed by sulfonic acid-functionalized ionic liquid in supercritical carbon dioxide ", The Journal of Supercritical Fluids, Vol.49(1), pp: 52-58, (2009), [PDF]

[277] Knez, Ž., "Enzymatic reactions in dense gases", The Journal of Supercritical Fluids, Vol.47(3), pp: 357-372, (2009), [PDF]

[278] Hartmann, T., Meyer, H.H., Scheper, T. " The enantioselective hydrolysis of 3-hydroxy-5-phenyl-4-pentenoicacidethylester in supercritical carbon dioxide using lipases ", Enzyme and Microbial Technology, vol. 28, pp:653–660,(2001), [PDF]

[279] Bornscheuer, U , Capewell, A. , Wendel, V. , Scheper, T.," On-line determination of the conversion in a lipase-catalyzed kinetic resolution in supercritical carbon dioxide ", Journal of Biotechnology, vol. 46, pp:. 139 – 143, (1996), [PDF]

[280] Capewell, A., Wendel, V., Bornscheuer, U., Meyer, H. H., Scheper, T., " Lipase-catalyzed kinetic resolution of 3-hydroxy esters in organic solvents and supercritical carbon dioxide. ", Enzyme and Microbial Technology, vol.19, pp:181-186, (1996), [PDF]

[281] Chulalaksananukul, W., Condoret, J.S., Combes, D.,"Geranyl acetate synthesis by lipase-catalyzed transesterification in supercritical carbon dioxide", Enzyme and Microbial Technology, Vol.15(8), pp: 691-698, (1993), [PDF]

[282] Ikushima, Y., Saito, N., Yokoyama, T., ***et.al.***, " Solvent effects on an enzymatic ester synthesis in supercritical carbon dioxide ", Chemistry Letters, Vol.22(1), pp. 109-112 ,(1993), [PDF]

[283] Martins, J. F.; Borges de Carvalho, I.; Correa de Sampaio, T.; Barreiros, S., " Lipase-catalyzed enantioselective esterification of glycidol in supercritical carbon dioxide ", Enzyme Microb. Technol.,vol.16(9), pp: 785–790, (1994), [PDF]

[284] Matsuda, T.; Harada, T.; Nakamura, K.," Alcohol dehydrogenase is active in supercritical carbon dioxide ", Chem. Commun., iss.15,pp: 1367–1368, (2000), [PDF]

[285] Matsuda, T., Kanamaru, R., Watanabe, K., Harada, T., Nakamura, K.," Control on enantioselectivity with pressure for lipase-catalyzed esterification in supercritical carbon dioxide ", Tetrahedron Letters, Vol. 42(47), pp: 8319-8321, (2001), [PDF]

[286] Matsuda, T., Harada, T., Nakamura, K., Ikariya, T., " Asymmetric synthesis using hydrolytic enzymes in supercritical carbon dioxide ", Tetrahedron Asymmetry, 16 (5): 909-915, (2005), [PDF]

[287] Mori, T., Okahata, Y., " Effective biocatalytic transgalactosylation in a supercritical fluid using a lipid-coated enzyme ", "Chem Commun., vol. 20, pp: 2215 – 2216 , (1998), [PDF]

[288] Overmeyer, A.; Schrader-Lippelt, S.; Kasche, V., Brunner, G.," Lipase-catalysed kinetic resolution of racemates at temperatures from 40 °C to 160 °C in supercritical CO2 ", Biotechnology Letters, Vol.21(1), pp. 65-69 , (1999), [PDF]

[289] Rantakylä, M., Aaltonen, O., " Enantioselective esterification of ibuprofen in supercritical carbon dioxide by immobilized lipase ", Biotechnology Letters, Vol.16(8), (1994), [PDF]

[290] Rantakylä, M., Alkio, M., Aaltonen, O., " Stereospecific hydrolysis of 3-(4-methoxyphenyl)glycidic ester in supercritical carbon dioxide by immobilized lipase", Biotechnol. Lett., vol.18(9), pp:1089–1094, (1996), [PDF]

[291] Russell, A.J., Beckman, E.J. " Should the high diffusivity of a supercritical fluid increase the rate of an enzyme-catalyzed reaction? ", Enzyme and Microbial Technology, Vol.13(12), p: 1007, (1991), [PDF]

[292] Ferraboschi, P., Grisenti,P., Manzocchi,A., Santaniello, E.," Regio- and enantioselectivity of *Pseudomonas cepacia* lipase in the transesterification of 2-substituted-1,4-butanediols ", Tetrahedron: Asymmetry, Vol.5(4) , pp:691-698, (1994), [PDF]

[293] Ferraboschi, P., Casati , S., Grisenti,P., Santaniello, E.," Enantioselective Pseudomonas fluorescens (P. cepacia) lipase-catalyzed irreversible transesterification of 2-methyl-1,2-diols in an organic solvent ", Tetrahedron: Asymmetry, Vol.5(10), pp: 1921-1924, (1994), [PDF]

[294] Ko, S-J, Lim, J-Y., Jeon, N-Y, ***et.al.***," Kinetic resolution of fluorinated propargyl alcohols by lipase-catalyzed enantioselective transesterification ", Tetrahedron: Asymmetry, Vol.20(10), pp: 1109-1114, (2009), [PDF]

[295] Matsuda, T., Harada, T., Nakamura, K. "Organic synthesis using enzymes in supercritical carbon dioxide ", Green Chem, Vol.6 , pp:440 – 444, (2004), [PDF]

[296] Pilissão, C., Carvalho, P.O., Nascimento, M.G.," Enantioselective acylation of (RS)-phenylethylamine catalysed by lipases ", Process Biochemistry, In Press, 25 July 2009, [PDF]

[297] Bornscheuer, U., Schapöhler, S., Scheper, T., Schügerl, K.," Influences of reaction conditions on the enantioselective transesterification using *Pseudomonas cepacia* lipase ", Tetrahedron: Asymmetry Vol. 2(10), pp. 1011-1014, (1991) , [PDF]

[298] Bornscheuer, U., Schapöhler, S., Scheper, T.H., Schügerl, K., König, W. A.," Application of enantioselective capillary gas chromatography in lipase-catalysed transesterification reactions in organic media", Journal of Chromatography A, Vol.606(2), pp: 288-290, (1992), [PDF]

[299] Bornscheuer, U., Capewell, A., Scheper, T., Meyer, H.H., Kolisis, F.," A Comparison of Enzymatic Reactions in Aqueous, Organic, and Supercritical Phasesa ", Annals of the New York Academy of Sciences, vol.672, Enzy.Eng.XI, Pages 336 – 342, (1992), [PDF]

[300] Bornscheuer, U., Stamatis, H., Xenakis, A., Yamane, T., Kolisis, F.N.,"A comparison of different strategies for lipase-catalyzed synthesis of partial glycerides", Biotech. Lett., Vol.16(7), pp.697- 702, (1994), [PDF]

[301] Knüttel, T., Meyer, H., Scheper, T.," Synthesis, test and application of chirale fluorescence substrates to evaluate enzymatic processes in different reaction media ", Enzyme and Microbial Technology, Vol.37(7), pp: 673-686, (2005), [PDF]

[302] Knüttel, T., Meyer, H., Scheper, T.," The application of two-dimensional fluorescence spectroscopy for the on-line evaluation of modified enzymatic enantioselectivities in organic solvents by forming substrate salts ", Enzyme and Microbial Technology, Vol.39(4), pp: 607-611, (2006), [PDF]

[303] Yildirim, A. Diplom, Institut für Technische Chemie, Leibniz Universitat Hannover, 2005,

[304] Temelli, F.," Perspectives on supercritical fluid processing of fats and oils" , The Journal of Supercritical Fluids, Vol.47(3), pp: 583 – 590, (2009); [PDF]

[305] Bornscheuer, U.T., Yamane, T., " Activity and stability of lipase in the solid-phase glycerolysis of triolein", Enzyme and Microbial Technology, vol.16(10), pp: 864-869, (1994), [PDF]

[306] Bornscheuer, U.T.," Lipase-catalyzed syntheses of monoacylglycerols ", Enzyme and Microbial Technology, vol.17(7), pp: 578-586, (1995), [PDF]

[307] Bornscheuer, U., Herar, A., Capewell, A., Wendel, V., Kreye, L., Scheper, T., ***et.al.***,," Lipase-catalyzed Kinetic Resolution of 3-Hydroxy Esters: Optimization, Batch, and Continuous Reactionsa ", Annals of the New York Academy of Sciences. Vol.750, Enzy.Eng.XII, Pages 215 – 221,(1995), [PDF]

[308] Hartmann, T.,, " Überkritisches Kohlendioxid als Reaktionsmedium für die Naturstoffsynthese – Die enantioselektive Hydrolyse von 3-Hydroxysäureester ", Vom Fachbereich Chemie, der Universität Hannover, (Nov. 1999), [PDF]

[309] Schwabe, E.,"Untersuchung einer neuartigen Extraktionsmethode mit überkritischem Kohlendioxid ",, Diplomarbeit im Fach Technische Chemie an der Universität Hannover, Aug. (1996), [PDF]

[310] Tservistas, M., " Untersuchungen zum Einsatz von Überkirtischem Kohlendioxid als medium für biokatalisierte Reaktionen ", Fachbereich Chemie der Universitat Hannover, (Mai 1997), [PDF]

[311] Bornscheuer, U., Reif, O.W., Lausch, R., Freitag, R., Scheper, T., ***et.al.*** ," Lipase of *Pseudomonas cepacia* for biotechnological purposes: purification, crystallization and characterization", Biochimica et Biophysica Acta (BBA) - General Subjects, Vol.1201(1), pp: 55-60, (1994), [PDF]

[312] Grunwaldt ,J.-D., Baiker, A.," In situ spectroscopic investigation of heterogeneous catalysts and reaction media at high pressure ", Phys. Chem. Chem. Phys., vol.7(20), pp: 3526 – 3539 , (2005), [PDF]

[313] Knez, Z., Habulin, M., Primozic, M.," Enzymatic reactions in dense gases ", Biochem. Eng. J., Vol.27(2), pp: 120-126, (2005), [PDF]

[314] McConnell, O., Bach II, A., Balibar, C., Byrne, N., ***et.al.***, " Enantiomeric separation and determination of absolute stereochemistry of asymmetric molecules in drug discovery - Building chiral technology toolboxes ", Chirality, Vol.19(9), pp: 658 – 682, (2007), [PDF]

[315] Zhang, X., Han, B., Hou, Z., Zhang, J., Liu, Z., Jiang, T., He, J., Li, H. , "Why Do Co-solvents Enhance the Solubility of Solutes in Supercritical Fluids? New Evidence and Opinion", Chemistry - A European Journal, Vol.8(22), pp: 5107-5111, (2002), [PDF]

[316] Baiker, A.," Supercritical Fluids in Heterogeneous Catalysis", Chem. Rev.,vol. 99,pp: 453-473, (1999), [PDF]

[317] Brennecke, J. F., Chateauneuf, J. E.,"Homogeneous Organic Reactions as Mechanistic Probes in Supercritical Fluids", Chem. Rev., vol. 99(2), pp: 433 – 452, (1999), [PDF]

[318] Ikushima, Y., "Supercritical fluids: an interesting medium for chemical and biochemical processes", Advances in Colloid and Interface Science, vol. 71-72, pp: 259-280, (1997), [PDF]

[319] Jessop, P.G., Ikariya, T., Noyori, R.," Homogeneous Catalysis in Supercritical Fluids", Chem. Rev.,vol. 99,pp: 475-493, (1999), [PDF]

[320] Kamat, S.V., Beckman, E.J. , Russell, A.J.,"Enzyme Activity in Supercritical Fluids", Critical Reviews in Biotechnology, vol.15(1), pp: 41 – 71, (1995), [PDF]

[321] Goddard, R., Bosley, J., , Al-Duri, B.," Esterification of oleic acid and ethanol in plug flow (packed bed) reactor under supercritical conditions: investigation of kinetics ", J. Supercrit. Fluids, vol. 18(2), pp. 121–130, (2000), [PDF]

[322] Hyde, J.R., Licence, P., Carter, D., Poliakoff, M.," Continuous catalytic reactions in supercritical fluids", Applied Catalysis A, Vol.222(1-2), pp: 119-131, (2001), [PDF]

[323] Kondo, M.; Rezaei, K.; Temelli, F.; Goto, M.," On-line Extraction-Reaction of Canola Oil with Ethanol by Immobilized Lipase in $scCO_2$ ", Ind. Eng. Chem. Res;vol. 41(23), pp: 5770-5774, (2002), [PDF]

