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CONTENTS

Chapter 1

RESEARCHES ON REMOVAL OF 6-AMINOPENICILLANIC ACID (6-APA) FROM WASTEWATER

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All life on earth depends on water, which is separated into four primary categories: drinking water, groundwater, surface water (lakes, rivers, rain, and springs), and sea water (oceans, seas). In the past century, getting access to affordable, clean water has become one of science's most fundamental responsibilities [1]. Agricultural, industrial, and medical activity have all greatly grown with the rise in world population, civilization, and industrialization. Sadly, this rise has caused the release of a number of hazardous substances into water sources (Figure 1). These pollutants include biological (bacteria, viruses, and fungus), chemical (heavy metals, aromatic compounds, dyes, pesticides, detergents, and insecticides, among others), and physical (microplastics, etc.) contaminants [2]. But there is also a greater need for drinking water. However, the need for potable water is increasing day by day.

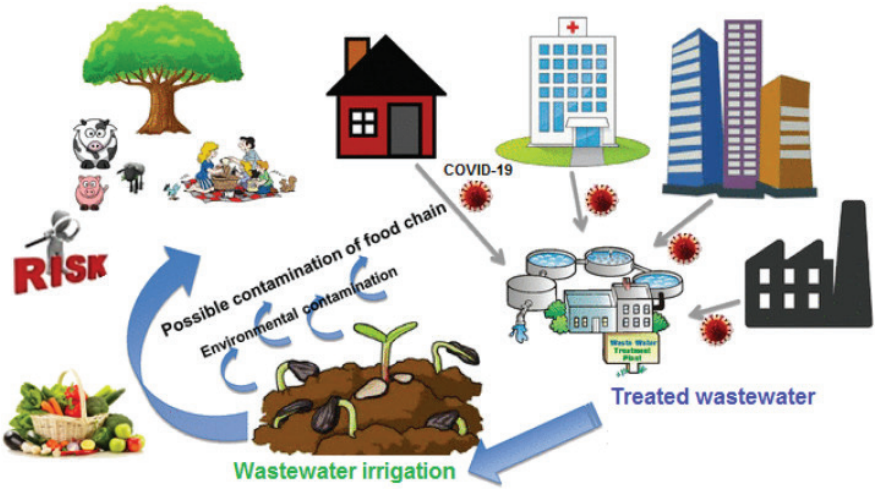

Figure 1: wastewater cycle

Water pollution from drug manufacturing facilities, hospitals, and residences has increased recently because to an increase in the production and usage of pharmaceuticals, especially during the COVID-19 crisis. This contaminated water poses a serious threat to sensitive habitats including the sea and soil [3]. The main class of pharmaceutical pollutants that seriously endanger both human life and ecosystems are antibiotics. Pharmaceutical residues are very toxic, persistent, and biodegradable substances, thus even very low amounts of them in drinking water can be harmful to people's health [4]. Pharmaceutical compounds can induce a variety of diseases by weakening the immune system when they enter the human body through food or accidently [5]. The consumption of antibiotics has expanded along with the population during the past few years. The majority of antibiotics consumed by the body are eliminated in the urine and feces, where they

combine with water. Hospitals and city wastewater have been shown to have antibiotic concentrations between 0.3 to 100 mg/L [6].

Penicillin, the first antibiotic developed by Alexander Fleming in 1928, is a class of drugs used to either treat or prevent bacterial infections. Today, a number of antibiotics, primarily penicillin, cephalosporin, tetracycline, and aminoglycosides, are used to treat ailments. These antibiotics were synthesized long ago, but they're still in use today. By inhibiting the biosynthetic enzymes that bacteria need to build their cell walls, β-lactam antibiotics are extremely powerful in killing both Gram-positive and Gram-negative bacteria [7]. Antibiotics are a class of medications that are frequently used to treat both humans and animals, and their presence in water promotes the growth of bacteria that are resistant to antibiotics [8]. As a result, the antibiotics that have built up in wastewater may contribute to the formation of antibiotic resistance genes and ecosystem harm. They are recognized as dangerous pollutants in water due to their stability and biodegradation resistance. These factors make the elimination of antibiotics from water extremely interesting [9] (Figure 2).

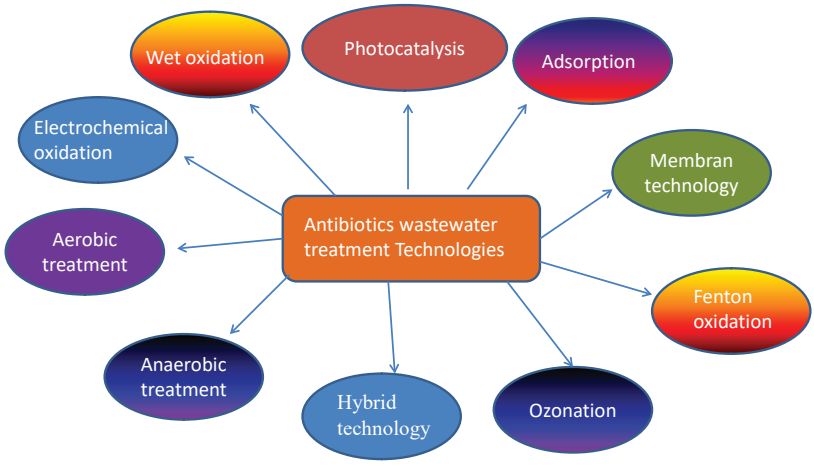

Figure 2: Treatment technologies for antibiotics wastewater

Pharmaceutical wastes in municipal wastewater were measured in parts per million in a study, and it was shown that the biological treatment methods now in use cannot get rid of them [10-12]. Such pharmaceuticals have been found to remain in the environment significantly [13]. Due to the presence of such persistent organic compounds in polluted streams, more efficient cleaning techniques must be created.

Due to their extensive application, β-lactam antibiotics represent the most valuable antibiotic class. And β-lactam antibiotics account for more than half of the global antibiotic market share. The skeletal core of 6-APA, which has the chemical formula $C_8H_{12}N_2O_3S$ and a molar mass of 216.26 g mol^{-1}, is made up of β-lactam antibiotics. It has been used to prevent a number of illnesses, frequently bacterial infections, as well as to promote food development [14] (Figure 3).

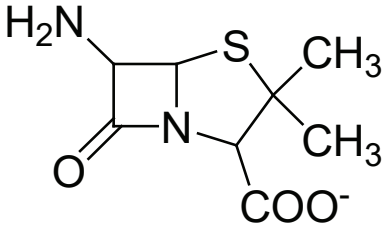

Figure 3: Chemical structure of 6-APA

In addition, 6-APA is also widely used in the pharmaceutical industry because it has high catalytic activity and is environmentally friendly [15, 16]. Currently, more than 75% of penicillin production is obtained from 6-APA [17]. The major starting component of the commercially important semisynthetic penicillins, ampicillin and amoxicillin, is 6-Aminopenicylanic acid (6-APA) [18-21]. Therefore, 6-APA is one of the most widely used β-lactam intermediary products [22]. β-lactam antibiotics are mainly cleaved by parent ring cleavage; however, ring-bound groups are active in this event, they can increase or decrease the potential for degradation [23]. 6-aminopenicylanic acid (6-APA) is one of the pharmaceutical compounds with the skeletal core of beta-lactam antibiotics, which are most commonly found in different concentrations in aquatic environments. According to a study, between 100,000 and 200,000 tons of antibiotics are consumed annually, and active metabolites like 6-Aminopenicylanic acid (6-APA) and ceftriaxone sodium are the main ones that are discharged into the environment [24]. The pharmaceutical sector has expanded significantly in recent years. Amoxicillin and 6-Aminopenicylanic Acid (6-APA) are more frequently found in pharmaceutical effluent. They are obtained by fermentation or chemical synthesis [25]. Wastes of 6-APA and ceftriaxone sodium pose a threat to public health. The reason for this is that their production and consumption are quite high and they have a disadvantage such as permanence in wastewater treatment processes [26]. Therefore, it is very important to purify wastewater from these compounds. Conventional treatment methods are available, including coagulation,

electrocoagulation, adsorption, and the activated sludge process. However, the application of these methods to pharmaceutical wastes did not give very good results. Bio-membrane processes such as nanofiltration and reverse osmosis are recommended for the removal of such substances, but these methods also have some limitations that greatly reduce their effectiveness due to contamination [27]. Various difficulties are encountered in the treatment of water containing pharmaceutical wastes such as 6-APA and amoxicillin [28]. Worldwide, industrial and domestic waste are treated using the upstream anaerobic sludge blanket (UASB). Modern anaerobic reactors use the UASB process [29-31] (Figure 4). This technique has the following benefits: a straightforward design, straightforward construction and maintenance, cheap operating costs, the capacity to endure variations in pH, temperature, and substrate concentration [32]. In many industrial wastewaters, such as pulp liquors [33], sulfur liquors [34], wastewater from the production of fiberboard [35], and wastewater from slaughterhouses, UASB is therefore an extensively employed technology. It is advertised as a cost-effective pretreatment system [36].

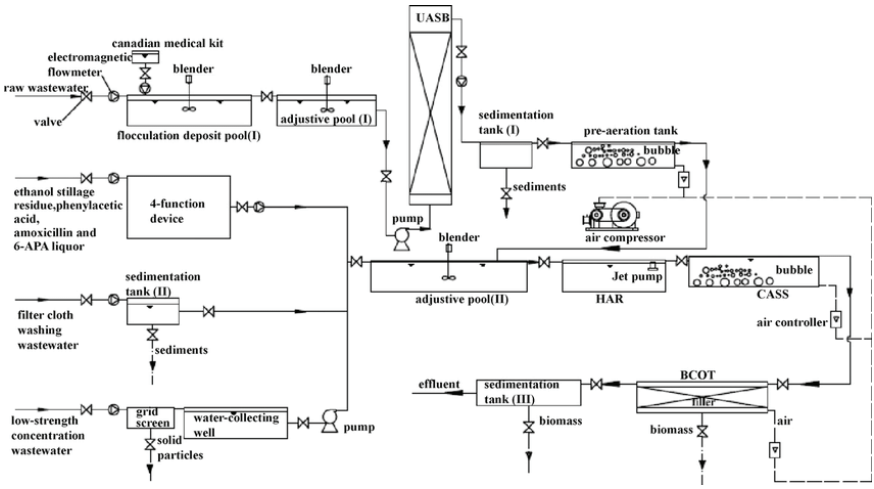

Figure 4: Process flow diagram with UASB

6-aminopenicylanic acid (6-APA), a fundamental raw material for the industrial manufacturing of semi-synthetic penicillins, is obtained with deacylated by using immobilized penicillin acylase from Penicillin G (Pen-G) in a commercial process (Figure 5). In this enzymatic hydrolysis, besides 6-APA, which is a starting material for the preparation of ampicillin and amoxicillin, phenoxyacetic acid is also produced as another by-product [19]. Amoxicillin is a penicillin class broad-spectrum β-lactam antibiotic used in the treatment of gastrointestinal and systemic bacterial infections

[37]. Environmentally harmful compounds such pyridine, phosphorus pentachloride, and nitrosylchloride must be used in the chemical processes used to produce 6-APA and amoxicillin [38]. These 6-APA and amoxicillin pharmaceutical effluents, which primarily come from cleaning equipment, contain a range of organic and inorganic components, including used solvents, catalysts, reactants, and trace amounts of intermediates or products [39]. The semi-synthetic penicillin synthesis relies heavily on 6-APA, one of these byproducts, as a key component. Penicillin G (Pen-G) is enzymatically hydrolyzed by penicillin acylase (EC 3.5.1.11) to create 6-APA, a byproduct of which is phenylacetic acid (PAA) [40]. The hydrolysis reaction is inhibited by both products. Since they should be kept in low concentrations, PAA and 6-APA should be rapidly removed from the process. It is crucial to keep the PAA concentration low while Pen-G is being enzymatically hydrolyzed. Because the enzyme reaction is severely inhibited by phenylacetic acid (PAA), which also lowers the pH of the reaction mixture. Product inhibitions can be removed using a variety of techniques. Ion exchange membrane electrodialysis has been described as one of them as a quick and effective method for ionic species separation. It has been used in desalination and concentration of seawater [41,42], in recovery or removal of toxic compounds and heavy metal ions in wastewater [43, 44], in desalination [46] and in the separation of proteins and amino acids [44] in the food and pharmaceutical industries.