[324] Savage, P.E., Gopalan, S., Mizan, T.I., Martino, C.J., Brock, E.E.," Reactions at supercritical conditions: Applications and fundamentals ", AIChE Journal, Vol. 41 (7), pp: 1723-1778, (1995), [PDF]

[325] Baiker, A., Wandeler, R.," Supercritical Fluids; Opportunities in Heterogeneous Catalysis", CATTECH , vol. 4 (2), pp: 128-143, (2000), [PDF]

[326] Celia, E., Cernia, E., Palocci, C., Soro, S., Turchet, T.,"Tuning Pseudomonas cepacea lipase (PCL) activity in supercritical fluids ", The Journal of Supercritical Fluids , Vol. 33 (2), pp: 193-199, (2005), [PDF]

[327] Matsuda, T., Kanamaru, R., Watanabe, K., Kamitanaka, T., Harada, T., Nakamura, K., "Control of enantioselectivity of lipase-catalyzed esterification in supercritical carbon dioxide by tuning the pressure and temperature", Tetrahedron: Asymmetry, vol. 14, pp: 2087–2091, (2003), [PDF]

[328] Matsuda, T. , Watanabe, K., Harada, T. , Nakamura, K., "Enzymatic reactions in supercritical CO2: carboxylation, asymmetric reduction and esterification ", Catalysis Today, vol. 96 , pp:103–111, (2004), [PDF]

[329] Cernia, E., Palocci, C., Soro, S.," The role of the reaction medium in lipase-catalyzed esterifications and transesterifications", Chemistry and Physics of Lipids, Vol. 93 (1-2), pp: 157-168, (1998), [PDF]

[330] Kmecz, I., Simandi, B., Poppe, L., et al., " Lipase-catalyzed enantio selective acylation of 3-benzyloxypropane-1,2-diol in supercritical carbon dioxide ", Biochemical Engineering Journal, Vol.28(3), pp: 275-280, (2006), [PDF]

[331] Liu, K-J.," Lipase-catalyzed synthesis and antibacterial activity of N-vanillylnonanamide", Journal of Molecular Catalysis B: Enzymatic, Vol.58(1-4), pp:181 – 186,(2009), [PDF]

[332] Oliveira, M.V., Rebocho, S.F., Ribeiro, A.S., Macedo, E.A., Loureiro, J.M.," Kinetic modelling of decyl acetate synthesis by immobilized lipase-catalysed transesterification of vinyl acetate with decanol in supercritical carbon dioxide ", J. of Supercritical Fluids, vol.50, pp:138 – 145, (2009) , [PDF]

[333] Palocci, C., Falconi, M., Chronopoulou, L., et al., " Lipase-catalyzed regioselective acylation of tritylglycosides in supercritical carbon dioxide ", Journal Supercritical Fluids, Vol. 45(1), pp: 88-93,(2008), [PDF]

[334] Kittikun, A.H, Kaewthong, W., Cheirsilp, B.," Continuous production of monoacylglycerols from palm olein in packed-bed reactor with immobilized lipase PS ", Biochemical Engineering J.,, Vol.40(1), pp:116-120, (2008), [PDF]

[335] Knez, M. H., Krmelj, V.," Enzyme catalyzed reactions in dense gases ", Journal of Supercritical Fluids, Vol.14(1), pp: 17-29, (1998), [PDF]

[336] Rosa, C.D., Morandim, M.B., Ninow, J.L., ***et.al.***,, "Continuous lipase-catalyzed production of fatty acid ethyl esters from soybean oil in compressed fluids ", Bioresource Technology, In Press, 18 July (2009), [PDF]

[337] Anderson, K.,, " Dyeing Textiles with Supercritical Carbon Dioxide: A Non-Aqueous Dyeing System", [TC]²,5651 Dillard Dr. Cary, NC 27518 USA, (2008), [PDF]

[338] Chi, Y. M, Nakamura, K., and Yano, T.," Enzymatic interesterification in supercritical carbon dioxide", Agric. Biol. Chem.,vol. 52(6), pp:1541-1550, (1988), [PDF]

[339] Dumont, T., Barth, D., Corbier, C., Branlant, G., Perrut, M., " Enzymatic reaction kinetic: Comparison in an organic solvent and in supercritical carbon dioxide ", Biotechnology and Bioengineering, Vol. 40(2), pp: 329-333, (1992), [PDF]

[340] Dumont T, Barth D and Perrut M.," Continuous synthesis of ethyl myristate by enzymatic reaction in supercritical carbon dioxide ", J Supercritical Fluids, vol. 6, pp: 85-89 (1993), [PDF]

[341] Goddard, R., Bosley, J., Al-Duri, B.," Lipase-catalysed esterification of oleic acid and ethanol in a continuous packed bed reactor, using supercritical CO2 as solvent: approximation of system kinetics", Journal of Chemical Technology & Biotechnology, Vol. 75(8), pp: 715-721, (2000), [PDF]

[342] Habulin, M., Knez, "Activity and stability of lipases from different sources in supercritical carbon dioxide and near-critical propane" , J. Chem. Technol.& Biotechnol., Vol.76(12), pp: 1260 – 1266, (2001), [PDF]

[343] Hauthal, W.H.," Advances with supercritical fluids ", Chemosphere, Vol.43(1), pp:123-135, (2001), [PDF]

[344] Kamat, S.V., Beckman, E.J., Russell, A.J.," Control of Enzyme Enantioselectivity with Pressure Changes in Supercritical Fluoroform ", J. Am. Chem. Soc., vol.115, pp: 8845-8846, (1993), [PDF]

[345] Karmee, S.K.," Lipase catalyzed synthesis of ester-based surfactants from biomass derivatives ", Biofuels, Bioproducts and Biorefining, Vol.2(2), pp: 144 – 154, (2008), [PDF]

[346] King, J.W., Srinivas, K.," Multiple unit processing using sub- and supercritical fluids", J. of Supercritical Fluids, vol. 47, pp: 598–610, (2009), [PDF]

[347] Knez, Z., Habulin, M.," Compressed gases as alternative enzymatic-reaction solvents: a short review", J. Supercrit. Fluids, vol. 23, pp. 29–42, (2002), [PDF]

[348] Krammer, P., Vogel, H., " Hydrolysis of esters in subcritical and supercritical water ", The Journal of Supercritical Fluids, Vol.16(3), pp: 189-206, (2000), [PDF]

[349] Kruse, A., Vogel, H.," Heterogeneous Catalysis in Supercritical Media: 1. Carbon Dioxide ", Chemical Engineering & Technology, Vol.31(1), pp: 23 – 32, (2008), [PDF]

[350] Lin, Bin, Akgerman, A.," Isoprene/Methyl Acrylate Diels-Alder Reaction in Supercritical Carbon Dioxide ", Ind. Eng. Chem. Res., vol.38, pp: 4525 – 4530, (1999), [PDF]

[351] Mase N, Sako T, Horikawa Y, Kunihiko T.," Novel strategic lipase-catalyzed asymmetrization of 1,3-propanediacetate in supercritical carbon dioxide ", Tetrahedron Lett., vol.44, pp:5175–5178, (2003), [PDF]

[352] Oakes, R. S.; Clifford, A. A.; Rayner, C. M.," The use of supercritical fluids in synthetic organic chemistry ", J. Chem.Soc., Perkin Trans. 1, iss.9, pp: 917 – 941, (2001), [PDF]

[353] Pichugin, A A., Tarasov, V V.," Supercritical extraction and prospects for the creation of new waste-free products ", Russ. Chem. Rev., vol. 60, pp: 1249 – 1254 , (1991), [PDF]

[354] Rehman, M., Shekunov, B. Y., York, P., Colthorpe, P., " Solubility and precipitation of nicotinic acid in supercritical carbon dioxide ", J. Pharma. Sci., Vol.90(10), pp: 1570 – 1582, (2001), [PDF]

[355] Ajzenberg, N., Trabelsi, F., Recasens, F.," What s New in Industrial Polymerization with Supercritical Solvents? A Short Review ", Chemical Engineering & Technology, Vol.23(10), pp:829-839, (2000), [PDF]

[356] Faigl, F., Thurner, A., Battancs, M., Farkas, F., ***et.al.***,"Efficient, scalable kinetic resolution of cis-4-benzyloxy-2,3-epoxybutanol ", Tetrahedron: Asymmetry, Vol.6(23),pp: 3841-3847, (2005), [PDF]

[357] Marty A, Chulalaksananukul W, Condoret JS, Willemot RMand Durand G,," Comparison of lipase-catalysed esterification in supercritical carbon dioxide and in *n*-hexane ", Biotech Lett, 12:11-16 (1990), [PDF]

[358] Marty, A., Chulalaksananukul, W., Willemot, R. M., Condoret, J. S.," Kinetics of lipase-catalyzed esterification in supercritical CO_2. ", Biotechnology and Bioengineering, Vol.39(3), pp: 273-280, (1992), [PDF]

[359] Marty, A., Combes, D., Condoret, J.S.," Continuous reaction-separation process for enzymatic esterification in supercritical carbon dioxide ", Biotechnology and Bioengineering, Vol. 43(6), pp: 497-504, (1994), [PDF]

[360] Marty, A. Manon, S., Dong, P.Y., Combes, D., Condoret,J.S.," The enzymic reaction–fractionation process in SC-CO2. ", Annals of the N.Y. Academy of Sciences, Enzyme Eng XII. Vol:750(12), pp: 408 – 411, (1995), [PDF]

[361] Marty, A., Dossat, V., Condoret, J.S.," Continuous Operation of Lipase-Catalyzed Reactions in Nonaqueous Solvents: Influence of the Production of Hydrophilic Compounds ", Biotechnol. Bioengr., Vol.56(2), pp: 232-237, (1997), [PDF]

[362] Randolph, TW, Blanch, HW, Prausnitz, JM, Wilke, CR,, " Enzymatic catalysis in a supercritical Fluid", Biotech. Lett. 7:325 - 328 (1985), [PDF]

[363] Randolph, T.W., Clark, D.S., Blanch, H.W., Prausnitz, J.M.," Cholesterol aggregation and interaction with cholesterol oxidase in supercritical carbon dioxide ", Proc. Nati. Acad. Sci., Vol. 85, pp. 2979-2983, (1988), [PDF]

[364] Randolph, T. W., Clark, D. S., Blanch, H. W., Prausnitz, J. M.,"Enzymatic oxidation of cholesterol aggregates in supercritical carbon dioxide. ", Science, vol. 238, pp: 387-390, (1988.), [PDF]

[365] Randolph, T. W., Blanch, H. W., Prausnitz, J. M.," Enzyme catalyzed oxidation of cholesterol in supercritical carbon dioxide.", AIChE J., vol. 34, pp: 1354-1360, (1988), [PDF]

[366] Muratov,G. Seo,K.-W. Kim, C.,"Application of supercritical carbon dioxide to the bioconversion of cotton fibers", Ind. Eng. Chem. 11 (1),pp: 42–46, (2005), [PDF]

[367] Rezaei, K., Temelli, F." On-line extraction-reaction of canola oil using immobilized lipase in supercritical CO_2. " , The Journal of Supercritical Fluids, vol.19(3), pp: 263-274,(2001), [PDF]

[368] Rezaei, K., Jenab, E., Temelli, F.," Effects of water on enzyme performance with an emphasis on the reactions in supercritical fluids", Critical Rewievs in Biotechnology, Vol.27(4), pp: 183-195, (2007), [PDF]

[369] Salgın, U., Salgın, S., Takaç, S., "The enantioselective hydrolysis of racemic naproxen methyl ester in supercritical CO2 using Candida rugosa lipase", The Journal of Supercritical Fluids, vol.43, pp: 310 – 316, (2007), [PDF]

[370] Kamat, S. Critchley, G., Beckman, E.J., Russell, A.J.," Biocatalytic Synthesis of Acrylates in Organic Solvents and Supercritical Fluids: 111. Does Carbon Dioxide Covalently Modify Enzymes? ", Biotechnology and Bioengineering, Vol.46(6), pp: 610 – 620, (1995), [PDF]

[371] Miller, D.A.; Blanch, H.W.; Prausnitz, J.M.," Enzymatic Interesterification of Triglycerides in Supercritical Carbon Dioxide ", Annals of the New York Academy of Sciences, vol. 613(1), Enzyme Eng.10, pp. 534-537, (1990), [PDF]

[372] Miller, D. A., Blanch, H.W., Prausnitz, J.M., " Enzyme-catalyzed interesterification of triglycerides in supercritical carbon dioxide ", Ind. Eng. Chem. Res.; 30(5) pp 939 - 946; (1991), [PDF]

[373] Pasta, P., Mazzola, G., Carrea, G., Riva, S." Subtilisin-catalyzed transesterification in supercritical carbon dioxide ", Biotechnology Letters, Vol.11(9), pp: 643 – 648, (1989), [PDF]