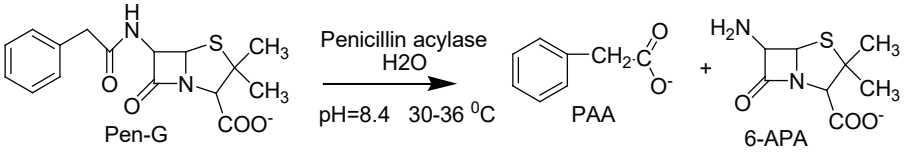

Figure 5: Deacylation of penicillin G (Pen-G) with immobilized penicillin acylase

In another work, constant voltage electrodialysis was used to examine the separation behavior of PAA, 6-APA, and Pen-G. On the separation behavior of PAA, 6-APA, and Pen-G, the effects of ionic concentrations and electric field intensity were examined. Investigated during electrodialysis were the adsorption of PAA, 6-APA, and Pen-G on the anion exchange membrane as well as variations in current and current efficiencies over time [47]. According to Andersson et al. [48], polymeric aqueous two-phase extraction should go hand in hand with Pen-G hydrolysis. Ishimura and Suga [49] suggested combining electrodialysis and Pen-G hydrolysis. While more PAA was retrieved in these experiments, less 6-APA was shown to be present. In a further experiment, Pen-G was hydrolyzed using penicillin acylase in a chromatographic reactor-separator (CRS) [50, 51].

Eliminating 6-APA inhibition in the hydrolysis step is the study's goal.

6-APA is also used in the production of Amoxicillin, one of the widely used antibiotics. However, in the hydrolysis of Amoxicillin, it also occurs as an impurity with 4-HPG (Figure 6).

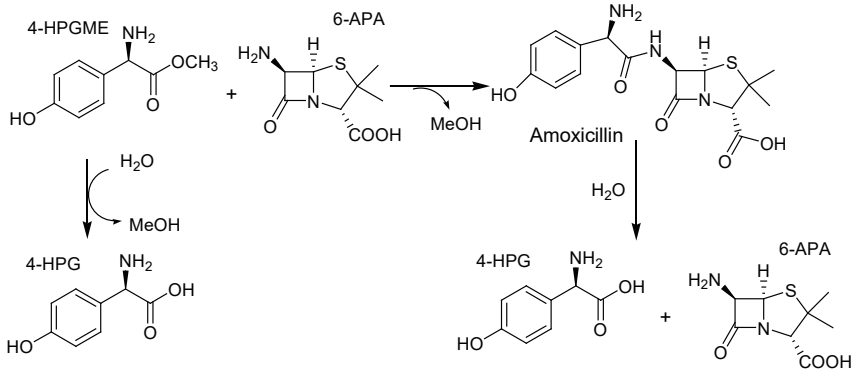

Figure 6: Amoxicillin production

In order to eliminate such pharmaceutical pollutants from aqueous solution, new procedures have been developed recently. These include, for instance, photocatalytic degradation, activated carbon adsorption, membrane filtration, chemical oxidation, and biodegradation [52-56]. Adsorption from the stated processes is favoured because it happens quickly, offers great efficiency, is simple to use, and has low running expenses. Additionally, this approach is offered as a special substitute when natural adsorbent vermiculite (VMT), which is widespread, is utilized [57]. Bentonite [58, 59], montmorillonite [60-63], kaolinite [52, 64] and halloysite [65, 66] are frequently used as adsorbent for wastewater treatment due to their bioavailability [52]. Vermiculite (natural and synthetic) is a low-cost organic adsorbent among clay minerals that has a wide surface area, a high cation exchange capacity, and good swelling properties [67, 68]. In a different study, the thorough investigation of 6-APA's adsorption on vermiculite (VMT) is done [69]. An eco-friendly clay called vermiculite is suggested as a potential contender for extracting 6-APA (6-aminopenicilanic) from pharmaceutical waste.

In the other study, response surface methodology (RSM) and adaptive neuro-fuzzy inference system (ANFIS) models, an efficient, quick, and simple technique, were successfully used with tetraethoxysilane (Si) . The goal of the study was to maximize the 6-APA's ability to adsorb substances by focusing on three factors: the amount of adsorbent (weight), the initial concentration (mg/mL), and the response time (min). The adsorption capacity was calculated as (mg/g) [70].

Another study used oxidizing substances (O_2, H_2O_2, and $K_2S_2O_8$) in the presence of nano ZnO catalyst to evaluate the degradation of 6-aminopenicillanic acid (6-APA) and cloxacillin in aqueous solution. The ideal experimental parameters, including temperature, treatment time, and oxidizing agent concentration, were found using the response surface methodology. H_2O_2, $K_2S_2O_8$, and O_2 were shown to have the highest rates of organic carbon (TOC) removal for 6-APA, with rates of 83.54%, 81.11%, and 42.42%, respectively [3].

In another purification method, the photocatalysis method was used in the presence of TiO2 and it was stated to be effective [71] (Figure 7).

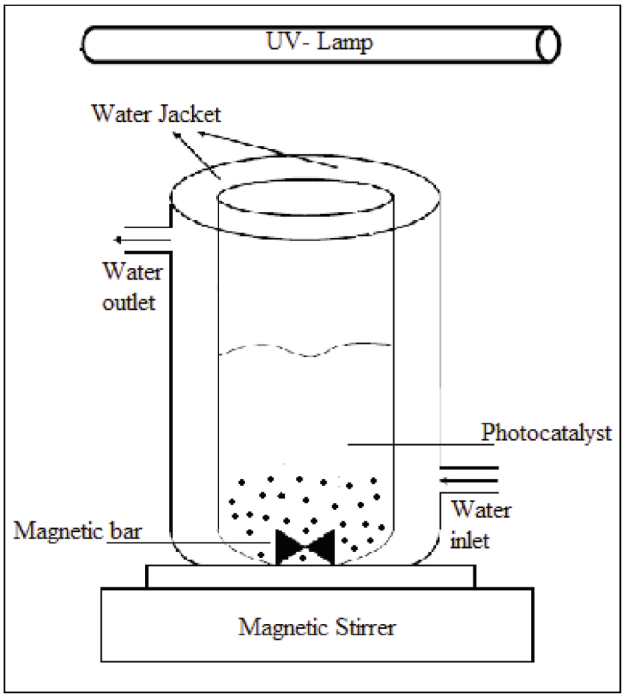

Figure 7: Experimental setup for photocatalysis process

A novel, high-speed reactor is be trying developed with the intention of treating pharmaceutical effluent. According to reports, Hu et al.'s membrane bioreactor (MBR) is superior to conventional reactors at removing pharmaceutical chemicals [72] (Figure 8). According to Prasertkulsak et al. [73] have also used MBR effectively; Chang et al. [74] used a MBR with an average COD removal of 95% to treat pharmaceutical wastewater. However, this approach uses more energy and is more expensive [75]. The primary enzyme activity is determined by dissolved oxygen (DO). The cost and rate of MBR adsorption are similarly determined by DO [76]. It has been stated that a certain amount of dissolved oxygen must be present

in the wastewater for an effective treatment. However, it consumes a lot of energy to provide this oxygen [77]. Additionally, it has been noted that the right amount of low dissolved oxygen might boost sludge production and promote better antibiotic solvent absorption [78]. The performance of the MBR was enhanced, and the airlift bioreactor (ALR) was discovered to address the issue of high energy consumption [79]. High biomass densities are achieved by ALR with minimal shear stresses and effective gas transfer. There are few uses for the multi-stage ALR system, when paired with membrane modules, in the treatment of pharmaceutical wastewater. In one study, a multi-stage vertical variable diameter membrane bioreactor was constructed to treat pharmaceutical wastewater including 6-APA and ceftriaxone sodium [72]. A photocatalyst comprised of semiconductors like ZnO, TiO_2, Fe_2O_3, CdS, SnO_2, ZnS, CeO_2, and WO is first exposed to light to initiate photocatalysis (infrared, visible or ultraviolet). TiO_2 excels over all other semiconductor photocatalysts in these categories due to its low cost, excellent stability, chemical penetration, photoreactivity, and non-toxicity [80].

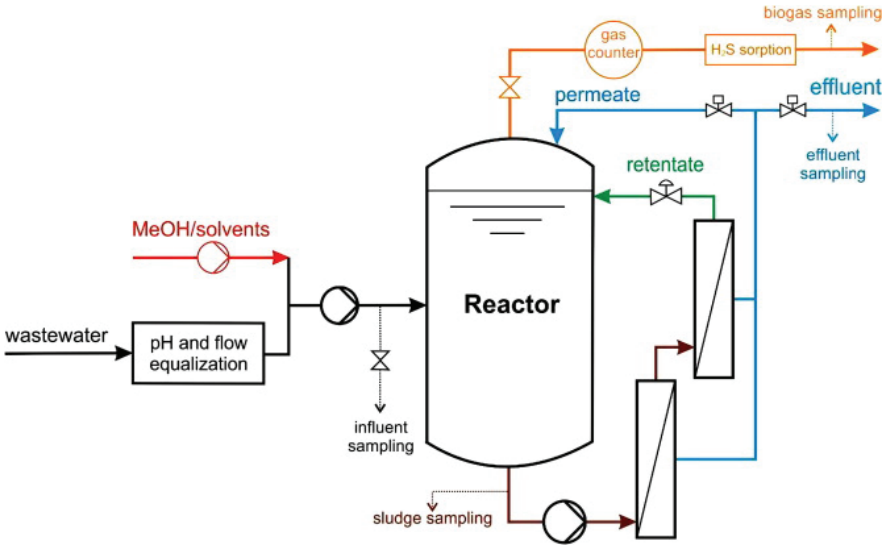

Figure 8: Process flow diagram with pilot MBR

Unsuccessful water treatment can lead to water loss, reduced oxygen levels and coastal closure [81]. The water treatment process aims to capture as much solid matter as possible before it is released into the environment through a two-stage process, primary and secondary treatment [82]. Membranes used in the purification process are used to separate contaminants according to their size or charge [83]. Membrane

technology is widely used due to its cheapness, low chemical consumption and easy availability [84]. The most common membrane procedures include microfiltration (MF), ultrafiltration (UF), reverse osmosis (RO), electrodialysis (ED), and nanofiltration (NF) [85]. These membranes, which are utilized for purification, function as barriers resembling the body's cell walls. While allowing water to pass through, they also prevent the passage of unwanted pollutants [86, 87]. This procedure is significant because it has the potential to develop into a ground-breaking and effective method for the treatment of wastewater and its safe release into the environment. A membrane barrier is used in the membrane process to filter or eliminate dangerous particles from water. In order for the liquid to travel through the membrane and the pollutants to stay on the other side, there must be a pressure difference between the two sides.