[374] Kim, S-J., Jung, S-M., Park, Y-C., Park, K.," Lipase catalyzed transesterification of soybean oil using ethyl acetate, an alternative acyl acceptor ", Biotechnology and Bioprocess Engineering, Vol.12(4), pp: (2007), [PDF]

[375] Nakamura K, Chi YM, Yamada Y and Yano T," Lipase activity and stability in supercritical carbon dioxide. ", Chem Eng. Commun, vol: 45,pp. 207 -212, (1986), [PDF]

[376] Olsen, T., Kerton, F., Marriott, R., Grogan, G., " Biocatalytic esterification of lavandulol in supercritical carbon dioxide using acetic acid as the acyl donor ", Enzyme and Microbial Technology, 39 (4), p.621-625, (2006), [PDF]

[377] Yasmin,T., Jiang, T., Han, B., Zhang, J., Ma, X., " Transesterification reaction catalysed by Novozym 435 in supercritical carbon dioxide ", Journal of Molecular Catalysis B: Enzymatic, Vol.41(1-2), pp: 27-31, (2006), [PDF]

[378] Williams, J.R., Clifford, A.A., Al-Saidi, S.H R., "Supercritical fluids and their applications in biotechnology and related areas. ", Molecular biotechnology, vol.22 (3), p.263-286, (2002), [PDF]

[379] Kroon, M.C., " Combined Reactions and Separations Using Ionic Liquids and Carbon Dioxide", Dissertation on Technische Universiteit Delft , (2006), [PDF]

[380] Debenedetti, P.G., Tom, J.W., Yeo, S-D., Lim, G-B., " Application of supercritical fluids for the production of sustained delivery devices ", Journal of Controlled Release, vol.24, pp: 21-44, (1993), [PDF]

[381] Gore, G., " On the Properties of Liquid Carbonic Acid. ", Proc.Roy.Soc., vol. 11, pp. 85-86, (1861), [PDF]

[382] Hannay, J. B., Hogarth, J., " On the Solubility of Solids in Gases ", Proc. Roy. Soc., vol. 29, pp: 324-326, (1879), [PDF]

[383] Hannay, J. B., Hogarth, J.," On the Solubility of Solids in Gases " , Proc. Roy. Soc., vol. 30, pp. 178-188, (1880) , [PDF]

[384] Hannay, J.B., " On the State of Fluids at Their Critical Temperatures " , Proc. Roy. Soc., Vol. 30, pp. 478-484, (1880), [PDF]

[385] Jessop, P.G.,"Homogeneous catalysis using supercritical fluids: Recent trends and systems studied " , The Journal of Supercritical Fluids, Vol.38(2), pp:211-231, (2006), [PDF]

[386] Matson, D.W., Fulton, J.L., Petersen, R.C., Smith, R.D.," Rapid expansion of supercritical fluid solutions: solute formation of powders,thin films, and fibers ", Ind. Eng. Chem. Res. Vol.26, pp: 2298 – 2306, (1987), [PDF]

[387] Matson, D.W., Petersen, R.C., Smith, R.D.," Production of powders and films from supercritical solutions ", J. Mater. Sci. vol.22(6), pp: 1919 – 1928, (1987), [PDF]

[388] McHugh, Mark A.; Krukonis, Val. J., " Supercritical Fluid Extraction" (2nd Ed.), (1994), Elsevier

[389] Palmer, M.V., Ting, S.S.T.,"Applications for supercritical fluid technology in food processing ", Food Chemistry, Vol.52(4), pp: 345-352, (1995), [PDF]

[390] Petersen, R.C., Matson, D.W., Smith, R.D. , " The formation of polymer fibers from the rapid expansion of supercritical fluid solutions ", Polym. Eng. Sci. vol.27, pp: 1693 – 1697 , (1987), [PDF]

[391] Ramsay, W.," On the Critical Point ", Proc.Roy.Soc, vol. 31, pp. 194-205, (1881), [PDF]

[392] Taylor, L.T.," Supercritical fluid chromatography for the 21st century ", The Journal of Supercritical Fluids, Vol.47(3), pp: 566-573, (2009), [PDF]

[393] Tom, J.W., Debenedetti, P.G.," Particle formation with supercritical fluids—a review " , J. Aerosol Sci., vol. 22, pp: 555 – 584. (1991), [PDF]

[394] Yeo, S.D., Kiran, E.," Formation of polymer particles with supercritical fluids: A review ", The Journal of Supercritical Fluids, Vol.34(3), pp: 287 – 308 , (2005), [PDF]

[395] Al-Jabari, M.," Kinetic models of supercritical fluid extraction ", Journal of Separation Science, Vol.25(8), pp: 477 – 489, (2002), [PDF]

[396] Tucker, S.C.," Solvent Density Inhomogeneities in Supercritical Fluids ", Chem. Rev., vol. 99(2), pp 391–418,(1999), [PDF]

[397] Debenedetti, P.G., Eeid, R.C.," Diffusion and mass transfer in supercritical fluids ", AIChEJ., vol.32(12), pp: 2034 – 2046, (1986), [PDF]

[398] Mongkholkhajornsilp, D., Douglas, S., Douglas, P.L., ***et.al.***, " Supercritical CO2 extraction of nimbin from neem seeds—a modelling study ", Journal of Food Engineering, Vol.71(4), pp: 331-340, (2005), [PDF]

[399] Müller, E.A., Estévez, L. A., „ Mixing expansivities and Grashof numbers in supercritical fluids using cubic equations-of-state ", The Journal of Supercritical Fluids, Vol.3(3), pp:136-142, (1990), [PDF]

[400] Shia, J., Kakudab, Y., Zhoua, X.„ ***et.al.***, "Correlation of mass transfer coefficient in the extraction of plant oil in a fixed bed for supercritical CO_2 .", Journal of Food Engineering, Vol.78(1), pp: 33-40, (2007), [PDF]

[401] Simões, P.C., Fernandes, J., Mota, J. P.," Dynamic model of a supercritical carbon dioxide heat exchanger ", The Journal of Supercritical Fluids, Vol.35(2), pp: 167-173, (2005), [PDF]

[402] Lim, G.B, Holder, G.D., Shah, Y.T.," Mass transfer in gas-solid systems at supercritical conditions ", The Journal of Supercritical Fluids, Vol.3(4), pp: 186-197, (1990), [PDF]

[403] Randolph, T.W.," Supercritical fluid extractions in biotechnology", Trends in Biotechnology, Volume 8, pp: 78-82, (1990), [PDF]

[404] Gordon, C.M., Leitner, W.,"Chp.8: Supercritical Fluids, Catalyst Separation, Recovery and Recycling , Vol.30 , ISBN 978-1-4020-4086-3 (Print) 978-1-4020-4087-0 (Online) , Springer, (2006)

[405] Maurizio Solinas, " New Strategies for Multiphase Catalysis using Supercritical Carbon Dioxide ",Von der Fakultät für Mathematik, Informatik und Naturwissenschaften der Rheinisch-Westfälischen Technischen Hochschule Aachen, (2004), [PDF]

[406] Bowyer, J.R., Pleil, J.D.," Supercritical fluid extraction as a means of cleaning and desorbing common air sampling sorbents ", Chemosphere, Vo.31(3), pp: 2905-2918, (1995), [PDF]

[407] Laintz, K.E., Sivils, L.D, Spall, W.D.," Supercritical Fluid Cleaning, 1998, Pages 22-37, Ed. by John McHardy, Samuel P. Sawan Noyes Publications,ISBN: 0-8155- 1416-6

[408] Porta, G.D., Volpe, M.C., Reverchon, E.," Supercritical cleaning of rollers for printing and packaging industry ", The Journal of Supercritical Fluids, Vol.37(3), pp: 409 – 416, (2006), [PDF]

[409] Tomioka, O., Enokida, Y., Yamamoto, I., Takahashi, T.," Cleaning of materials contaminated with metal oxides through supercritical fluid extraction with CO_2 containing TBP ", Progress in Nuclear Energy, Vol.37(1-4), pp: 417-422, (2000), [PDF]

[410] van Roosmalen, M.J.E., Woerlee, G.F., Witkamp, G.J., " Dry-cleaning with high-pressure carbon dioxide—the influence of process conditions and various co-solvents (alcohols) on cleaning-results ", The Journal of Supercritical Fluids, Vol.27(3), pp:337-344, (2003), [PDF]

[411] van Roosmalen, van Diggelen, M., M.J.E., Woerlee, G.F., Witkamp, G.J.," Dry-cleaning with high-pressure carbon dioxide—the influence of mechanical action on washing-results ", The Journal of Supercritical Fluids, Vol.27(1), pp:97-108, (2003), [PDF]

[412] Ventosa, C., Rébiscoul, D., Perrut, V., ***et.al.***," Copper cleaning in supercritical CO2 for the microprocessor interconnects ", Microelectronic Engineering, Vol.85(7), pp: 1629-1638, (2008), [PDF]

[413] Weibel, G.L., Ober, C.K.," An overview of supercritical CO2 applications in microelectronics processing ", Microelectronic Engineering, Vol.65(1-2), pp: 145 – 152, (2003), [PDF]

[414] Biscaia, D., Ferreira, S.R.S.," Propolis extracts obtained by low pressure methods and supercritical fluid extraction ", The Journal of Supercritical Fluids, In Press, 8 August 2009, [PDF]

[415] Braida, I., Mattea, M., Cardarelli,D.," Extraction–adsorption–desorption process under supercritical condition as a method to concentrate antioxidants from natural sources ", The Journal of Supercritical Fluids, Vol.45(2), pp: 195-199, (2008), [PDF]

[416] Chai, J., Zhang, X., Huang, J., Tan, X., Da,i G.," Microscopic model of nano-scale particles removal in high pressure CO_2-based solvents ", The Journal of Supercritical Fluids, Vol.49(2),pp: 182-188, (2009), [PDF]

[417] Davarnejad, R., Kassim, K.M., Zainal, A., Sata, S.A., „ Phase Equilibrium Study of Triacylglycerols from Crude Palm Oil Using CO_2 ", World Applied Sciences Journal 5 (1): 68-75, (2008), [PDF]

[418] Lee, S-H., Ha, Sng Ho, Sun Bok Lee and Yoon-Mo Koo, " Adverse effect of chloride impurities on lipase-catalyzed transesterifications in ionic liquids ", Biotechnology Letters, vol.28(17), pp: 1335-1339, (2006), [PDF]

[419] Wang, S-P., Lee, W-T.," Determination of benzophenones in a cosmetic matrix by supercritical fluid extraction and capillary electrophoresis ", Journal of Chromatography A, Vol.987(1-2), pp: 269-275, (2003), [PDF]

[420] Antczak, M.S., Kubiak, A., Antczak, T., Bielecki, S.," Enzymatic biodiesel synthesis – Key factors affecting efficiency of the process ", Renewable Energy, Vol.34(5), pp: 1185-1194, (2009), [PDF]

[421] Catchpole, O.J., Tallon, S.J., Eltringham, W.E. ***et.al.***, " The extraction and fractionation of specialty lipids using near critical fluids ", The Journal of Supercritical Fluids, Vol.47(3), pp: 591-597, (2009), [PDF]

[422] Chen, Y., Xiao, B., Chang, J., Fu, Y., ***et.al.***, " Synthesis of biodiesel from waste cooking oil using immobilized lipase in fixed bed reactor ", Energy Conversion and Management, Vol.50(3), pp: 668-673, (2009), [PDF]

[423] Clark, J. H., Deswarte, F.E.I., Farmer, T.J.," The integration of green chemistry into future biorefineries", Biofuels, Bioproducts and Biorefining, Vol.3(1), pp: 72-90, (2009), [PDF]

[424] Demirbas, A.," Biodiesel fuels from vegetable oils via catalytic and non-catalytic supercritical alcohol transesterifications and other methods: a survey ", Energ. Convers. Manage, 44, pp: 2093-2109, (2003), [PDF]

[425] Demirbas, A.," Biorefineries: Current activities and future developments ", Energy Conversion and Management, In Press, , Available online 30 July 2009, [PDF]

[426] Demirbas, A.," Progress and recent trends in biodiesel fuels", Energy Conversion and Management, Vol.50(1), pp: 14-34, (2009), [PDF]

[427] Demirbas, A.," Political, economic and environmental impacts of biofuels: A review ", Applied Energy, Vol.86(1), pp: S108-S117, (2009), [PDF]

[428] Demirbas, M. F.," Biorefineries for biofuel upgrading: A critical review ", Applied Energy, Vol. 86(1), pp: S151-S161, (2009), [PDF]

[429] Alonso, E., Cantero, F.J., Garcia, J., Cocero, M..J.," Scale-up for a process of supercritical extraction with adsorption of solute onto active carbon. Application to soil remediation ", Journal of Supercritical Fluids, vol. 24, pp: 123–135, (2002), [PDF]

[430] Anitescu, G., Tavlarides, L.L.," Supercritical extraction of contaminants from soils and sediments ", The Journal of Supercritical Fluids, Vol.38(2), pp: 167-180, (2006), [PDF]

[431] Balaban MO, Arreora AG, Marshall M, Peplow A, Wei CI, Cornell J.," Inactivation of pectinesterase in orange juice by supercritical carbon dioxide ", J. Food Sci., vol. 56(3), pp:743-746, (1991), [PDF]

[432] Dillow AK, Dehghani F, Hrkach JS, Foster NR, Langer R., " Bacterial inactivation by using near- and supercritical carbon dioxide ", Proc Natl Acad Sci.,vol.96(18), pp:10344-10348, (1999), [PDF]

[433] Erkmen, O.," Inactivation of *Salmonella typhimurium* by high pressure carbon dioxide ", Food Microbiol 17(2):225-232, (2000), [PDF]

[434] Erkmen,O., " Effect of carbon dioxide pressure on *Listeria monocytogenes* in physiological saline and foods ", Food Microbiol 17(6):589-596, (2000), [PDF]

[435] Erkmen, O.," Antimicrobial effect of pressurized carbon dioxide on *Enterococcus faecalis* in physiological saline and foods ", J Sci Food Agric 80(4):465-470, (2000), [PDF]

[436] Erkmen, O.," Antimicrobial effect of pressurized carbon dioxide on *Brochothrix thermosphacta* in broth and foods ", J. Sci. Food Agric., vol. 80(9):1365-1370, (2000), [PDF]

[437] Erkmen, O.," Effect of high-pressure carbon dioxide on *Escherichia coli* in nutrient broth and milk ", Int J Food Microbiol 65(1-2):131-135, (2001), [PDF]

[438] Erkmen ,O., Dogan, C.," Kinetic analysis of *Escherichia coli* inactivation by high hydrostatic pressure in broth and foods ", Food Microbiology, Vol.21(2), pp:181-185, (2004), [PDF]

[439] Fu,,Y-J., Liu, W., Zu,Y-G, ***et.al.***, ,, Breaking the spores of the fungus Ganoderma lucidum by supercritical CO_2 ", Food Chemistry, Vol.112(1), pp: 71-76, (2009), [PDF]

[440] İçen, H., Gürü, M.," Extraction of caffeine from tea stalk and fiber wastes using supercritical carbon dioxide ", The Journal of Supercritical Fluids, Vol.50(3), pp: 225-228, (2009), [PDF]

[441] Araus, K., Uquiche,,E., del Valle, J.M.," Matrix effects in supercritical CO2 extraction of essential oils from plant material ", Journal of Food Engineering, Vol.92(4),pp: 438-447, (2009), [PDF]

[442] Bensebia, O., Barth, D., Bensebia, B., Dahmani, A.," Supercritical CO2 extraction of rosemary: Effect of extraction parameters and modeling ", The Journal of Supercritical Fluids, Vol.49(2), pp: 161-166, (2009), [PDF]

[443] Gasperi, F., Aprea, E., Biasioli, F. ***et.al.***," Effects of supercritical CO2 and N2O pasteurisation on the quality of fresh apple juice ", Food Chemistry, Vol.115(1), pp: 129-136, (2009), [PDF]

[444] Liang, X., Gao, S., Yang, J., He, M.," Highly efficient procedure for the transesterification of vegetable oil ", Renewable Energy, Vol.34(10), pp: 2215-2217, (2009), [PDF]

[445] Norhuda, I, Jusoff, K.," Supercritical carbon dioxide (SC-CO2) as a clean technology for palm kernel oil extraction ", J Biochem Tech , vol.1(2):XX-XX, in press, (2009), [PDF]

[446] Sahena, F., Zaidul, I.S.M, Jinap, S., ***et.al.***, " Application of Supercritical CO2 in Lipid Extraction - A Review ", Journal of Food Engineering, in press, (2009), [PDF]

[447] Shekarchizadeh, H., Kadivar, M., Ghaziaskar, H.S., Rezayat, M., " Optimization of enzymatic synthesis of cocoa butter analog from camel hump fat in supercritical carbon dioxide by response surface method (RSM) ", The Journal of Supercritical Fluids, Vol.49(2), pp: 209-215, (2009), [PDF]

[448] Bahrami, M., Ranjbarian, S., " Production of micro- and nano-composite particles by supercritical carbon dioxide ", The Journal of Supercritical Fluids, Vol.40(2), pp: 263-283, (2007), [PDF]

[449] Cansell, F., Aymoniera, C.," Design of functional nanostructured materials using supercritical fluids ", J. of Supercritical Fluids, vol. 47, pp: 508–516, (2009), [PDF]

[450] Chehroudi, B.I.," Supercritical Fluids: nanotechnology and select emreging applications ", Combustion Science and Technology, Vol.178(1-3), pp: 555-621(67), (2006), [PDF]

[451] Chih, Y-W., Cheng, W-T.," Supercritical carbon dioxide-assisted synthesis of silver nano-particles in polyol process ", Materials Science and Engineering: B, Vol.145(1-3), pp: 67-75, (2007), [PDF]

[452] Erriguible, A., Marias, F., Cansell, F., Aymonier, C.," Monodisperse model to predict the growth of inorganic nanostructured particles in supercritical fluids through a coalescence and aggregation mechanism " , J. of Supercritical Fluids , vol.48, pp: 79–84, (2009), [PDF]

[453] Ibeh, C.C., Bubacz, M.," Current Trends in Nanocomposite Foams ", Journal of Cellular Plastics, vol. 44: pp. 493 – 515, (2008) , [PDF]

[454] Manoharan, M., " Research on the frontiers of materials science: The impact of nanotechnology on new material development ", Technology in Society, Vol.30(3-4), pp: 401-404, (2008), [PDF]

[455] Merisko-Liversidge, E.M., Liversidge, G.G.," Drug Nanoparticles: Formulating Poorly Water-Soluble Compounds ", Toxicologic Pathology; vol. 36: pp. 43 - 48. , (2008), [PDF]

[456] Reverchon, E., Adami, R.," Nanomaterials and supercritical fluids ", The Journal of Supercritical Fluids, Vol.37(1), pp: 1-22, (2006), [PDF]

[457] Sharma, V.K., Yngard, R.A., Lin, Y., " Silver nanoparticles: Green synthesis and their antimicrobial activities ", Advances in Colloid and Interface Science, vol.145, pp: 83–96, (2009), [PDF]

[458] Varshosaz, J., Hassanzadeh, F., Mahmoudzadeh, M., Sadeghi, A.," Preparation of cefuroxime axetil nanoparticles by rapid expansion of supercritical fluid technology ", Powder Technology, vol. 189, pp: 97–102, (2009), [PDF]

[459] Barna, L., Blanchard, J-M., Rauzy, E., Berro, C.," Solubility of Flouranthene, Chrysene, and Triphenylene in Supercritical Carbon Dioxide ", J. Chem. Eng. Data, vol.41 (6), pp 1466–1469, (1996), [PDF]

[460] Dadashev, M. N., Stepanov, G. V.," Supercritical extraction in petroleum refining and petrochemistry", Chemistry and Technology of Fuels and Oils, Vol.36(1), pp: 8 – 13, (2000), [PDF]

[461] Goto, M.," Chemical recycling of plastics using sub- and supercritical fluids ", J. of Supercritical Fluids, vol. 47, pp: 500 – 507, (2009), [PDF]

[462] Heaton, D.M., Bartle, K.D., Clifford, A.A., *et.al.*," Rapid separation of polycyclic aromatic hydrocarbons by packed column supercritical fluid chromatography ", Chromatographia, Vol.39(9-10), pp: 607 – 611, (1994), [PDF]

[463] Karakhanov, E.A., Maksimov, A.L.," Biphasic catalysis in petrochemical processes ", Russian Journal of General Chemistry, Vo.79(6), pp: 1370-1383, (2009), [PDF]

[464] Lee, L-s, Fu, J-h., Hsu, H-l., " Solubility of Solid 1,4-Dimethoxybenzene in Supercritical Carbon Dioxide ", J. Chem. Eng. Data, vol.45(2), pp 358–361,(2000), [PDF]

[465] Leitner, W.," Recent advances in catalyst immobilization using supercritical carbon dioxide ", Pure Appl. Chem., Vol. 76(3), pp. 635–644, (2004), [PDF]

[466] Meehan, N. J., Sandee, A.J., Reek, J.N.H., ***et.al.***," Continuous, selective hydroformylation in supercritical carbon dioxide using an immobilised homogeneous catalyst ", Chem. Commun., pp: 1497–1498,(2000), [PDF]

[467] Pilat, S. and Godlewicz, M. (1940), " Method of separating high molecular mixtures ", U.S.Patent 2,188,013, (1040), [PDF]

[468] Rahmanian, A., Ghaziaskar, H.S.," Selective extraction of maleic acid and phthalic acid by supercritical carbon dioxide saturated with trioctylamine ", The Journal of Supercritical Fluids, Vol.46(2), pp: 118-122, (2008), [PDF]

[469] Salgın, U., Yıldız, N., Köroğlu, F., Çalımlı, A.," A parametric study of phenolic compounds desorption performance from organobentonite by supercritical fluids ", The Journal of Supercritical Fluids, Vol.39(3), pp: 296-303, (2007), [PDF]

[470] Selva, M., Perosa,,A., Massimo Fabrisa and Patrizia Cantonb, " The metathesis of a-olefins over supported Re-catalysts in supercritical CO_2 ", Green Chem., vol.11, pp: 229–238, (2009), [PDF]

[471] Tan, C-S., Lee, P-L.," Supercritical CO2 desorption of toluene from activated carbon in rotating packed bed ", The Journal of Supercritical Fluids, Vol.46(2), pp: 99-104, (2008), [PDF]

[472] Carvalho, P.O., Cass, Q.B., Calafatti, S.A., Contesini, F.J., Bizaco, R.," Reviwe- Alternatives for the separation of drug enantiomers iboprufen as a model compound ", Brazilian Journal of Chemical Engineering, Vol. 23(3), pp. 291 - 300, (2006), [PDF]

[473] Garlapati, C., Madras, G.," Temperature independent mixing rules to correlate the solubilities of antibiotics and anti-inflammatory drugs in $SCCO_2$ ", Thermochimica Acta, in press, (2009), [PDF]

[474] Junco, S., Casmiro, T., Ribero, N., Da Pontes, M., Mariques, H.M.C.," Optimisation of Supercritical Carbon Dioxide Systems for Complexation of Naproxen : Beta-Cyclodextrin ", Journal of Inclusion Phenomena and Macrocyclic Chemistry, vol 44, pp: 69–73, (2002), [PDF]

[475] Li, D-C, Zhong, X-K., Zeng, Z-P., ***et.al.***," Application of targeted drug delivery system in Chinese medicine ", Journal of Controlled Release, Vol.138(2), pp: 103-112, (2009), [PDF]

[476] Reverchon, E., Adami, R., Cardea, S., Porta, G.D., " Supercritical fluids processing of polymers for pharmaceutical and medical applications ", J. of Supercritical Fluids, vol. 47, pp: 484–492,(2009), [PDF]

[477] Stolnik S., Shakesheff. K., " Formulations for delivery of therapeutic proteins ", Biotechnology Letters, Vol.31(1), (2009), [PDF]

[478] Subramaniam, B., Rajewski, R.A., Snavely, K., " Pharmaceutical Processing with Supercritical Carbon Dioxide ", Journal of Pharmaceutical Sciences, Vol. 86(8), pp: 885 – 890, (1997), [PDF]

[479] Temtem, M., Lígia M.C. Silva, Andrade, P.Z., dos Santos, F., ***et.al.***, " Supercritical CO2 generating chitosan devices with controlled morphology. Potential application for drug delivery and mesenchymal stem cell culture ", J. of Supercritical Fluids, vol. 48, pp: 269–277, (2009), [PDF]

[480] Türk, M., " Manufacture of submicron drug particles with enhanced dissolution behavior by rapid expansion processes ", J. of Supercritical Fluids, vol. 47, pp: 537–545, (2009), [PDF]

[481] Ganapathy, H.S.,, Hwang, H S., Lee, M Y., ***et.al.***," Stabilizer architectures based on fluorinated random and block copolymers for the dispersion polymerization of 2-hydroxyethyl methacrylate in supercritical carbon dioxide ", Journal of Materials Science, Vol.43(7), pp: :2300–2306, (2008), [PDF]