Water resources and quality are becoming more and more important day by day [88]. It is very important to recover pharmaceutical compounds from water in order to gain both health and economic benefits.

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Chapter 2

CHROMATOGRAPHY OF BETA-LACTAM ANTIBIOTICS

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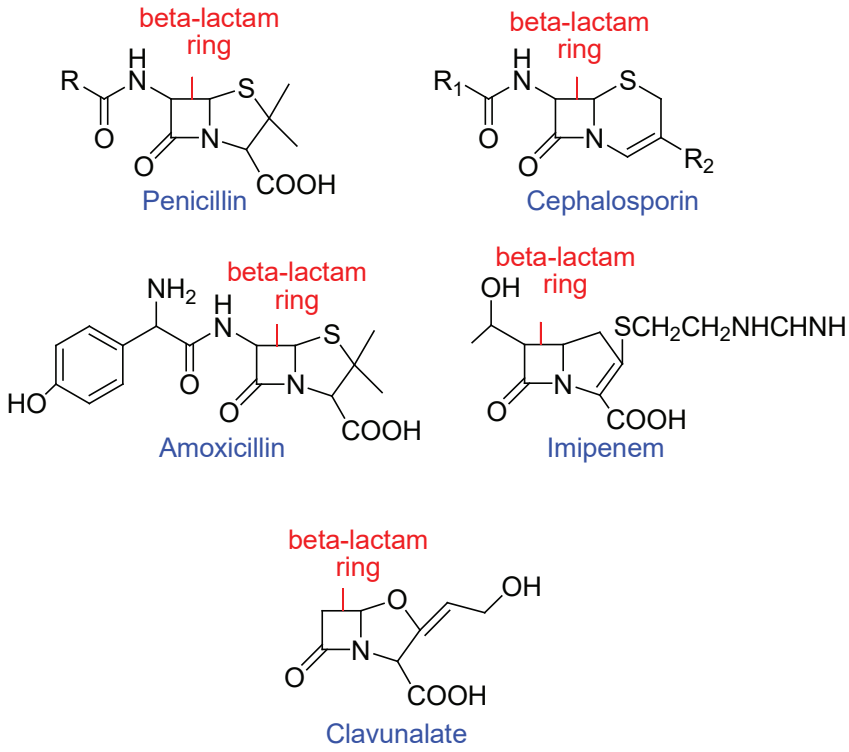
Beta-lactam antibiotics

Beta-lactam antibiotics; It is a large category of antibiotics with different antibacterial effects, chemical structures, and pharmacokinetic properties.[1] The common characteristics of the members of this group are the presence of the beta-lactam ring in the structure of all of them, the mechanisms of action, and the forms of resistance developed against them. Antibiotics in this group; are penicillins, carbapenems, monobactams, cephalosporins, and beta-lactam/beta-lactamase inhibitor combinations.



Scheme 1. *Antibiotics containing beta-lactam rings in their structure*

Common beta-lactam antibiotics

1. Penicillins

1.1. Narrow spectrum penicillins

Benzylpenicillin

Phenoxymethylpenicillin

1.2. Common penicillinase-resistant beta-lactam antibiotics

Methicillin

Dicloxacillin

Flucloxacillin

1.3. Medium spectrum penicillins

Amoxicillin

Ampicillin

1.4. Broad spectrum penicillins

Augmentin

1.5. Extended Spectrum Penicillins

Piperacillin

Ticarcillin

Azlocillin

Carbenicillin

2. Cephalosporins

2.1. First-generation cephalosporins

Cephalothin

Cephalexin

Cefazolin

2.2. Second-generation cephalosporins

Cefaclor

Cefuroxime

Cefamandol

2.3. Second generation cefamycins

Cefotetane

Cefoxitin

2.4. Third-generationlosporins

Ceftriaxone

Cefixime

Cefotaxime

Ceftazidime

2.5. Fourth-generation cephalosporins
Cefapim
Cefpirome

3. Carbapenems
Mipenem in combination with cilastatin
Meropenem
Ertapenem
Doripenem

4. Monobactams
Aztreonam

5. Beta-lactamase inhibitors
Clavulanic acid
Tazobactam
Sulbactam
Avibactam

Among all antibiotics, the beta-lactam group ranks first both in terms of the number of compounds on the market and in terms of their use in the treatment of infectious diseases. The very commonly applied beta-lactams are cephalosporins and penicillins respectively.[2]

The best popular analytical methods for the specification of beta-lactams are chromatographic methods, bearing high-performance liquid chromatography (HPLC) and thin layer chromatography (TLC). HPLC provides separation efficiency and high susceptibility, establishing itself as the primary selection method for the analysis of beta-lactams; but, it is costly and requires complex devices. TLC is a cheaper and less complex chromatographic method that can be used successfully in the prescreening of pharmaceuticals.[3,4]

A number of publications have appeared in the literature on the differentiation and isolation of beta-lactams by TLC, but few of them debate contemporaneous isolation from complex mixtures of structurally related derivatives.[5-8]

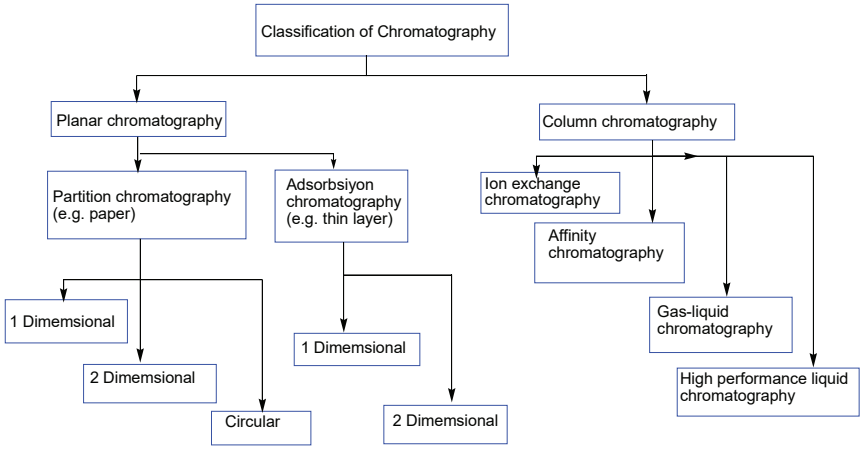
The isolation and purification[9] methods of antibiotics are highly dependent on the antibiotic's properties, chemical nature, and environment of accumulation.[10] Separation methods have been developed. Natural chemistry greatly encouraged the development of refined techniques applied today. Chromatographic methods, including column chromatography,[11-13] thin layer chromatography (TLC),[14-16] gas chromatography (GC),[17] and high-performance liquid chromatography

(HPLC),[18] have made it possible to separate compounds present in highly small amounts.

Chromatography

It is the process of separating and purifying the components in a mixture between two phases, one mobile, and the other stationary. The stationary phase can be solid and the mobile phase liquid and gas. Separation; It takes place according to the relevance of the substances in the mixture to the mobile and stationary phase. Today, chromatographic analysis methods have become one of the most widely used instrumented analysis methods, which implements the processes of determining the species that make up a mixture separately and quantifying the components that make up the mixture. It has become possible to separate pure substances that are difficult or even impossible to separate from each other with other methods with the help of this method.

Although chromatography can be classified in different ways, it mainly proceeds through adsorption (adsorption) and partitioning (dispersion) mechanisms. We can also represent the classification of chromatography with the following diagram:

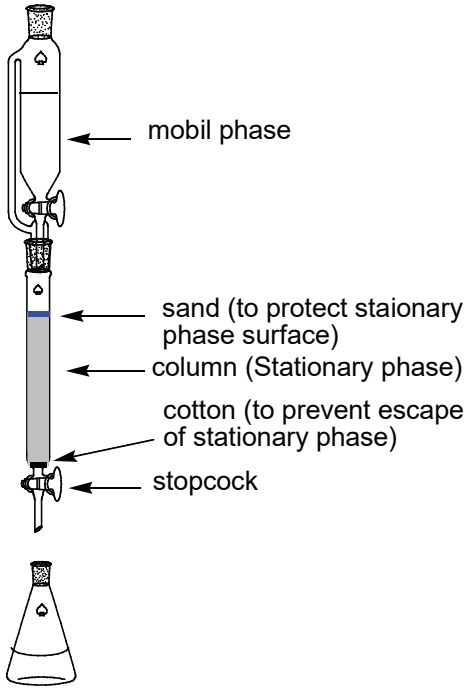


Scheme 2. *Classification of chromatography*

Column Chromatography

Column chromatography is the first applied chromatographic method and is the beginning of chromatography.[11-13] In this chromatography, active surface materials such as calcium carbonate, cellulose, aluminum oxide, silica gel, zeolite, etc. are used as the stationary phase. Organic

solvents are used as the mobile phase. In this method, the mixture to be separated is dissolved in a suitable solvent and passed through the solid stationary phase filled into a column. In the column, the components are adsorbed by the stationary phase. Then the solvent or solvent mixtures with different polarity are passed through the column and the components are taken separately from the column base. A pure substance is obtained by evaporation of the solvent (Scheme 3).



Scheme 3. *Column chromatography*

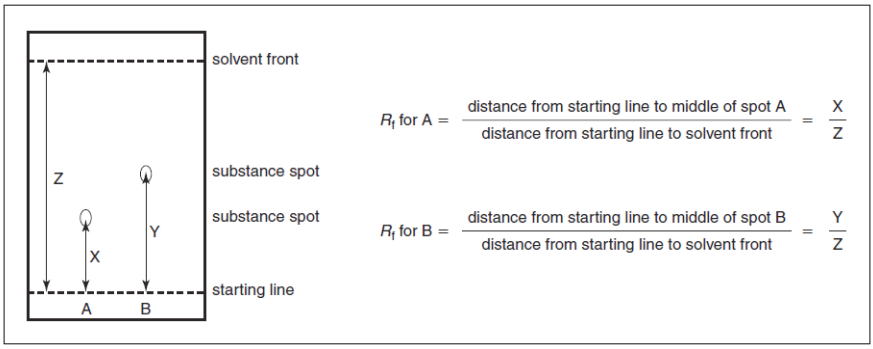
Affinity Chromatography

This method is based on the specific and reversible adsorbing of the target biomolecule by ligands that are immobilized on an indissoluble backing material and contain binding ends complementary to the target molecule. This method, which is highly selective, is the newest chromatography technique.[19,20] It is based on highly specific interactions such as antigen-antibody, enzyme–substrate, receptor–drug. It is a sort of ion exchange chromatography.

Thin layer chromatography (TLC)

TLC is a solid-liquid adsorption chromatography. In this process, the stationary phase is a solid adsorbent material that is plastered as a thin layer on glass plates of various sizes (20x20, 20x5, and 5x2 or microscope

glass, etc.). Alumina, silica gel, cellulose etc. can be used as adsorbent material. At the method the mobile phase moves from the bottom up over the stationary phase. The walking speed depends on the polarity of the substance, solid phase and solvent. Polar substances interact more closely with those that are more polar than the solvent/adsorbent pair. In TLC, the ratio of the level reached by the sample to the highest level of the solvent (solvent front) is called the retention factor (Retardation factor, Rf). In TLC, the retention factor is used to discriminate substances.[21,22]



Scheme 4. *Calculation of Rf value*

Flash Column Chromatography (FCC)

Flash Chromatography offers great advantages in post-synthesis purification, separation of impurities and isolation of substances. FCC is a popular method of preliminary separation in drug discovery.[23] The significance of flash chromatography is predominantly due to its low operating compression,[23] simple packing procedure,[24] and low cost for instrumentation.[24] Speed, ease of use, and high-quality products are some of the advantages of flash chromatography, an automated chromatography system.[25] The fundamental hypothesis is the significance of partitioning between a stationary phase and a mobile phase to separate the compounds in a mixture.