[482] Gang-sheng, T., Liu, T., Zhao ,L., Li-xia, H., Wei-kang, Y., " Supercritical carbon dioxide-assisted preparation of polypropylene grafted acrylic acid with high grafted content and small gel percent ", The Journal of Supercritical Fluids, Vol.48(3), pp: 261-268, (2009), [PDF]

[483] Guo, Z., Xu, X., " Lipase-catalyzed glycerolysis of fats and oils in ionic liquids: a further study on the reaction system ", Green Chem., vol.(1),pp: 54-62, (2006), [PDF]

[484] Kelly, C., Howdle, S., " Supercritical processing ", Education in Chemistry , Vol 45(6), (2008), [PDF]

[485] Kikic, I., Vecchione, F., " Supercritical impregnation of polymers ", Current Opinion in Solid State and Materials Science, vol. 7, pp: 399–405, (2003), [PDF]

[486] Kikic, I., " Polymer–supercritical fluid interactions ", J. of Supercritical Fluids, vol. 47, pp: 458–465, (2009), [PDF]

[487] Kumar, R., Tyagi, R., Parmar, V. S., Samuelson, L.A., Kumar, J., Watterson, A.C., " Biocatalytic ''green'' synthesis of PEG-based aromatic polyesters: optimization of the substrate and reaction conditions ", Green Chem . , vol.6, pp:516 – 520, (2004), [PDF]

[488] Meziani, M.J., Pathak, P., Desai, T., Sun, Y-P., " Supercritical Fluid Processing of Nanoscale Particles from Biodegradable and Biocompatible Polymers ", Ind. Eng. Chem. Res., vol. 45 (10), pp: 3420–3424, (2006), [PDF]

[489] Nalawade, S.P., Picchioni,,F., Janssen L.P.B.M.," Supercritical carbon dioxide as a green solvent for processing polymer melts: Processing aspects and applications ", Prog. Polym. Sci., vol. 31 pp:19–43, (2006), [PDF]

[490] Natu, M.V., Gil, M.H., Sousa, H.C., " Supercritical solvent impregnation of poly(-caprolactone)/poly(oxyethylene-b-oxypropylene-b-oxyethylene) and poly(-caprolactone)/poly(ethylene-vinyl acetate) blends for controlled release applications ", J. of Supercritical Fluids, vol. 47, pp:93–102, (2008) , [PDF]

[491] Nobelen, M., Hoppe, S., Fonteix, C., Pla, F., Dupire, M., Jacques, B., " Modeling of the rheological behavior of polyethylene/supercritical CO2 solutions ", Chemical Engineering Science, Vol.61(16), pp: 5334-5345, (2006), [PDF]

[492] Wu, K., Li, J., " Precipitation of a biodegradable polymer using compressed carbon dioxide as antisolvent ", The Journal of Supercritical Fluids, Vol.46(2), pp: 211 – 216, (2008), [PDF]

[493] Cazenave-Gassiot, A., Boughtflower, R., Caldwell, J., *et.al.*," Effect of increasing concentration of ammonium acetate as an additive in supercritical fluid chromatography using CO2–methanol mobile phase ", Journal of Chromatography A, Vol.1216(36), pp: 6441-6450, (2009), [PDF]

[494] Hühnerfuss, H., Shah, M.R.," Enantioselective chromatography—A powerful tool for the discrimination of biotic and abiotic transformation processes of chiral environmental pollutants ", Journal of Chromatography A, Vol.1216(3), pp: 481-502, (2009), [PDF]

[495] Klesper, E., Corwin, A.H., Turner, D.A.," High pressure gas chromatography above critical temperatures ", J. Org. Chem. 27(2), pp: 700–701, (1962), [PDF]

[496] Strife, R.J., Mangels, M.L,. Skare, J.A., " Separation and Analysis of Dimethylaniline Isomers by Supercritical Fluid Chromatography - Electrospray Ionization Tandem Mass Spectrometry ", Journal of Chromatography A, In Press, 30 July 2009, [PDF]

[497] Su, B., Bao, Z., Xing, H., Yang, Y., Ren, Q.," Enantioseparation of paroxetine intermediate on an amylose-derived chiral stationary phase by supercritical fluid chromatography ", Journal of Chromatography A, Vol.1216(26), pp: 5140-5146, (2009), [PDF]

[498] Wenda, C., Rajendran, A.," Enantioseparation of flurbiprofen on amylose-derived chiral stationary phase by supercritical fluid chromatography ", Journal of Chromatography A, In Press, 21 February 2009 ,[PDF]

[499] Drews, M.J.," An Investrgation into the use of Supercritical fluid technology for analytical, process and environmental applications in textiles ", National Textile Center Research Briefs:, pp: 20 – 21, May 1995, [PDF]

[500] Drews, M. J.," An Investigation Into the Use of Supercritical Fluid Technology for Analytical, Process and Environmental Applications in Textiles ", National Textile Center Annual Report: c95-3, pp: 109 – 117 , Aug. 1995, [PDF]

[501] Drews, M. J.," An Investigation Into the Use of Supercritical Fluid Technology for Analytical, Process and Environmental Applications in Textiles ", National Textile Center Annual Report: c95-3, pp: 81 – 90, November 1996, [PDF]

[502] Hendrix, W. A." Progress in Supercritical CO_2 Dyeing ", Journal of Industrial Textiles, vol. 31(1), pp: 43 – 56, (2001), [PDF]

[503] Marsal, A., Celma, P. J., Cot ,J., Cequier, M., " Supercritical CO2 extraction as a clean degreasing process in the leather industry ", The Journal of Supercritical Fluids, Vol.16(3),pp: 217-223, (2000), [PDF]

[504] Mileo, R.R., Gelabert, M. M., Peral,I.G., ***et.al.***," Ceramides Extracted from Wool: Supercritical Extraction Processes ", Textile Research J., vol. 79: pp. 721 – 727, (2009), [PDF]

[505] Montero, G., Carl, A., Smith, B., Hendrix, W.A., Butcher, D. L., " Supercritical Fluid Technology in Textile Processing: An Overview ", Ind. Eng. Chem. Res, vol.39 (12), pp 4806–4812, (2000), [PDF]

[506] Ramachandran, T, Guruprasad, S S., " Role of Supercritical Carbon Dioxide (SC-CO2) in Wet Processing ", IE (I) Journal—TX, Vol 86, pp: 12-15, (2005), [PDF]

[507] Ramírez, R., Martí, M., Manich, A., Parra, J. L., Coderch, L., " Ceramides Extracted from Wool: Pilot Plant Solvent Extraction ", Textile Research Journal; vol. 78: pp. 73 – 80, (2008), [PDF]

[508] Van der Kraan, M., Bayrak, Ö., Fernandez Cida, M.V., *et.al.*, „ Textile Dyeing in Supercritical Carbon Dioxide ", Laboratory for Process Equipment, Delft University of Technology, [PDF]

[509] Antonov, E. N., Bagratashvili, V. N., Howdle, S. M., Konovalov A. N., *et.al.*, " Fabrication of polymer scaffolds for tissue engineering using surface selective laser sintering ",
Laser Physics, , Vol. 16(5), pp. 774–787, (2006), [PDF]

[510] Aydin, H.M., Piskin, E., Çalimli, A., " Microporous Scaffolds from Poly(Lactide-Co--Caprolactone) Composites with Hydroxyapatite and Tricalcium Phosphates Using Supercritical CO2 for Bone Tissue Engineering ", Journal of Bioactive and Compatible Polymers, vol. 19, pp. 383 – 394, (2004), [PDF]

[511] Boissard, C.I.R., Bourban, P.-E., Tami A.E., Alini, M., Eglin D.," Nanohydroxyapatite/poly(ester urethane) scaffold for bone tissue engineering ", Acta Biomaterialia, In Pres, 13 May 2009, [PDF]

[512] Collins, N J., Bridson, R.H., Leeke, G.A., Grover, L.M.," Particle seeding enhances interconnectivity in polymeric scaffolds foamed using supercritical CO_2 ", Acta Biomaterialia, In Press, 9 August 2009, [PDF]

[513] Duarte, A.R.C., Mano, J.F., Reis, R.L.," Preparation of chitosan scaffolds loaded with dexamethasone for tissue engineering applications using supercritical fluid technology ", European Polymer Journal, vol. 45(1), pp: 141 – 148, (2009), [PDF]

[514] Duarte, A. R. C., Caridade, S.G., João F. Mano, Rui L. Reis., " Processing of novel bioactive polymeric matrixes for tissue engineering using supercritical fluid technology ", Materials Science and Engineering , in press (2009) , [PDF]

[515] Duarte, A.R.C., Mano, J.F., Reis, R.L., " Perspectives on: Supercritical Fluid Technology for 3D Tissue Engineering Scaffold Applications ", Journal of Bioactive and Compatible Polymers, vol.24, pp: 385 – 400, (2009), [PDF]

[516] Duarte, A.R.C., Mano, J.F., Reis, R.L., " Separation of starch-based scaffolds for tissue engineering by supercritical immersion precipitation ", The Journal of Supercritical Fluids, Vol.49(2), pp: 279-285, (2009), [PDF]

[517] Garcıa-Gonzalez, C.A., Vega-Gonzalez, A., Lopez-Periago, A.M., *et.al.*," Composite fibrous biomaterials for tissue engineering obtained using a supercritical CO2 antisolvent process ", Acta Biomaterialia, vol. 5, pp: 1094–1103, (2009) ,[PDF]

[518] Gualandi, C.,, White, L.J., Chen, L., ***et.al.***," Scaffold for tissue engineering fabricated by non-isothermal supercritical carbon dioxide foaming of a highly crystalline polyester ", Acta Biomaterialia, In Press, (2009), [PDF]

[519] Lee, L.Y., Wu, S.C., Fu, S.S., Zeng, S.Y., Leong, W.S., Tan, L.P., " Biodegradable elastomer for soft tissue engineering ", European Polymer Journal, In Press, Corrected Proof, online 29 July 2009, [PDF]

[520] Léonard, A., Calberg,,C., Kerckhofs, G., *et.al.*, " Characterization of the porous structure of biodegradable scaffolds obtained with supercritical CO2 as foaming agent ", Journal of Porous Materials, Vol.15(4), pp: 397–403, (2008) , [PDF]

[521] López-Periago, A.M., Vega, A., Subra, P., *et.al.*, " Supercritical CO2 processing of polymers for the production of materials with applications in tissue engineering and drug delivery ", Journal of Materials Science, Vol.43(6), pp: 1939–1947, (2008) , [PDF]

[522] Salerno, A., Netti, P.A., Maio, E. D., Iannace, S.," Engineering of Foamed Structures for Biomedical Application ", Journal of Cellular Plastics, vol. 45: pp. 103 – 117, (2009), [PDF]

[523] Walter, A., Leicher, S., Wintermantel, E.," Cell 3D: Kunststoffschäume für dreidimensionale Zellkultivierung „, Medizintechnik Life Science Engineering, Pages 675-705, Springer Berlin Heidelberg, ISBN 978-3-540-74924-0 (Print) , (2008)

[524] Wang, X.,, Li, W., Kumar, V., "Creating Open-celled Solid-state Foams Using Ultrasound ", Journal of Cellular Plastics, vol. 45: pp. 353 – 369, (2009), [PDF]

[525] Habulin, M., Primoži, M., Knez, Ž.," Enzymatic Reactions in High-Pressure Membrane Reactors ",
Ind. Eng. Chem. Res., vol.44 (25), pp 9619–9625, (2005) , [PDF]

[526] Habulin, M., Šabeder, S., Paljevac, M., *et.al.*, " Lipase-catalyzed esterification of citronellol with lauric acid in supercritical carbon dioxide/co-solvent media ", The Journal of Supercritical Fluids, Vol.43(2), pp: 199-203, (2007), [PDF]

[527] Habulin, M., Primozic, M., Knez, Z.," Supercritical Fluids as Solvents for Enzymatic Reactions ",
Acta Chim. Slov. Vol.54, pp: 667–677, (2007), [PDF]

[528] Habulin, M., Šabeder, S., Sampedro, M.A., Knez, Ž., " Enzymatic synthesis of citronellol laurate in organic media and in supercritical carbon dioxide ", Biochemical Engineering Journal, Vol.42(1), pp: 6-12, (2008), [PDF]

[529] King, J.W., Snyder, J.M., Frykman, H., Neese, A., " Sterol ester production using lipase-catalyzed reactions in supercritical carbon dioxide ", Eur. Food Res. Technol., vol.212, pp: 566–569, (2001), [PDF]