In one resource, a sensitive densitometric TLC method was established and validated for the analysis of eight β-lactam antibiotics. Chromatographic separation of eight β-lactam antibiotics was performed on silica gel F254 plates using various mixtures of chloroform-ethyl acetate-glacial acetic acid-water as mobile phase. Densitometric detection at 275 nm was also measured.[26]

One manuscript relates to a simple and sensitive method for rapid separation and detection of selected β-lactams. In the method, the

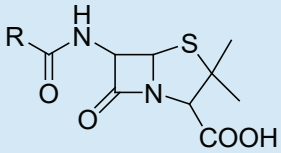
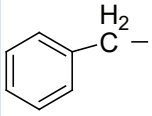
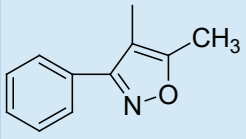
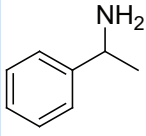
impregnation of the silica gel thin layer chromatography layer with 0.2% ammonium chloride was carried out. Various propanol-acetic acid and butanol-acetic acid mixtures were used as mobile phases in the purification of penicillins and cephalosporins.[27]

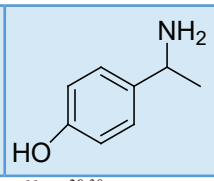
One study defines some TLC techniques for the separation and identification of cephalosporins and penicillins from complex mixtures. It has been reported that they succeeded in separating the studied beta-lactamines by different mobile phases and silica gel as the stationary phase.

All beta-lactam derivatives examined in the article were separated by silica gel with a suitable mobile phase. They reported that using a mobile phase, a combination of two mobile phases and a color reaction, the investigated substances can be identified from complex mixtures.

Researchers have reported that, with some limitations on the stability of beta-lactam antibiotics, the solvents and fixation system mentioned in the article can quickly and conveniently separate and detect large numbers of penicillins and cephalosporins.[28]

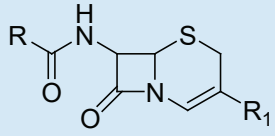
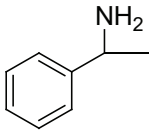
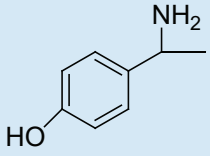
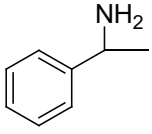
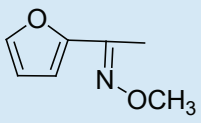
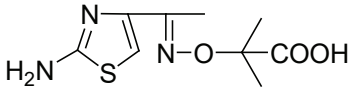
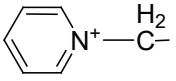
In this study, benzylpenicillin with different structural properties is the first natural penicillin to enter treatment; ampicillin and amoxicillin–two semisynthetic aminopenicillins; they analyzed oxacillin–a semi-synthetic isoxazolylpenicillin. The chemical structures of the investigated penicillins are seen in Scheme 5.

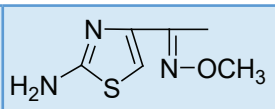
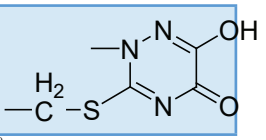
Penicillin Derivative	R
	
Benzylpenicillin	
Oxacillin	
Ampicillin	

Amoxicillin	

Scheme 5. *The studied penicillins*[29,30]

In addition, among the six commonly used cephalosporin derivatives, cephalexin, cefadroxil, cefaclor–first generation oral cephalosporins, cefuroxime–second generation parenteral cephalosporin, ceftazidime, ceftriaxone–third generation parentosporins were analyzed. The chemical structures of the working cephalosporins are seen in Scheme 6.

Cephalosporin Derivative	R	R_1
		
Cefalexin		$-CH_3$
Cefadroxil		$-CH_3$
Cefaclor		-Cl
Cefuroxim		$-CH_2$-O-CO-NH_2
Ceftazidim		

Ceftriaxon		

Scheme 6. *The studied cephalosporins*[29,30]

In another study, The antimicrobial substance produced in S. violatus was isolated and purified. The isolated extract was subjected to column chromatography. Various mixtures of ethyl acetate- n-hexane and ethanol-ethyl acetate were used as mobile phases. Fractions with high antimicrobial activity obtained here were purified by subjecting them to a second column chromatography.[31,32]

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Chapter 3

IMPORTANT WORKS ON THE ASYMMETRIC SYNTHESIS OF CHIRAL KETO ALCOHOLS VIA BIOCATALYSTS

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The utilization of enzymes or whole cells for chemical conversions of organic compounds is named biocatalysis. In spite of enzymes having a long past in the fermentation and food industries, their applications have risen in number in order to supplement traditional chemical catalysis. Enzymes are exceptionally complicated frameworks that catalyzed most chemical reactions in the living cell (Robertson & Bornscheuer, 2005). Enzymes catalyze the chemical reactions that take place in living cells, making them faster and thus keeping the metabolism alive (Beisson et al., 2000).

Biocatalysis offers many advantages over chemocatalysis for example lower cost and energy spending, moderate conditions, high stereo- and regio-selectivity, and fewer numbers of synthetic reaction steps and byproducts (Borse et al., 2012; Humble & Berglund, 2011; Woodley, 2008). Moreover, in most reactions, well-known biocatalysts are used in the name of green chemistry. Due to their environmentally friend and attachment to green chemistry laws, the implementation of biocatalytic processes like using yeast or enzyme is usually used in so many chemical industries, especially the pharmaceutical industries. (Bornscheuer & Kazlauskas, 2004; Gotor-Fernández et al., 2006; Svedendahl et al., 2005). However, there are also some disadvantages to using biocatalysts. Reaction rates for an enzyme with non-native substrates are usually slower than those monitored for natural substrates or chemocatalysis. High concentrations of reagents can also adversely affect enzyme stability, and enzymes are often unstable and easily inactivated in organic or biphasic organic/aqueous media.

Owing to their commercial presence, superior catalytic capacity, well constancy, and widespread substrate selectivity, especially lipase class enzymes are supremely and growing implemented in various industrial areas for example pharmaceutical chemistry, agricultural chemistry, and native products (Robertson & Bornscheuer, 2005). The most important feature that distinguishes enzymes from chemical reactions is their selectivity. Lipases can be obtained in animals, plants, fungi, and single-celled organisms and are among industrially important enzymes. Lipids make up a wide fragment of the world's biological mass, and lipid-processing biocatalysts have a very important act in the cycle of substances that do not like water and are insoluble. Hydrolases, especially lipases are among industrially important enzymes. Lipids make up a very large part of the world's biomass, and lipolytic enzymes play a very important role in the cycle of these water-insoluble compounds. Lipases are very important characters for lipid metabolism in hydrolysis and esterification reactions in eukaryotic and prokaryotic cells. The structure of the enzyme, the nature of the substrate, and the agents that influence the linking of the enzyme to the substrate are affected the selectivity of lipase. The fatty

acid selectivity of lipases is used for the production of oil for medicinal foods and for enrichment with fatty acids to increase the nutritional value of the oil. Lipase specificity is collected in three basic groups; position, substrate and stereo selectivity. Lipases from different sources are not similar to other hydrophilic enzymes, because of their stability in apolar organic solvents and admitting a large number of substrates of different sizes and properties. Their elastic protein structures allow them to catalyze many reactions such as hydrolysis, interesterification, transesterification, aminolysis, oxymolysis, and thio transesterification. Apart from hydrolysis and transesterification reactions, lipases are also used as catalysts in other catalytic organic methods such as the Michael reaction (Cai et al., 2011; Guan et al., 2015), Mannich reaction (Li et al., 2009), Markovnikov addition (Wu et al., 2005), Henry reaction (Wang et al., 2010), polymerization (Monsalve et al., 2010) and epoxidation (Svedendahl et al., 2008).

Since chiral keto alcohols are valued and useful fragments, they can be employed to obtain organic products with polyfunctional groups besides native matters such as some drugs used in the pharmaceutical industry (Evans et al., 1995; Mukaiyama, 1999; Nicolaou et al., 2000). They have two important functional groups (keto group and hydroxyl group) and are convenient interim for advanced combination proposes of worthy products. Optically pure keto alcohols are also very beneficial in the production of new compounds of biological importance diols, amino alcohols, and amino acids, thanks to their favorable structure (Acetti et al., 2010; Andreu & Del Olmo, 2013; Kohls et al., 2015; Vishnumaya & Singh, 2009).

In this book chapter, the syntheses of chiral keto alcohols (which can be 1,2- or 1,3) using biochemical methods are compiled in a general framework. For the biochemical synthesis of chiral keto alcohols, those that provide high enantiomeric selectivity, as in chiral alcohols, come to the fore. If it is listed these methods under the headings; a) enzymatic hydrolysis, b) enzymatic resolution, c) biocatalytic reduction, d) enzymatic aldol reaction and e) enzymatic benzoin condensation methods. The biocatalytic methods mentioned are summarized in figure 1.

Considering these biocatalysis methods applied for keto alcohols, two types of biocatalysts come to the fore. The first type is the lipase enzymes and is used in enzymatic hydrolysis, kinetic resolution and aldol reaction methods. The most used lipase enzymes as biocatalysts are; *Lipase from Candida cylindracea* (Majewska et al., 2016; Melais et al., 2016; Strohalm et al., 2010), *Lipase from Candida antartica* (Andrade & Barcellos, 2009; Ciou et al., 2011; Kao et al., 2012; Sembayeva et al., 2017; Trapp et al., 2021)*, Lipase from Candida rugosa* (de Almeida et al., 2020; Ozyilmaz et al., 2021; Sayin et al., 2020; Zheng et al., 2019), *Porcine pancreatic lipase* (Husain et al., 2010; Jia et al., 2013; Kücük & Yusufoğlu, 2013; Ochiai

et al., 2019; Yaşa & Yusufoğlu, 2018), *Amano lipase from Burkholderia cepacia* (*Pseudomonas cepacia*) (Borowiecki & Dranka, 2019; Daramwar et al., 2014; Kołodziejska & Studzińska, 2016; Poterała et al., 2017; Sugai et al., 2020; Yildiz & Yusufolu, 2011; J. Zhang et al., 2013), *Amano Lipase A from Aspergillus niger* (Dunne & Palomo, 2016; Yıldız, 2019; Yıldız et al., 2017), *Amano Lipase from Pseudomonas fluorescens* (Harvey & Crout, 1993; Izquierdo et al., 1999; Krishna et al., 2020; Panja et al., 2014), and *Rhizopus oryzae lipase* (Ayinla et al., 2022; Cabrera & Palomo, 2011; Kumar et al., 2016; Ochiai et al., 2019; Yousefi et al., 2014). The other type is oxido reductase enzymes, and baker's yeast (García-Urdiales et al., 2005; Kaluzna et al., 2004; Moore et al., 2007) used for biocatalytic reduction.

Enzyme-catalyzed kinetic resolution or biocatalytic reductions of carbonyl compounds by oxidoreductases have been frequently mentioned in previous reviews and book chapters (Ghanem, 2007; Hari Krishna & Karanth, 2002; Klibanov, 2001; Patel, 2002, 2004). However, in the literature search, there are very few reviews focusing on the use of biocatalytic procedures for the synthesis of chiral (1,2- or 1,3-)-keto alcohols.