[530] Kwon, C.H., Jeong, .Y., Kang, J.W., " Molecular modeling and experimental verification of lipase-catalyzed enantioselective esterification of racemic naproxen in supercritical carbon dioxide ", Korean J. Chem. Eng., vol.26(1), pp: 214 – 219, (2009), [PDF]

[531] Tsitsimpikou, C., Stamatis, H., Sereti, V., Daflos, H., Kolisis, F.N., " Acylation of glucose catalysed by lipases in supercritical carbon dioxide ", J. Chem. Technol. & Biotechnol., vol.71(4), pp: 309-314, (1998), [PDF]

[532] Varma, M.N., Madras, G., " Kinetics of synthesis of butyl butyrate by esterification and transesterification in supercritical carbon dioxide ", Journal of Chemical Technology & Biotechnology, Vol.83(8), pp: 1135 – 1144, (2008), [PDF]

[533] Zhang, J., Han, B., " Supercritical CO_2-continuous microemulsions and compressed CO_2-expanded reverse microemulsions ", The Journal of Supercritical Fluids, Vol.47(3), pp: 531-536, (2009), [PDF]

[534] Jiménez-Carmona, M.M., Luque de Castro, M.D., " Reverse-micelle formation: a strategy for enhancing CO2-supercritical fluid extraction of polar analytes ", Analytica Chimica Acta, Vol.358(1), pp: 1-4, (1998), [PDF]

[535] Kawakami, K., Urakawa, T., Oda, Y., Iwai, Y., " Activation of lipase by sol–gel coating with hydrophobic alkyl-substituted silicates in supercritical carbon dioxide ", J. Chem. Technol. Biotechnol., vol.84, pp: 1412–1417, (2009), [PDF]

[536] Lozano, P., Víllora, G., Gómez, D., *et.al.* , " Membrane reactor with immobilized Candida antarctica lipase B for ester synthesis in supercritical carbon dioxide ", The Journal of Supercritical Fluids, Vol.29(1-2), pp: 121-128, (2004), [PDF]

[537] Lozano, P., De Diego, T., Sauer, T., *et.al.*," On the importance of the supporting material for activity of immobilized Candida antarctica lipase B in ionic liquid/hexane and ionic liquid/supercritical carbon dioxide biphasic media ", The Journal of Supercritical Fluids, Vol.40(1), pp: 93 – 100 , (2007), [PDF]

[538] Maury, S., Pierre, A. C. " Hydrolysis Behaviour of Lipase from *Pseudomonas cepacia* Encapsulated in Silica Aerogels with Different Characteristics ", Macromolecular Bioscience, Vol.1(4), pp: 119-125, (2001), [PDF]

[539] Maury, S., Buisson, P., Perrard, A., Pierre, A.C., " Influence of the sol–gel chemistry on the activity of a lipase encapsulated in a silica aerogel ", Journal of Molecular Catalysis B: Enzymatic, Vol.29(1-6), pp: 133-148, ,(2004), [PDF]

[540] Maury, S., Buisson, P., Perrard, A., Pierre, A.C., " Compared esterification kinetics of the lipase from *Burkholderia cepacia* either free or encapsulated in a silica aerogel ", Journal of Molecular Catalysis B: Enzymatic, Vol.32(5-6), pp: 193-203, (2005), [PDF]

[541] Oliveira, D., Feihrmann, A.C., Rubira, A.F., *et.al.*," Assessment of two immobilized lipases activity treated in compressed fluids ", The Journal of Supercritical Fluids, Vol.38(3), pp: 373-382, (2006), [PDF]

[542] Romero, M.D., Calvo, L., Alba, C., Habulin, M., Primožič, M., Knez, Ž., " Enzymatic synthesis of isoamyl acetate with immobilized Candida antarctica lipase in supercritical carbon dioxide ", The Journal of Supercritical Fluids, Vol.33(1), pp: 77-84, (2005), [PDF]

[543] Bruns, N., Bannwarth, W., Tiller, J.C.," Amphiphilic conetworks as activating carriers for the enhancement of enzymatic activity in supercritical CO_2 ", Biotechnology and Bioengineering, Vol.101(1), pp: 19 – 26, (2008), [PDF]

[544] Frykman, H.B., Snyder, J.M. , King, J.W. " Screening Catalytic Lipase Activities with an Analytical Supercritical Fluid Extractor ", JAOCS, Vol. 75(4), pp: 517 – 520, (1998), [PDF]

[545] Ghanem, A.," Trends in Lipase-Catalyzed Asymmetric Access to Enantiomerically Pure/Enriched Compounds ", Tetrahedron, vol 63(8), pp: 1721-1754 ; (2007), [PDF]

[546] Glowacz, G., Bariszlovich, M., Linke, M., Richter, P., Fuchs, C., Morsel, J.T. , ,, Stereoselectivity of lipases in supercritical carbon dioxide. I. Dependence of the regio- and enantioselectivity of. porcine pancreas lipase on the water content during the hydrolysis of triolein and its partial glycerides. ", Chemistry and Physics of Lipids, 79 (2), p.101-106, (1996), [PDF]

[547] Guo, Y., Akgerman, A., " Determination of selectivity for parallel reactions in supercritical fluids ", Journal of Supercritical Fluids, The, Vol.15(1), pp: 63 – 71, (1999), [PDF]

[548] Hlavsová, K., Wimmer, Z., Xanthakis, E., *et.al.*," Lipase activity enhancement by SC-CO_2 treatment ", Zeitschrift fur Naturforschung – Sec. B J. Chem. Sci.,vol. 63(6), pp. 779-784, (2008), [PDF]

[549] Noel, M., Combes, D., " Effects of temperature and pressure on Rhizomucor miehei lipase stability ", Journal of Biotechnology, Vol.102(1), pp: 23-32, (2003), [PDF]

[550] Rozzi, N.L., Singh, R.K.," Supercritical Fluids and the Food Industry ", Comp.Rev. Food Sci., vol 1(1), pp: 33- 44, (2002), [PDF]

[551] Rezaei, K., Jenab, E., Temelli, F.," Effects of water on enzyme performance with an emphasis on the reactions in supercritical fluids ", Critical Rewievs in Biotechnology, Vol.27(4), pp: 183-195, (2007), [PDF]

[552] Kasche, V., Schlothauer, R., Brunner, G.," Enzyme denaturation in supercritical CO2: Stabilizing effect of S-S bonds during the depressurization step ", Biotechnology Letters, Vol.10(8), pp: 525 – 608, (1988), [PDF]

[553] Celebi, N., Yildiz, N., Demir, A.S., Calimli, A.," Enzymatic synthesis of benzoin in supercritical carbon dioxide ", The Journal of Supercritical Fluids, Vol.41(3), pp: 386-390, (2007), [PDF]

[554] Erickson, J.C., Schyns, P., Cooney,C.L.," Effect of pressure on an enzymatic reaction in a supercritical fluid, ", AIChE J. 36(2), pp: 299–301, (1990), [PDF]

[555] Laudani, C.G., Habulin, M., Knez, Ž., *et.al.*," Lipase-catalyzed long chain fatty ester synthesis in dense carbon dioxide: Kinetics and thermodynamics ", The Journal of Supercritical Fluids, Vol. 41, Iss.1, pp: 92-101, (2007), [PDF]

[556] Francisco, J.C., Gough, S.P., Deyi, E.S.," Chp. 20: Use of Lipases in the Synthesis of Structured Lipidsin Supercritical Carbon Dioxide, pp: 341–354. " ,Author: Julio Polaina and Andrew P. MacCabe, Industrial Enzymes ,Structure, Function and Applications, ISBN: 978-1-4020-5376-4, 2007, Springer Berlin / Heidelberg

[557] Kamat, S., Barrera, J., Beckman, J., Russell, A.J. " Biocatalytic synthesis of acrylates in organic solvents and supercritical fluids: I. Optimization of enzyme environment ", Biotechnology and Bioengineering, Vol.40(1), pp: 158 – 166, (2004), [PDF]

[558] Kamat, S., Critchley, G., Beckman, E.J., Russell, A.J.," Biocatalytic synthesis of acrylates in organic solvents and supercritical fluids: III. Does carbon dioxide covalently modify enzymes? ", Biotechnology and Bioengineering, Vol.46(6), pp: 610 – 620, (2004) , [PDF]

[559] Radzi, S.M., Basri, M., Salleh, A. B., *et.al.*, " High performance enzymatic synthesis of oleyl oleate using immobilized lipase from Candida antartica" , Electronic Journal of Biotechnology, vol.8(3), pp: 291 – 298, (2005), [PDF]

[560] Laudani, C.G., Habulin, M., Knez, Ž., Porta, G.D., Reverchon, E., " Immobilized lipase-mediated long-chain fatty acid esterification in dense carbon dioxide: bench-scale packed-bed reactor study ", The Journal of Supercritical Fluids, Vol.41(1), pp: 74-81, (2007), [PDF]

[561] Smith , L:D.,The engineering of Chemical Reactions, Oxford University press, ISBN O-19-510588-5, (1998)

[562] Levenspiel, O.,Chemical Reaction Engineering , 3rd ed. John Wiley & Sons, ISBN : 0-471-25424-X,

[563] Schließmann, A., Hidalgo, A., Berenguer, J., Bornscheuer, U.T. "Increased Enantioselectivity by Engineering Bottleneck Mutants in an Esterase from Pseudomonas fluorescens ", ChemBioChem 0000, 00, 1 – 5, in press, [PDF]

[564] Chen, C-S., Fujimoto, Y., Girdaukas, G., Sih, C.J.," Quantitative Analyses of Biochemical Kinetic Resolutions of Enantiomers?", J. Am. Chem. Soc. Vol.104, pp: 1294 – 1299, (1982), [PDF]

[565] Bornscheuer, UT , "Methods to increase enantioselectivity of lipases and esterases ", Curr. Opin. Biotech., vol. 13 (6), pp: 543-547, (2002), [PDF]

[566] Anthonsen, H.W., Hoff, B.H., Anthonsen, T.," Calculation of enantiomer ratio and equilibrium constants in biocatalytic ping-pong bi-bi resolutions ", Tetrahedron: Asymmetry, vol.7(9), pp: 2633-2638, (1996), [PDF]

[567] Balavoine, G.; Moradpour, A.; Kagan, H. B.," Preparation of Chiral Compounds with High Optical Purity by Irradiation with Circularly Polarized Light, a Model Reaction for the Prebiotic Generation of Optical Activity ", J. Am. Chem. Soc., vol. 96, pp. 5152–5158, (1974), [PDF]

[568] Martin, V.S., Woodard, S.S., Katsuki, T., Yamada, Y., Ikeda, M., Sharpless, K. B., " Kinetic resolution of racemic allylic alcohols by enantioselective epoxidation. A route to substances of absolute enantiomeric purity? J. Am. Chem. Soc.. vol. 103(20), pp: 6237-6240, (1981), [PDF]

[569] Chen,C.S., Wu, S.H., Girdaukas, G., Sih, C.J., "Quantitative analyses of biochemical kinetic resolution of enantiomers. 2. Enzyme-catalyzed esterifications in water-organic solvent biphasic systems", J. Am. Chem. Soc., Vol. 109(9), pp: 2812 – 2817, (1987), [PDF]

[570] Chen, C.S., Sih, C.J., "General Aspects and Optimization of Enantioselective Biocatalysis in Organic Solvents: The Use of Lipases ", Angew. Chemie Int. Ed. Eng.,Vol. 28(6), pp: 695-707, (1989), [PDF]

[571] Van Tol, J.B., Geerlof, J.A., Duine, J.A.,"Kinetic Resolution of Racemic GlycerolDerivatives with Lipase and Quinohemoprotein Alcohol Dehydrogenasea", Annals of the New York Academy of Sciences, vol. 672, pp: 462 – 470, (1992), [PDF]

[572] Straathof, A. J. J.; Rakels, J. L. L.; Heijnen, J. J. , "Strategies in the Preparation of Homochiral Compounds Using Combined Enantioselective Enzymes ", Biocatalysis and Biotransformation, vol. 4(2&3), pp: Pages 89 – 104, (1990), [PDF]

[573] Straathof, A.J.J., Rakels, J.L.L., Heijnen, J.J.,"Kinetics of the Enzymatic Resolution of Racemic Compunds in Bi-Bi Reactions" Biocatalysis, Vol.7, pp. 13-27, (1992), [PDF]

[574] Straathof, A. J. J.; Rakels, J. L. L.; van Tol, J. B. A., Heijnen, J. J., "Improvement of lipase-catalyzed kinetic resolution by tandem transesterification", Enzyme and Microbial Technology, Vol.17(7),pp: 623-628, (1995), [PDF]