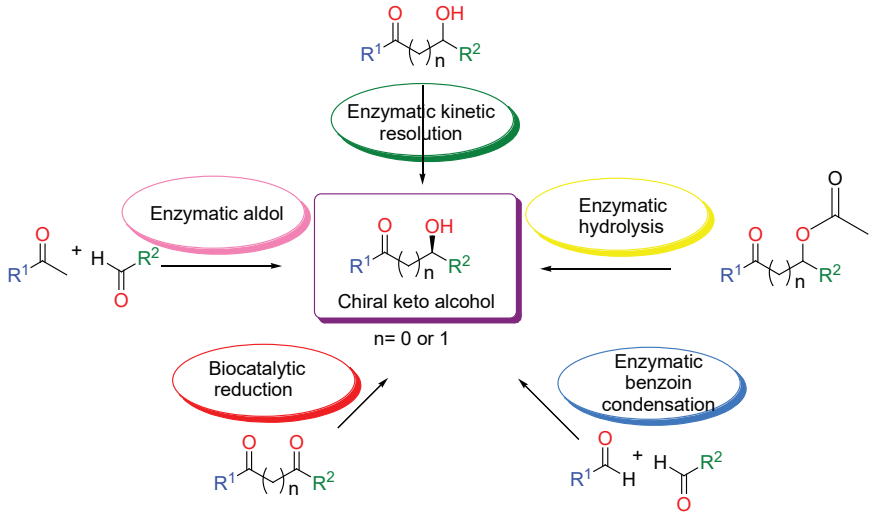


Figure 1. *Biocatalytic methods for synthesizing chiral keto alcohols*

a) Enzymatic hydrolysis of keto acetates

Biocatalytic hydrolysis of some keto alcohols is described by Joly and Nair in 2003 (Joly & Nair, 2003). Different 1,3-keto alcohols were obtained with very good enantioselectivity handling vinyl acetate as the acyl donor in the existence of lipase from CCL (*Candida cylindracea lipase*).

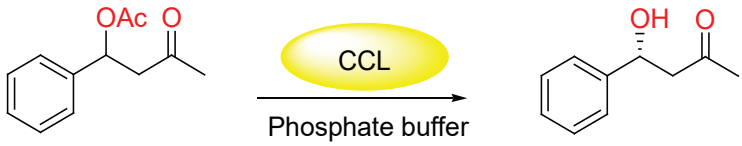


Figure 2. *Enzymatic hydrolysis of racemic ketoacyl compounds by lipase*

In another work, Griengl et al discovered Lyophilized yeast (*Saccharomyces cerevisiae*) for enzymatic hydrolysis reaction of ketoacyl compounds in 1988 (Glänzer et al., 1988). They succeeded to obtain some chiral keto alcohols with high optical purity (Figure 3).

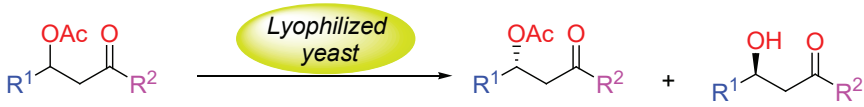


Figure 3. *Asymmetric hydrolysis of racemic ketoacyl compounds by Lyophilized yeast*

In our recent study, we succeed enzymatic hydrolysis of acyl-derived (±) β-hydroxy ketones using the lipase AL-AN (Amano Lipase A from *Aspergillus niger*) for the first time (Yıldız, 2019). This study presented the preparation of (*S*)-1,3-keto alcohols with good ee by an environmentalist and green procedure.

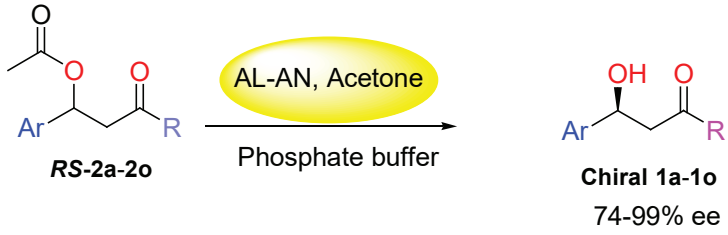


Figure 4. *Synthesis of chiral 1,3-keto alcohols by Aspergillus niger (AL-AN)*

b) Enzymatic kinetic resolution of keto alcohols

Zhang et al developed a two-step consecutive biocatalytic method in order to synthesize chiral keto alcohols that consolidate a lipase-catalyzed kinetic resolution (W. W. Zhang et al., 2014). In this study, the best results were obtained using CRL (lipase from C. rugosa) lipase enzyme (Figure 2).

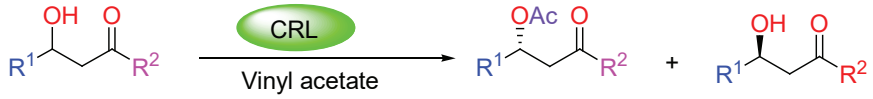


Figure 5. *Enzymatic kinetic resolution of racemic keto alcohol compounds by CRL*

There is a new report on the progress and practice of both chemical and enzymatic reactions (Dynamic Kinetic Asymmetric Transformation= DYKAT) of 1,3-keto alcohol, in the method, it was used Candida Antarctica lipase B (CALB) for resolution, and ruthenium complex for epimerization. (Hilker et al., 2021).

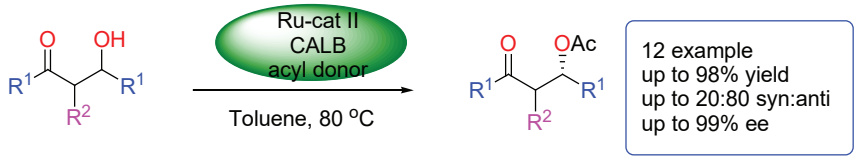


Figure 6. *Chemoenzymatic transesterification of racemic keto alcohol compounds by CALB*

Wu et al. developed a double enzymatic sequential and one-pot Cascade reaction method for the synthesis of (*S*)-1,3-keto alcohols and acyl derivative of (*R*)-1,3-keto alcohols (Xu et al., 2016). In this study, while an immobilized lipase from Mucor miehei (MML) was used for the decarboxylative aldol reaction, CAL-A or CAL-B (a lipase A or B from Candida antarctica) in the same vessel catalyzed the enzymatic kinetic resolution of the racemic 1,3-keto alcohol. With the developed bienzymatic method, twelve chiral 1,3-keto alcohols and the identical number of corresponding acylated derivatives were synthesized with very high ee values and good yields (Figure 7).

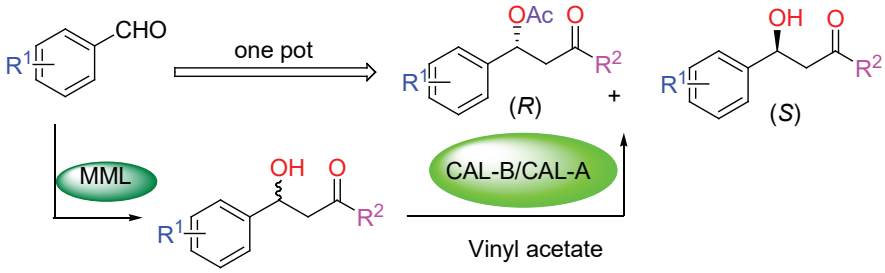


Figure 7. *Synthesis of the (S) β-keto alcohol by enzymatic transesterification with CALB*

In another study (Joly & Nair, 2003), different aldol products derived from acetone were subjected to transesterification reaction in the presence of *Candida cylindracea lipase* (CCL) handling vinyl acetate as the acyl donor (Figure 8). It was obtained various acetone derived chiral 1,3-keto alcohols and 1,3-ketoacyl compounds with very high ee values up to 96%.

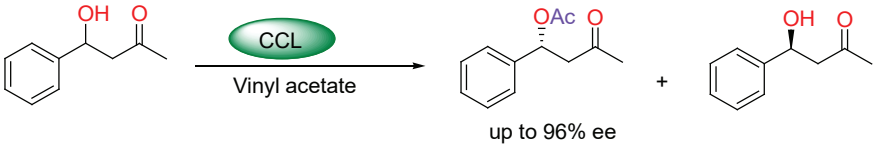


Figure 8. *Synthesis of the chiral β-keto acetates by CCL*

A research article published in 1996 (Adam et al., 1996) studied the enzymatic kinetic resolution of racemic alpha-hydroxy ketones in the existence of isoprenyl acetate using various lipases in organic media. In this study, various lipases were investigated and the best yields were obtained with *Amano PS* and *Amano AK lipases* when methyl-t-butyl ether was preferred as a solvent. Therefore some chiral alpha-hydroxy ketones (1-4) and their esters were obtained with enantiomeric excesses up to 99% using the enzymatic kinetic resolution method (Figure 9).

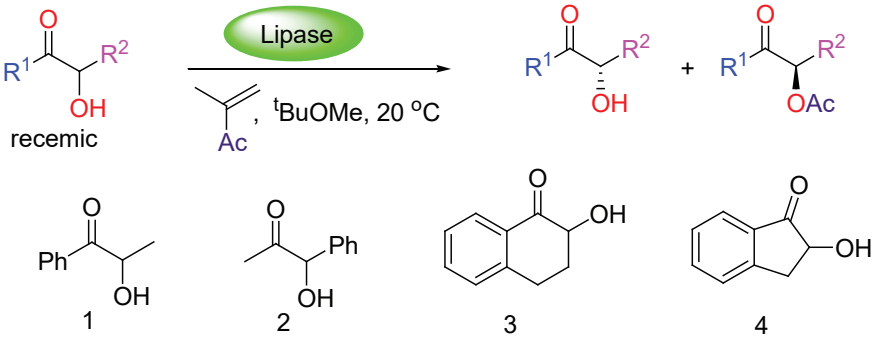


Figure 9. *Kinetic resolution of some α-hydroxy ketones 1-4 using lipase.*

In another study conducted in 2006, chiral aldol compounds synthesized under proline catalysis were converted to acyl derivatives using the enzymatic kinetic resolution method. Excellent enantiomeric excesses of up to 99% were obtained by using a tandem asymmetric model (Edin et al., 2004). In this study, proline was preferred as the catalyst for the asymmetric aldol reaction, while lipase enzymes such as P. cepacia lipases Amano I and II, Candida cylindracea lipase (CCL), and Candida Antarctica lipase B (CAL-B) were tested for the second step. As a result, in the study performed with the combination of organo- and biocatalysts, Amano I was determined as the best biocatalyst, vinyl acetate was determined as the

acyl reagent and the reaction was carried out in a solvent-free environment (Figure 10).

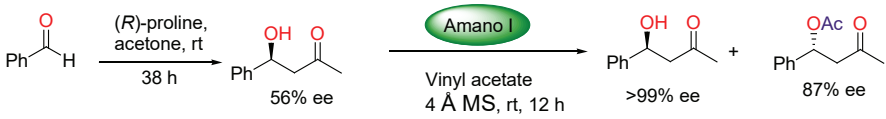
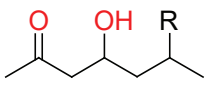


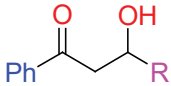
Figure 10. *Sequential asymmetric synthesis of β-hydroxy ketones piroline and Amano I.*

In 2004, Abate et al declared the production of whole optically active isomers of some floral odorants synthesized by biocatalysis methods (Abate et al., 2004). In this study, some chiral 1,3-keto alcohols were synthesized using biocatalysts as intermediates for the production of valuable fragrance molecules. The chiral 1,3-keto alcohols synthesized as intermediates and the biocatalysts used are summarized in table 1 below.