[575] Straathof, A.J.J., Jongejan, J.A., "The enantiomeric ratio: origin, determination and prediction", Enzyme and Microbial Technology, Vol.21(8), pp: 559-571, (1997), [PDF]

[576] Alwash, M.A.M., "Periodic solutions of Abel differential equations", Journal of Mathematical Analysis and Applications, Vol.329(2), pp:1161-1169, (2007), [PDF]

[577] Mak, M. K., Harko, T.," New method for generating general solution of Abel differential equation", Computers & Mathematics with Applications, Vol.43(1-2),pp: 91-94, (2002), [PDF]

[578] Panayotounakos, D.E.,"Exact analytic solutions of unsolvable classes of first and second order nonlinear ODEs (Part I: Abel's equations)", Applied Mathematics Letters, Vol.18(2), pp:155-162, (2005), [PDF]

[579] Tomic, S., Dobovicnik, V., Sunjic, V., Kojic-Prodic, B., "Enantioselectivity of *Pseudomonas cepacia* Lipase towards 2-Methyl-3(or 4)-arylalkanols: An Approach Based on the Stereoelectronic Theory and Molecular Modeling" Croatica Chmica ACTA, CCACAA 74 (2) 343¿357 (2001), [PDF]

[580] Tomic, Sanja, Kojic-Prodic ,B., "A quantitative model for predicting enzyme enantioselectivity: application to Burkholderia cepacia lipase and 3-(aryloxy)-1,2-propanediol derivatives", Journal of Molecular Graphics and Modelling, Vol.21(3), pp: 241-252, (2002), [PDF]

[581] Tomic, S., Bertosa, B., Kojic-Prodic, B., Kolosvary, I., "Stereoselectivity of *Burkholderia cepacia* lipase towards secondary alcohols: molecular modelling and 3D QSAR approach", Tetrahedron: Asymmetry, vol. 15, Iss. 7, pp: 1163-1172, (2004), [PDF]

[582] Tomić, S., Ramek, M.,"Quantum mechanical study of *Burkholderia cepacia* lipase enantioselectivity" Journal of Molecular Catalysis B: Enzymatic, Vol.38(3-6), pp: 139 – 147, (2006), [PDF]

[583] Yadav, G.D., Trivedi, A.H.," Kinetic modeling of immobilized-lipase catalyzed transesterification of n-octanol with vinyl acetate in non-aqueous media ", Enzyme and Microbial Technology, Vol. 32, Iss. 7, pp: 783-789, (2003), [PDF]

[584] Xing – Quan, L., Zhao – Xia, J., Wu – Tang, J., Yu-Tang, W., Zuo – Long, Y., "Application of triethylamine and imidazole to the transesterfication reaction ", J. Nat. Gas Chem., Vol.8(1), pp: 76 – 92 , (1999), [PDF]

[585] Demirbas, A., "Comparison of transesterification methods for production of biodiesel from vegetable oils and fats" Energy Conversion and Management, vol. 49, pp: 125–130, (2008), [PDF]

[586] Miyazawa, T., Hamada, M., Morimoto, R., Murashima, T., Yamada, T., " Secondary alcohols act as better nucleophiles than primary alcohols in the lipase-catalyzed regioselective deacylation of dihydroxybenzenes acylated at both phenolic hydroxyls " Tetrahedron Letters, Vol. 48(47), pp: 8334-8337, (2007), [PDF]

[587] Santaniello, E., Casati, S., Ciuffreda, P., Meroni, G., Pedretti, A., Vistoli, G., " A molecular modelling approach to rationalize the stereochemical outcome of the *Burkholderia cepacia* lipase-catalyzed transesterification of aromatic primary alcohols with vinyl esters with different chain lengths in chloroform", Tetrahedron: Asymmetry, In Press, Corrected Proof, (3 Sept. 2009), [PDF]

[588] Schoffers, E., Kohler, L., " Efficient alcoholysis of 5,6-dihydro-1,10-phenanthroline-5,6-epoxide with ytterbium(III) triflate and subsequent enantioselective transesterification with lipases ", Tetrahedron: Asymm., Vol.20(16), pp: 1897-1902, (2009), [PDF]

[589] Singh, M., Singh, S., Singh, R.S., Chisti, Y., Banerjee, U.C., " Transesterification of primary and secondary alcohols using *Pseudomonas aeruginosa* lipase ", Bioresource Technology, vol. 99(7), pp: 2116–2120, (2008), [PDF]

[590] Berendsen, W.R. , Gendrot, G. , Resnick, S., Reuss, M. „Kinetic modeling of lipase catalyzed hydrolysis of (R/S)-1-methoxy-2-propyl-acetate as a model reaction for production of chiral secondary alcohols ", J. Biotechnol.vol. 121 , pp: 213–226, (2006), [PDF]

[591] Garcia, T., Coteron, A., Martinez, M., Aracil, J. "Kinetic model for the esterification of oleic acid and cetyl alcohol using an immobilized lipase as catalyst ", Chemical Engineering Science, Vol.55(8), pp: 1411-1423, (2000), [PDF]

[592] Hirata, H., Kawanishi, M., Iwata, Y., Sakaki, K., Yanagishita, H. " Kinetic Studies on Lipase-Catalyzed Acetylation of 2-Alkanol with Vinyl Acetate in Organic Solvent ", Journal of Oleo Science, Vol. 56(6), pp.309-317,(2007) , [PDF]

[593] Yadav, G.D., Borkar, I.V. , "Kinetic modeling of immobilized lipase catalysis in synthesis of n-butyl levulinate ", Ind. Eng. Chem. Res., 47(10), pp:3358-3363, (2008), [PDF]

[594] Arcos, J.A., Hill C.G. Jr., Otero, C."Kinetics of the lipase-catalyzed synthesis of glucose esters in acetone" Biotechnology and Bioengineering, Vol.:73(2), pp: 104-110, (2001), [PDF]

[595] Al-Zuhair, S., Ramachandran, K.B., Hasan, M. "High enzyme concentration model for the kinetics of hydrolysis of oils by lipase" Chemical Engineering Journal, Vol. 103, Iss. 1-3, pp: 7-11, (2004) [PDF]

[596] BerendsenW.R., Gendrot, G, Freund, A., Reuss. M, " A kinetic study of lipase-catalysed reversible kinetic resolution involving verification, at miniplant-scale. ", Biotechnol. Bioeng., vol. 95, pp: 883–892, (2006). [PDF]

[597] Malcata, F.X., Reyes, H.R., Garcia, H.S., Hill Jr. C.G., Amundson, C.H., " Kinetics and mechanisms of reactions catalysed by immobilized lipases ", Enzyme and Microbial Technology, Vol.14(6), pp: 426-446, (1992), [PDF]

[598] Torres, C.F., Fornari, T., Tenllado, D., Señoráns, F.J., Reglero, G., " A predictive kinetic study of lipase-catalyzed ethanolysis reactions for the optimal reutilization of the biocatalyst ", Biochemical Engineering Journal, Vol.42(2), pp:105-110, (2008), [PDF]

[599] Torres, C.F., Vázquez, L., Señoráns, F.J., Reglero, G.," Enzymatic synthesis of short-chain diacylated alkylglycerols: A kinetic study ", Process Biochemistry, Vol.44(9), pp: 1025-1031, (2009), [PDF]

[600] Taylor, K.B., " Enzyme Kinetics and Mechanisms ", Print ©2002 Kluwer Academic Publishers, Dordrech, ISBN: 1-4020-0728-0

[601] Irwin H. Segel, "Enzyme Kinetics", Wiley-Interscience, ISBN-10: 0471303097 (1993)

[602] Berendsen, W.R., Lapin A., Reuss, M., „Non-isothermal lipase-catalyzed kinetic resolution in a packed bed reactor: Modeling, simulation and miniplant studies “, Chemical Engineering Science, vol. 62, pp:2375 – 2385, (2007), [PDF]

[603] Yildirim, N., Ankaralioglu, N., Yildirim, D. and Akcay, F. ,”Application of Grobner basis theory to derive rate equations for enzyme catalyzed reactions with two or more substrates or products, “,
Applied Mathematics and Computation, 137(1), 67-76, (2003), [PDF]

[604] Yildirim, N. , “Use of symbolic and numeric computation techniques in analysis of biochemical reaction networks,”
International Journal of Quantum Chemistry, 106,256-265, (2006), [PDF]

[605] Yildirim, N., Akcay, F. , Okur, H., Yildirim, D. “Parameter estimation of nonlinear models in biochemistry: a comparative study on optimization methods “, Applied Mathematics and Computation, vol.140, pp: 29–36, (2003), [PDF]

[606] Yildirim, N., Çiftçi, M., Küfrevioglu, Ö.I., “Kinetic analysis of multi enzyme systems:A case study of the closed system of creatine kinase, hexokinase and glucose 6-phosphate dehydrogenase”,
Journal of Mathematical Chemistry, Vol. 31(1), pp: 121 – 130, (2002), [PDF]

[607] Heidt, M., Bornscheuer, U., Schmid ,R. D. „ Studies on the enantioselectivity in the lipase-catalyzed synthesis of monoacylglycerols from isopropylidene glycerol “, Biotechnology Techniques, 10(1), pp:25-30, (1996), [PDF]

[608] Tservistas, M., Köneke, R., Comte, A. and Scheper, T. “ Oxygen monitoring in supercritical carbon dioxide using a fibre optic sensor “, Enzyme and Microbial Technology , Vol. 28(7-8), pp: 637-641, (2001), [PDF]

[609] Crosby, J. , “Synthesis of optically active compounds: A large scale perspective “,
Tetrahedron, Vol.47(27), pp: 4789 – 4846,(1991), [PDF]

[610] Stinson, S.C. .”Chiral drugs “, Chem Engineering News, vol.78(43), pp: 55 -78, (2000), [PDF]

[611] Tucker, G.T. ,”Chiral switches. “,Lancet, vol.355(9209),pp: 1085-1087, (2000), [PDF]

[612] Lee, S. B.; Mitchell, D. T.; Trofin, L.; Nevanen, T. K.; Söderlund, H.,Martin, C. R. ,”Antibody-Based Bio-Nanotube Membranes for Enantiomeric Drug Separations”, Science, , 296(5576), pp: 2198 - 2200, (2002), [PDF]

[613] Miyako, E, “Highly Enantioselective Separation Using a Supported Liquid Membrane Encapsulating Surfactant-Enzyme Complex”,J. Am. Chem. Soc., vol. 126(28), pp: 8622 - 8623, (2004), [PDF]

[614] Yoon, T.H., Kim, In-Ho., “Chiral separation of ketoprofen racemate by using chirex® 3005 and kromasil® CHI-II chiral column “,
Korean Journal of Chemical Engineering, vol. 21(2), pp: 521-526, (2004), [PDF]

[615] Xia, J., Hui, Y.Z.,”The efficient synthesis of mixed diacyl phospholipids with polyunsaturated fatty acid in sn-2 position of glycerol”, Tetrahedron: Asymmetry, Vol. 8(28), pp. 3019--3021, (1997), [PDF]

[616] Baer, E., Mourukas, J., Russel, M.,“Synthesis of Enantiomeric α-Cephalins”,
J.Am.Chem.Soc., vol.74(1), pp 152 – 157, (1952), [PDF]

[617] Baer, E., Staneer, H.C., “An Improved Procedure for the Synthesis of Enantiomeric α-Lecithins”,
J.Am.Chem.Soc., vol. 74(1), pp 158 – 160, (1952), [PDF]

[618] Nelson, W.L., Burke, T.Jr.,”Absolute configuration of glycerol derivatives. 5. Oxprenolol enantiomers”
J. Org. Chem.,vol. 43(9), pp: 3641 - 3645, (1978), [PDF]

[619] Bhatia, S.K., Hajdu, J.,”Stereospecific synthesis of PAF analogues “, Tetrahedron Lett., vol.28, pp:1729- 1732, (1987), [PDF]

[620] Peters, U., Bankova, W., Welzel, P.”Platelet activation factor synthetic studies”,
Tetrahedron, vol.43, pp: 3803 - 3816 , (1987), [PDF]

[621] Stork, G., Takahasi, T., “Chiral synthesis of prostaglandins (PGE1) from D-glyceraldehyde”,
J. Am. Chem. Soc., vol. 99, pp 1275 – 1276, (1977), [PDF]

[622] Hanson, R.M.,”The Synthetic Methodology of Nonracemic Glycidol and Related 2,3-Epoxy Alcohols”
Chemical Revlews, Vol. 91, No. 4, pp: 437 – 475, (1991), [PDF]