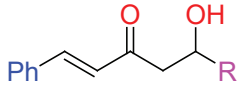
Table 1. *Enzymatic Transesterification for the Synthesis of Enantiomerically Enriched Odorants*



4 R= H
5 R= Me



6 R= Me
7 R= Et
8 R= Pr



9 R= Me
10 R= Me_2CH
11 R= Pr

Substrate	Enzyme[a]	Acetate (ee [%]),Chemical yield [%]	Alcohol (ee [%]),Chemical yield [%]
4	CCL	(*S*)(60), 18	Racemic
5	Lipase PS	(*S*)(95), 30	(*R*)(91), 35
6	Lipase PS	(*R*)(97), 33	(*S*)(93), 37
7	Lipase PS	(*R*)(96), 41	(*S*)(94), 37
8	Lipase PS	(*R*)(86), 28	(*S*)(50), 55
9	Lipase PS	(*R*)(46)	(*S*)(82)
	PPL	(*R*)(98), 39	(*S*)(76), 33
10	PPL	Racemic	Racemic
11	PPL	Racemic	Racemic

[a] CCL: *Candida cylindracea lipase*; lipase PS: *Pseudomonas cepacia lipase*; PPL: *Porcine pancreas lipase*.

In another study of the same group (Abate et al., 2003), enantiomerically enriched diastereoisomers of chiral 1,3 dioxane odorants (Floropal® and Magnolan®) were produced using 1,3-chiral keto alcohols obtained by kinetic resolution using lipase enzyme. In this study, odor properties, and differences in odor perception for stereoisomers were investigated (Figure 11).

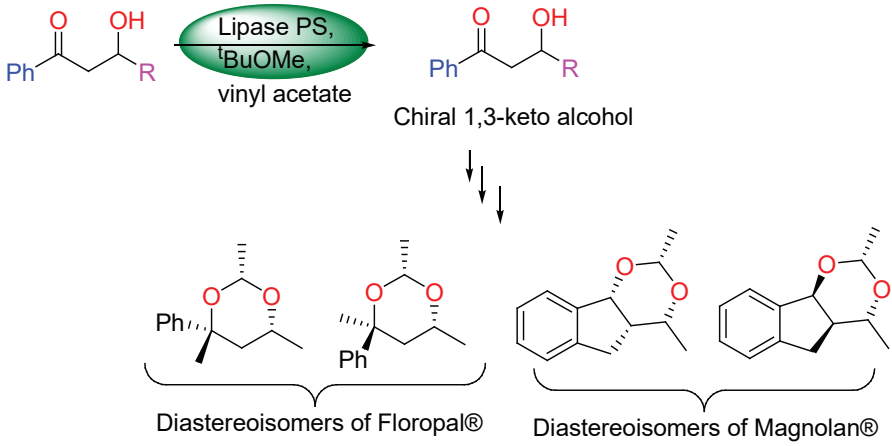


Figure 11. *Asymmetric synthesis of enantiomerically enriched diastereoisomers of chiral 1,3 dioxane odorants (Floropal® and Magnolan®).*

c) Biocatalytic reduction of diketones

Dehydrogenase enzymes are also very useful enzymes in biocatalytic reductions. The most important dehydrogenase enzymes, which are widely applied for their synthesis under mild reaction conditions and provide high enantiomeric selectivity, are NAD(P)H-linked dehydrogenases, and the synthesis of chiral alcohols by asymmetric transfer hydrogenation is possible using them (Goldberg et al., 2007; Moore et al., 2007). Shanati et al have recently designated the isolation of two complements each other dehydrogenases from *Arthrobacter sp.* TS-15 (DSM 32400) (Shanati et al., 2019). The two enzymes obtained were pseudoephedrine dehydrogenase (PseDH) and ephedrine dehydrogenase (EDH) enzymes, and they catalyzed the reduction of 1-phenyl-1,2-propandione, providing the product with both the *R* and *S* enantiomers with up to 99% ee (Figure 12).

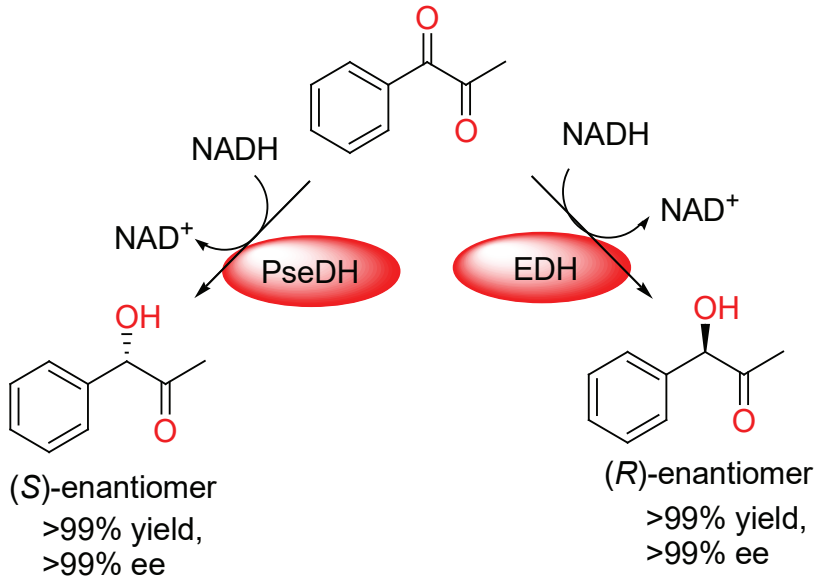


Figure 12. *Biocatalytic reduction of 1,2-diketones with dehydrogenase enzymes*

Hoyos et al reported in 2008 that lyophilized cells from *Pichia glucozyma* were used for enantio and regio-selective reduction of aromatic diketones for the first time (Hoyos et al., 2008). In this work, the enantioselective induction to constructionally dissimilar symmetric benzil compounds was established in order to obtain optically active benzoins (Figure 13) with high yields, very good ee, and in a quick time. They investigated the reduction of symmetric and asymmetric diaryl ketones to understand the regio- and stereoselectivity of this biocatalyst.

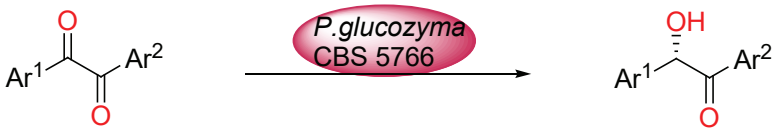


Figure 13. *Synthesis of α-diaryl-benzoins by biocatalytic reduction with Pichia glucozyma*

In another study, the enzymes *Aspergillus oryzae* OUT5048 and Fusarium roseum OUT4019 were found to be useful enzymes for the enantio- and regio-selective reduction of 1,2-diketones to 1,2-keto alcohols (Demir et al., 2008). In this work, it was investigated various species of *Aspergillus* and *Fusarium* genus for the bioreduction of diaromatic 1,2-diketones to corresponding keto alcohols. Two kinds were selected from each type: *Aspergillus oryzae* OUT5048 and *Fusarium roseum* OUT4019 (Figure 14). So they succeeded in the bioreduction of the symmetrical diaromatic 1,2-diketones to 1,2-keto alcohols with excellent yields and

ee's up to 99%. In another study by Demir et al., *Rhizopus oryzae* (ATCC 9363) enzyme was also used for the reduction of benzyl to benzoin (Demir et al., 2004).

Figure 14. *Bioreduction of symmetric 1,2-diaryl-ethanediones using Aspergillus oryzae OUT5048 and Fusarium roseum OUT4019*

In a 2015 study (Mahajabeen & Chadha, 2015), optically pure 1,3-keto alcohols were obtained by the bioreduction of the suitable diketones with high ee's (98%) and well yields (up to 75%) trying whole cells of *Candida parapsilosis* (Figure 15).

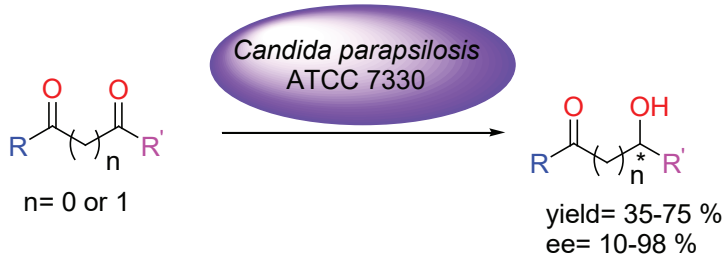


Figure 15. *Bioreduction of some 1,2- or 1,3-diketones by Candida parapsilosis ATCC 7330*

Baker's yeast (*Saccharomyces cerevisiae*), which has been used for the bioreduction of ketone groups for a long time, has also been used for enantioselective reduction of dicarbonyl compounds (especially α- and β-diketones and keto esters) to high ee chiral alcohols (Johanson et al., 2005). Additionally, in 2008, we developed a new method using baker's yeast (Yildiz et al., 2014). We reported the asymmetric synthesis of chiral both α- and β-keto alcohols with different structures via bioreduction of diketones using baker's yeast. In this study, α- and β-keto alcohols were obtained with good yield and ee values up to >99% (Figure 16).

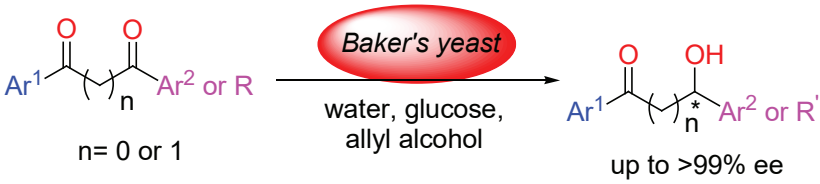


Figure 16. *An efficient bioreduction of some 1,2- or 1,3-diketones by baker's yeast*

Here, we showed that bioreduction with baker's yeast is a very effective, environmentally friendly, and inexpensive method for the preparation of chiral α- and β-keto alcohols in particular.

d) Enzymatic aldol reaction

The aldol reaction is among the reactions catalyzed by the lipase enzyme. In recent years, good results have been obtained with biocatalysts as well as organocatalysts such as proline in asymmetric aldol reactions. Xie et al have published many studies in this area. They reported many lipases for enzymatic aldol reaction. Firstly the direct aldol reactions were carried on between aromatic aldehydes and cyclic ketones in acetonitrile (MeCN) in the presence of water using AUAP (acidic protease from *Aspergillus usamii*) (B. H. Xie et al., 2012). Then in 2013, they (Z. B. Xie, Wang, Zhou, et al., 2013) first reported that PPL, *lipase from pig pancreas*, catalyzes direct asymmetric aldol reactions between aromatic aldehydes and cyclic ketones. In the reaction with various aromatic aldehydes and cyclic ketones, aldol products were synthesized with yields up to 99% and enantiomeric excesses up to 90% (Figure 17).

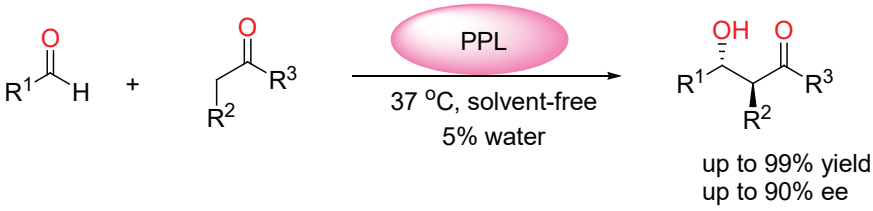


Figure 17. *Enzymatic asymmetric aldol reaction catalyzed by PPL*

The same group (Z. B. Xie, Wang, Jiang, et al., 2013) reported also that *bovine pancreatic lipase* (BPL) catalyzed the aldol condensation and they used buffer for the enzymatic aldol procedure first time. In this study, although the enantioselectivity was not sufficient, this method was one of the first important investigations into enzyme promiscuity study (Figure 18).