[623] Jurczak, J., Pikul, S., Bauer, T.,“(R)- and (S)- 2,3-O-Isopropyledeneglyceraldehyde in Streoselective Synthesis”,
Tetrahedron, vol.42(2), pp: 447 – 488, (1986), [PDF]

[624] Molinari, F., Romano, D., Gandolfi, R., Kroppenstedt, R.M., Marinelli, F.,”Newly isolated Streptomyces spp. as enantioselective biocatalysts: hydrolysis of 1,2-O-isopropylidene glycerol racemic esters “
‚Journal of Applied Microbiology, 99(4), p. 960, (2005), [PDF]

[625] Molinari, F., Brenna, O., Valenti, M. and Aragozzini, F. ,”Isolation of a novel carboxylesterase from Bacillus coagulans with high enantioselectivity toward racemic esters of 1, 2-O-isopropylideneglycerol.”,
Enzyme Microb Technol, vol.19(7), pp:551–556, (1996), [PDF]

[626] Molinari, F.,Gandolfi, R.,”Enzymatic synthesis of (S)-1,2-O-isopropylidene glycerol”, European Patent EP1413627, [PDF]

[627] Aragozzini, F., Maconi, E., Potenza, D., and Scolastico, C. " Enantioselective microbial reduction of monoesters of 1,3-dihydroxypropanone: Synthesis of (R) and (s)-1.2 - O-isopropylideneglycerol. ", Synthesis, vol.3, pp: 225-227,(1989), [PDF]

[628] Maconi, E., Gualandris, R., Aragozzini, F.,"Hydrolytic Microbial Resolution of 1,2-O-Isopropylidenglycerol: Improvements by using Ester with Homologous Fatty Acids", Biotechnology Letters Vol 12 No 6 415-418 (1990), [PDF]

[629] Romano, D., Falcioni, F., Mora, D., Molinari, F., Buthe, A., Ansorge-Schumacher, M.," Enhanced enantioselectivity of Bacillus coagulans in the hydrolysis of 1,2-O-isopropylidene glycerol esters by thermal knock-out of undesired enzymes", Tetrahedron Asymmetry,vol. 16, pp: 841-845, (2005), [PDF]

[630] Vänttinen, E. and Kanerva, E.L. ,"Optically active 1, 3-dioxolane-4-methanols. Lipase-catalysed acylation of racemic ketals and diastereomeric acetals. ",Tetrahedron Asymmetry, vol. 7(10), pp:3037–3046, (1996), [PDF]

[631] Vänttinen, E., Kanerva, L. T.,"Optimized double kinetic resolution for the preparation of (S)-solketal", Tetrahedron: Asymmetry,vol.8(6), pp:923-933, (1997), [PDF]

[632] Lundh, M., Nordin, O., Hedenström, E., Högberg, H.E.,"Enzyme catalysed irreversible transesterifications with vinyl acetate. Are they really irreversible?", Tetrahedron: Asymmetry, Vol.6(9), pp: 2237-2244,(1995), [PDF]

[633] Secundo, F., Ottolina, G., Riva, S., Carrea, G."The enantioselectivity of lipase PS in chlorinated solvents increases as a function of substrate conversion", Tetrahedron: Asymmetry, Vol.8(13), pp: 2167-2173, (1997), [PDF]

[634] Zanoni, G., Agnelli, F., Meriggi, A., Vidari, G. , „ Enantioselective syntheses of isoprostane and iridoid lactones intermediates by enzymatic transesterification ",Tetrahedron: Asymmetry , Vol.12(12), pp: 1779-1784, (2001), [PDF]

[635] Guieysse, D., Cortés, J., Puech-Guenot, S., *et.al.*,"A Structure-Controlled Investigation of Lipase Enantioselectivity by a Path-Planning Approach", ChemBioChem, Vol.9(8), pp: 1308-1317, (2008), [PDF]

[636] Guieysse, D., Salagnad, C., Monsan, P., Remaud-Simeon, M., "Lipase-catalyzed enantioselective transesterification toward esters of 2-bromo-tolylacetic acids", Tetrahedron: Asymmetry, vol.14(3), pp: 317–323, (2003), [PDF]

[637] Mezzetti, A., Schrag, J. D., Cheong, C. S., and Kazlauskas, R. J. Mirror-image packing in enantiomer discrimination: molecular basis for the enantioselectivity of B. cepacia lipase toward 2-methyl-3-phenyl-1-propanol. Chem. Biol., vol. 12, pp: 427-437, (2005), [PDF]

[638] Brady, L., Brzozowski, A.M., Derewenda, Z. S., Dodson, E., *et al.*, "A serine protease triad forms the catalytic centre of a triacylglycerol lipase. ", Nature, vol. 343, pp: 767 – 770,(1990) , [PDF]

[639] van Tilbeurgh, H., Egloff, M.P., Martinez, C., *et.al.*, "Interfacial activation of the lipase−procolipase complex by mixed micelles revealed by X-ray crystallography", Nature, vol. 362, pp:814 - 819, (1993), [PDF]

[640] Lang, D.A., Mannesse, M.L., de Haas, G.H., Verheij, H.M., and Dijkstra, B.W. ,"Structural basis of the chiral selectivity of *Pseudomonas cepacia* lipase", Eur. J. Biochem. Vol.254, pp: 333-340, (1998), [PDF]

[641] Ollis , D.L., Cheah, E., et. al.,"The /ß hydrolase fold ",Protein Eng. Vol.5, pp: 197-211, (1992), [PDF]

[642] Schrag, J. D., Cygler, M.,"1.8 A refined structure of the lipase from Geotrichum candidum.", J Mol Biol. Vol.230(2), pp:575-591, (1993), [PDF]

[643] Warshel A, Narayszabo G, Sussman F, Hwang JK., "How do serine protease really work?", Biochemistry, vol.28, pp: 3629-3637, (1989), [PDF]

[644] Grabuleda, X., Jaime C., Guerrero, A. "Estimation of the lipase PS (*Pseudomonas cepacia*) active site dimensions based on molecular mechanics calculations ",Tetrahedron: Asymmetry, Vol. 8, Iss. 21, pp: 3675-3683, (1997), [PDF]

[645] Lavandera, I., Fernández, S., Magdalena, J., Ferrero, M., Grewal, H., Savile, C.K., Kazlauskas, R.J., Gotor, V. „Remote Interactions Explain the Unusual Regioselectivity of Lipase from *Pseudomonas cepacia* toward the Secondary Hydroxyl of 2-Deoxynucleosides", ChemBioChem, Vol.7 (4), pp: 693-698, (2006), [PDF]

[646] Kim, KK, Song, HK, Shin, DH, Hwang, KY, Suh, SW.,"The crystal structure of a triacylglycerol lipase from *Pseudomonas cepacia* reveals a highly open conformation in the absence of a bound inhibitor",Structure, Vol 5 No 2, pp:173-185, (1997), [PDF]

[647] Cortes, J., Simeon, T., *et.al.*,"A path planning approach for computing large-amplitude motions of flexible molecules" Bioinformatics, vol.21, pp: 116-125, (2005), [PDF]

[648] Guieysse, D., Salagnad, C., Monsan, P., Remaud-Simeon, M., Tran, V.," Towards a novel explanation of *Pseudomonas cepacia* lipase enantioselectivity via molecular modelling of the enantiomer trajectory into the active site", Tetrahedron: Asymmetry, vol.14(13), pp: 1807–1817, (2003), [PDF]

[649] Tuomi, W. V., Kazlauskas, R.J.,"Molecular Basis for Enantioselectivity of Lipase from *Pseudomonas cepacia* toward Primary Alcohols. Modeling, Kinetics, and Chemical Modification of Tyr29 to Increase or Decrease Enantioselectivity", J. Org. Chem., Vol.64, pp: 2638-2647., (1999),[PDF]

[650] Ohno, M., Kamo, N., Amoto, T., *et.al.*, "Kinetic Studies on the Optical Resolution of 2-Alkanol by *Pseudomonas cepacia* Lipase Catalyzed Transesterification with Vinyl Acetate in Organic Solvent", Journal of Oleo Science, Vol. 51(10), pp: 643-653,(2002), [PDF]

[651] Hirata, H., Kondo, G., Kawauchi, K., *et.al.*,"Kinetic Studies on Lipase-Catalyzed Acetylation of 1-Alkanol with Vinyl Acetate in Organic Solvent ",Journal of Oleo Science, Vol. 55(5), pp: 239-248, (2006) , [PDF]

[652] Sahin, S., Warnå,J., Maki-Arvela, P., Salmi, T., Murzin, D.Y.,"Kineticmodeling of lipase-mediated one-pot chemo-bio cascade synthesis of R-1-phenyl ethyl acetate starting from acetophenone ", J. Chem. Technol. Biotechnol., 16 September (2009), [PDF]

[653] Faber, K., Riva, S., "Enzyme-catalyzed irreversible acyl transfer. ",Synthesis, iss.(10), pp: 895–910. (1992), [PDF]

[654] Ghanem, A., Aboul-Enein, H.Y. "Application of lipases in kinetic resolution of racemates", Chirality,Volume 17 Issue 1, Pages 1 – 15,(2004), [PDF]

[655] Ghanem, A.,, Hoenen, H., Aboul-Enein, H.Y.,"Application and comparison of immobilized and coated amylose tris-(3,5-dimethylphenylcarbamate) chiral stationary phases for the enantioselective separation of β-blockers enantiomers by liquid chromatography", Talanta, Vol.68(3), pp:602-609, (2006), [PDF]

[656] Yu, Y., Lutz, S., "Improved triglyceride transesterification by circular permuted *Candida antarctica* lipase B", Biotechnology and Bioengineering, Vol. xxx, No. xxx, (2009), [PDF]

[657] Braiuca, P., Lorena, K., Ferrario, V., Ebert, C., Gardossi, L., "A Three-Dimensional Quanititative Structure-Activity Relationship (3D-QSAR) Model for Predicting the Enantioselectivity of Candida antarctica Lipase B", Advanced Synthesis & Catalysis, vol. 351(9), pp: 1293-1302, (2009), [PDF]

[658] Bayram, M., "A novel method for analyzing enzyme kinetic systems", Applied Mathematics and Computation, vol. 87(2-3), pp: 161-174, (1997), [PDF]

[659] Bayram, M., Yildirim, N., "Metabolic control analysis of trio enzymes system", Applied Mathematics and Computation, vol. 170, pp: 948–957, (2005), [PDF]

[660] Çelik, E., Bayram, M., "Application of Gröbner basis techniques to enzyme kinetics" Applied Mathematics and Computation, Vol.153(1), pp: 97-109, (2004), [PDF]

[661] Yildirim, N., Bayram, M., "An analysis of the kinetics of unstable enzymatic systems using MAPLE" Applied Mathematics and Computation, vol. 112, pp: 41-48, (2000), [PDF]

[662] Yildirim, N., Bayram, M., "Derivation of conservation relationships for metabolic networks using MAPLE" Applied Mathematics and Computation, vol. 112, pp: 255 – 263, (2000), [PDF]

[663] Yildirim, N., "Quasi-steady state kinetics of simple sequential multienzyme reactions with single substrates" Journal of Mathematical Chemistry Vol. 32(3), pp: 271 – 280 , (2002), [PDF]

[664] Arrhenius, S., "On the Reaction Velocity of the Inversion of Cane Sugar by Acids," Zeitschrift für physikalische Chemie 4, 226ff (1889), [PDF]

[665] Oliveira, M.V., Rebocho, S.F., Ribeiro, A.S., Macedo, E.A., Loureiro, J.M. ,"Kinetic modelling of decyl acetate synthesis by immobilized lipase-catalysed transesterification of vinyl acetate with decanol in supercritical carbon dioxide" J. of Supercritical Fluids, vol.50, pp:138 – 145, (2009) , [PDF]

[666] Salcedo, R.L. ,"Solving nonconvex nonlinear programming and mixed-integer nonlinear programming problems with adaptive random search, ", Ind. Eng. Chem. Res. 31(1), pp: 262–273., (1992), [PDF]

[667] Al-Zuhair, S., Ling, F.W., Jun, L.S. " Proposed kinetic mechanism of the production of biodiesel from palm oil using lipase" Process Biochemistry, vol. 42 , pp: 951–960, (2007), [PDF]

[668] Shin, J.S., Kim, B.G. "Kinetic Modeling of v-Transamination for Enzymatic Kinetic Resolution of a-Methylbenzylamine", Biotechnol.& Bioengr., Vol 60(5), Dec. 5, pp. 534 – 540,(1998), [PDF]

[669] Bezerra, R.M. F., Dias, A.A.,, „Utilization of integrated Michaelis-Menten equation to determine kinetic constants" Biochemistry and Molecular Biology Education, vol. 35(2), pp: 145- 150, (2007), [PDF]

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