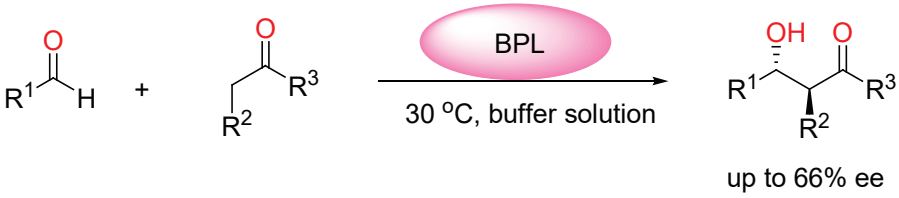


Figure 18. *Enzymatic asymmetric aldol reaction catalyzed by BPL*

In another biocatalytic aldol study, the direct asymmetric aldol

reaction of aromatic aldehydes with cyclic or acyclic ketones was catalyzed by a proteinase from *Aspergillus melleus* (AMP) in acetonitrile in the presence of water (Yuan et al., 2013). A broad range of substrates could be converted into the corresponding aldol products in yields up to 89%, enantioselectivities up to 91% ee, and diastereoselectivities up to >99:1 (anti/syn) (Figure 19).

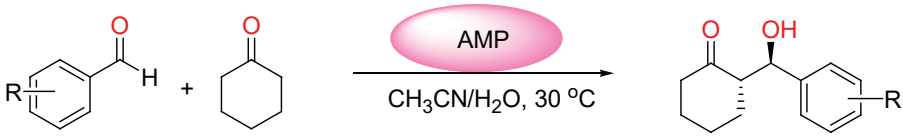


Figure 19. *AMP catalyzed biocatalytic direct asymmetric aldol reaction*

For the enzymatic asymmetric aldol reaction *lipase from pig pancreas* type II (PPL II) enzyme was also investigated and good results were obtained (Guan et al., 2012). In this work, it was reported to direct the unsymmetric aldol reaction of heterocyclic ketones with aromatic aldehydes in MeCN-water in the presence of lipase (PPL II). Then this method was applied to a series of substrates and average yields with an ee of up to 87% were obtained (Figure 20).

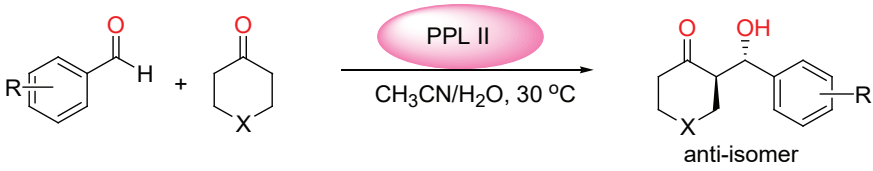


Figure 20. *Enzymatic direct asymmetric aldol reaction catalyzed by PPL II*

The last example we will show for the enzymatic asymmetric aldol reaction is our own study that we reported in 2017 (Yıldız et al., 2017). In this study, we investigated the effect of the combination of enzymes and Lewis acids with different structures on the asymmetric aldol reaction. We discovered that Lewis acid used with the enzyme has a positive effect on yield and ee. In the method we developed, the best results were obtained by combining Amano lipase A from *Aspergillus niger* (AL-AN) and porcine pancreatic lipase (PPL) with $CoCl_2$. PPL or AL-AN/CoCl2-catalyzed reaction method gave anti-products with higher ee in the range of 80 to 100%ee if checked against other biocatalytic procedures (Figure 21).

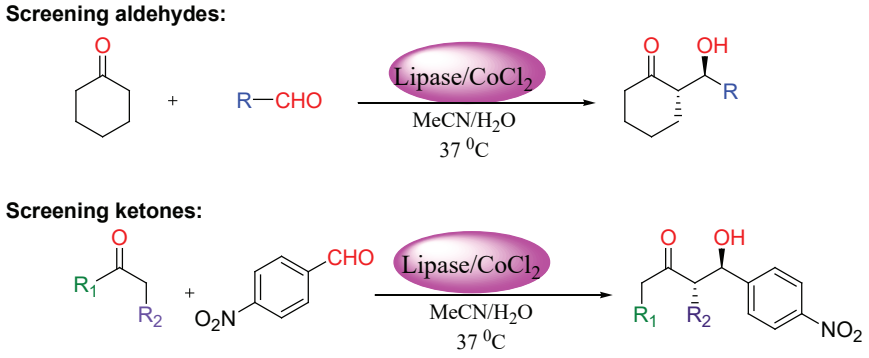


Figure 21. *Enzymatic direct asymmetric aldol reaction catalyzed by PPL or AL-AN/Lewis acid*

e) Enzymatic benzoin condensation

Benzaldehyde lyase (BAL, EC 4.1.2.38) is a thiamin diphosphate (ThDP) dependent enzyme from Pseudomonas fluorescens Biovar I and it was used before for benzoin condensation in the 2000s. It is demonstrated that BAL can catalyze benzoin condensation reaction as a biocatalyst and energy source starting from benzaldehyde, because of the capability of BAL to split the acyloin connection. Feature Demir et al. made very important contributions to the literature on this subject. One of the first works of them, they succeeded in synthesizing the enantioselective synthesis of keto alcohols with excellent ee via enzymatic C–C bond cleavage (Demir et al., 2001). The enantiopure hydroxy ketones were gained in excellent yield beginning from elementary aldehydes using BAL (Figure 22).

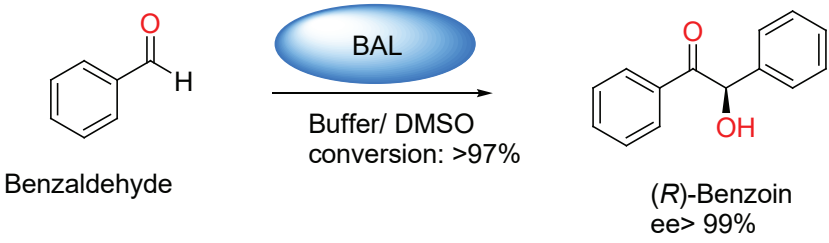


Figure 22. *Enzymatic benzoin condensation catalyzed by BAL*

In another study by the same group, BAL catalyzed the reaction of aromatic aldehydes with (di)methoxy acetaldehydes and provided methoxy derivatives of hydroxy ketones in high yields and enantiomeric excess by acyloin bond (Demir et al., 2003). With this developed method, the synthesis of methoxy-derived hydroxy ketones with high enantioselectivity was achieved for the first time (Figure 23).

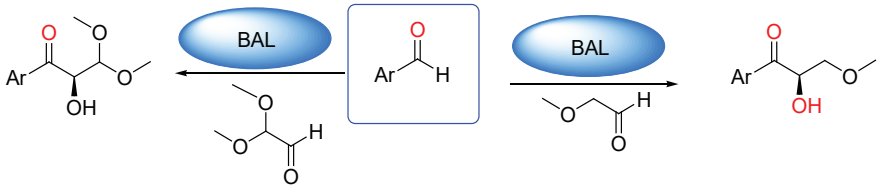


Figure 23. *The synthesis of chiral methoxy derived-hydroxy ketones by BAL*

Drager et al. were shown in 2007 that o new tyrosine-linked polyvinyl pyrrolidinone-based matrix was used for the biocatalytic synthesis of benzoin derivatives in a flow-through mode (Dräger et al., 2007). BAL was preferred as a model enzyme for attaching Ni-nitrilotriacetic acid (Ni-NTA) via the polymer-based matrix (Figure 24).

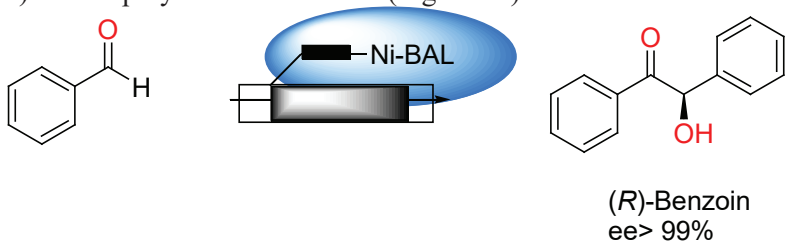


Figure 24. *Flow-through synthesis of (R)-benzoin by BAL*

In recent years, Hernandez et al. studied with benzaldehyde lyase from *Pseudomonas fluorescens biovar I* for catalyzing the benzoin reaction of some aromatic aldehydes to dimethyl acetaldehyde (Hernández et al., 2015). Then, they reduced the 1,2- hydroxy ketones obtained by using $NaBH_4$ to obtain the anti-diol (Figure 25).

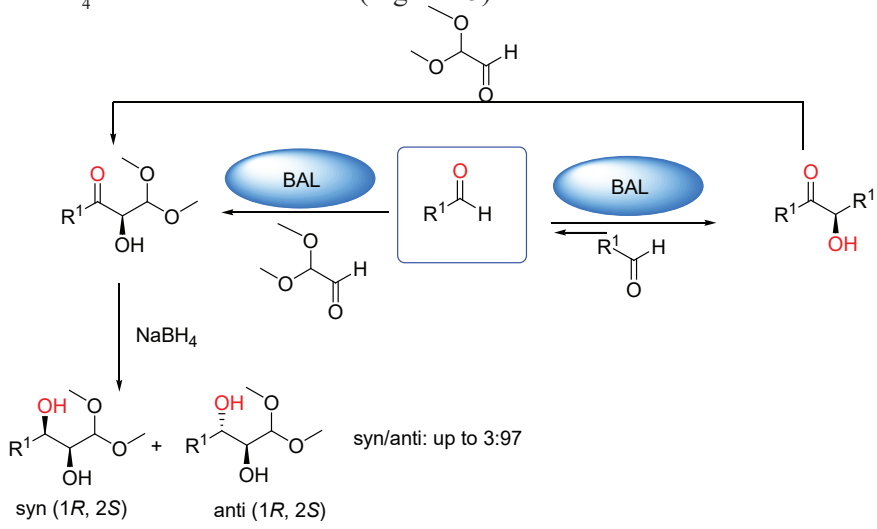


Figure 25. *The synthesis of chiral dimethoxy derived-hydroxy ketones by BAL and transfer to anti diols*

In 2016 (Beigi et al., 2016), it was studied that the synthesis of chiral α-hydroxy ketones by using various benzaldehyde lyase from Pseudomonas fluorescens (PfBAL), In this study, very good stereoselectivities (>98% ee) were achieved. The regio- and stereoselective of the product in the asymmetric aliphatic or aromatic cross benzoin reaction was observed only by the selection of the suitable biocatalyst or its variant (Figure 26).

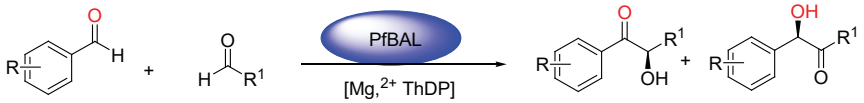


Figure 26. *Enzymatic Cross-Benzoin Reaction by PfBAL*

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Chapter 4

THERAPEUTIC POTENTIALS OF GENUS ORIGANUM

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Introduction

Information on medicinal aromatic plants dates back to about 5000 years ago in India, China and Egypt, and 2500 years ago in Greece and Central Asia. From ancient times to the present, people have tried to heal their own diseases by using nature. Since the use of animals was instinctive in the beginning, the use of plants was also used in the same way. In ancient times, the use of medicinal plants was almost by trial and error due to the lack of knowledge and experience. As the benefits of plants for diseases were discovered over time, trial and error left its place to pure knowledge (Ang-Lee et al., 2001; Lindberg –Madsen and Bertelsen , 1995; Mohammed et al., 2019a; Mohammed et al., 2020a; Rios and Recio, 2005; Schippmann et al., 2006; Sevindik et al., 2017; Stojanoski, 1999; Qiu, 2007).

Medicinal and aromatic plants have many uses such as food, medicine, cosmetics and spices. It has been used for these purposes since the existence of humanity (Mohammed et al., 2020b; Akgül et al., 2022). The most notable and researched medicinal and aromatic plants are those used for therapeutic purposes. A significant portion of those used as treatments are supplied from their natural habitat (Mohammed et al., 2019b; Uysal et al., 2021). The World Health Organization reported that medicinal plants are still present in the traditional health systems of developing countries (Mohammed et al., 2020c). After the 1990s, new uses of medicinal and aromatic plants emerged. With the increase in these usage areas, the demand for aromatic plants has increased (Mohammed et al., 2021; Pehlivan et al., 2021). Total trade in medicinal and aromatic plants has increased from 2.4 billion United States dollars (US) in 1996 to approximately 6.2 billion US dollars, with an annual growth rate of 5.4% in recent years. More than half of total medicinal and aromatic plant exports in terms of trade value are China (27.1%), Hong Kong (7.6%), USA (7%), India (6.5%) and Germany (6.1%). known. Today, the USA, European Union and Japan are the leading consumers of natural products (Dzoyem et al., 2013; Mohammed et al., 2019c; Tripathi et al., 2017; Vasisht et al., 2016).

15 plant species in *Origanum, Thymus, Thymbra, Satureja* and *Coridotymus* genera were subjected to the scope of thyme. Among the important traded species, *O. vulgare* is the species with the most diversity (Başer et al., 1993). The species containing carvacrol/thymol essential oil in the Lamiaceae family are called thyme. While carvacrol essential oil is abundant in *Thmybra* and *Origanum* species, thymol essential oil is abundant in *Thymus* species (Baydar et al., 2007; Özhatay and Koyuncu, 1998).

Kingdom: Plantae

Subkingdom: Tracheobionta

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Asteridae

Order: Lamiales

Family: Lamiaceae

Genus: *Origanum*

Anatomical Structure and Classification of Origanum

When we look at the general structure and classification of *Origanum* species, there are quite a lot of glandular hairs in their vegetative organs and these glandular hairs are not observed in the flower part. The glandular hairs secrete the characteristic essential oils. While most of the studies on the anatomical structure of origanum were on glandular hairs, studies on leaves were limited. There are stomata on the upper and lower epidermis of the leaf part. These stomas are more dense in the lower epidermis. The number of stomata ranges from 144 to 1200 per mm2. Diastic type stoma was observed in Origanum species in general. Parenchymas usually have a rounded appearance. Chloroplasts are located in the cytoplasm. No intercellular space was observed. Leucoplasts have irregular shapes. The trichomes found in *Origanum* species, on the other hand, remove harmful insects from the plant and prevent a decrease in productivity. Roots in *Origanum* species have a square cross-sectional appearance. The cortex part in the roots has a relatively thin structure. In the vascular bundles, the phloem is arranged uniformly, while the xylem is not. Due to the regional structure of the plant in the roots, it does not extend to the depths (Chishti et al., 2013).

Biological activities of *Origanum* species

In some studies on Origanum species that naturally spread in different parts of the world, essential oil, hydrodistillation, methanol, ethanol, subcritical water, water extracts were used to determine biological activities. Some biological activity studies on *Origanum* species are shown in Table 1.

Table 1. *Biological activity studies on some Origanum species*

Origanum plants	Pharmacological effect	Extraction	References
Origanum boissieri Ietsw.	Antibacterial, Anticandidal, Antimicrobials, Antioxidant,Anticholinesterase	Essential oils, Hydrodistillation	(İşcan et al., 2020; Özer et al., 2020)
Origanum munzurense Kit Tan & Sorger	Antioxidant, Antibacterial, DNA damage protecting activity	Essential oils, Methanol, Ethanol, Subcritical water, Soxhlet	(Yabalak et al., 2020)
Origanum saccatum P.H.Davis	Antioxidant,Anticholinesterase, Antibacterial,Antispasmodic	Essential oils, Solvent-Free Microwave, Hydrodistillation, Methanol	(Çam et al., 2020; Özer et al., 2020; Sozmen et al., 2011)
Origanum solymicum P.H.Davis	Anticholinesterase, Antioxidant, Antimicrobials	Essential oils, Methanol	(Dulger, 2006; Erenler et al., 2017;Özer et al., 2020)
Origanum hypericifolium O.Schwarz & P.H.Davis	Antispasmodic, Antimicrobials,Antioxidant, Antifungal	Essential oils, Methanol, Hydrodistillation	(Çam et al., 2020; Çelik et al., 2010; Ocak et al., 2014)
Origanum sipyleum L.	Antioxidant,Cytotoxic activity, Antibacterial	Essential oils, Ethanol	(Kaska, 2018; Özkan et al., 2007)
Origanum rotundifolium Boiss.	Antioxidant, Anticancer	Essential oils	(Erenler et al., 2017)
Origanum acutidens (Hand.-Mazz.) Ietsw.	Antagonistic activity, Antioxidant, Antimicrobials	Essential oils	(Goze et al., 2010)
Origanum haussknechtii Boiss.	Antioxidant, Antimicrobial,Cytotoxic activity	Methanol, Water extracts, Essential oils	(Ayaz et al., 2021;Küçükboyacı et al., 2014)
Origanum bargyli Mouterde	Antimicrobial	Essential oils	(Karaman et al., 2004)
Origanum brevidens (Bornm.) Dinsm.	Antioxidant	Essential oils, Methanol	(Özer et al., 2020)
Origanum husnucan-baseri H.Duman, Aytac & A.Duran	Antioxidant , Antibacterial	Essential oils, Methanol, Hydrodistillation, Microwave	(Özer et al., 2020; Uysal et al., 2010)
Origanum amanum Post	Antioxidant,Anticholinesterase	Hydrodistillation, Essential oils	(Özer et al., 2020)
Origanum bilgeri P.H.Davis	Antimicrobials, Acaricidal *activity*	Methanol, Essential oils	(Başer et al., 1996; Dulger, 2006;)
Origanum micranthum Colla	Antioxidant	Essential oils, in aqueous phases	(Taghi-Khani and Kırıcı, 2018)
Origanum minutiflorum O.Schwarz & P.H.Davis	Antimicrobials,Cytotoxic effects, Antioxidant	Essential oils, in aqueous phases	(Albayrak and Aksoy, 2019; Anlas et al., 2017; Şarer et al., 1996)
Origanum majorana L.	Antiasthmatic, Antiparlytic, Antidiuretic, Anticancer	Essential oils	(Johannes et al., 2002; Leung and Steven, 2003; Yadava and Khare, 1995)
Origanum onites L.	Antispasmodic, Antibacterial, Antifungal	Essential oils	(Burt, 2004; Daferera et al., 2000; Ultee et al., 1997)
Origanum syriacum L.	Antiseptic, Antibacterial, Antimycotic	Essential oils	(Gardner, 1989; Letchamo et al., 1995;Yanar et al., 2016)

Origanum vulgare L.	Antimicrobial, Antioxidant, Antiradical, Antifungal, Antihyperglycaemic, Antibacterial, Antithrombin	Essential oils	(Cervato et al.,2000; Cleff et al., 2010; 2000; Dorman and Deans, 2000; Goun et al., 2002; Lemhadri et al., 2004; Mastelic et al., 2008; Yanishlieva et al., 1999)
Origanum laevigatum Boiss.	Antioxidant	Essential oils, Methanol	(Çarıkçı et al., 2018)
Origanum ehrenbergii Boiss	Antioxidant, Antiinflammatory , Anticholinesterase	Essential oils	(Loizzo et al., 2009)
Origanum virens L.	Antioxidant, Antimicrobial	Essential oils, Hydrodistillation	(Arantes et al., 2019)
Origanum elogatum L.	Antioxidant	Essential oils	(Oualili et al., 2018)
Origanum glandulosum L.	Antioxidant	Essential oils	(Bouaziz et al., 2019)
Origanum compactum L.	Antioxidant, Antimicrobials Antifungal	Essential oils	(Bellakhdar et al., 1998;Bouhdid et al., 2008; Chebli et al., 2003)
Origanum ayliniae Dirmenci & Yazıcı	Antioxidant ,	Essential oils	(Özer et al., 2020)

Antioxidant activity

Antioxidant activities of *O. saccatum, O. brevidens, O. husnucanbaseri, O. amanum, O. ayliniae* and *O. boisseri* were reported using DPPH free radical scavenging activity, β-carotene linoleic acid and CUPRAC test (Özer et al., 2020). The antioxidant activity of *O. munzurense* has been reported using the DPPH and CUPRAC tests (Yabalak et al., 2020). The antioxidant activity of *O. solymicum* has been reported using DPPH free radical scavenger, ABTS radical cation scavenger and FRAP reducing potency tests (Erenler et al., 2017). The antioxidant activity of *O. hypericifolium* has been reported using ABTS radical cation scavenger (Çelik et al., 2010). The antioxidant activity of *O. sipyleum* has been reported using the DPPH free radical scavenger and ABTS radical cation scavenger tests (Kaska, 2018). The antioxidant activity of *O. rotundifolium* has been reported using the ABTS radical cation scavenger test (Erenler et al., 2017). The antioxidant activity of *O. acutidens* has been reported using DPPH and beta-carotene/linoleic acid tests (Goze et al., 2010). The antioxidant activity of *O. haussknechtii* has been reported using DPPH free radical scavenger (Küçükboyacı et al., 2014). The antioxidant activity of *O. micranthum* has been reported using the DPPH, CUPRAC, and FOLIN tests (Taghi-Khani and Kırıcı, 2018). The antioxidant activity *of O. minutiflorum* has been reported using phosphomolybdenum, DPPH, hydrogen peroxide scavenging, beta-carotene bleaching activity, and FRAP tests (Albayrak and Aksoy, 2019). The antioxidant activity of *O.*

vulgare has been reported according to TGL and TGSO (Yanishlieva et al., 1999). The antioxidant activity of *O. laevigatum* has been reported using DPPH, CUPRAC, and β-carotene linoleic acid tests (Çarıkçı et al., 2018). The antioxidant activity of *O. ehrenbergii* has been reported using the DPPH test (Loizzo et al., 2009). The antioxidant activity of *O. virens* has been reported using DPPH, total reducing power, and β-carotene/ linoleic acid tests (Arantes et al., 2019). The antioxidant activity of *O. elogatum* has been reported using the DPPH test (Oualili et al., 2018). The antioxidant activity of *O. glandulosum* has been reported using DPPH and hydroxyl radical tests (Bouaziz et al., 2019). The antioxidant activity of *O. compactum* has been reported using DPPH, beta-carotene bleaching activity, and total reducing power tests (Bouhdid et al., 2008).

Antimicrobial activity

The antimicrobial activity of *O. boisseri* has been reported by studies on *Bacillus cereus, B. subtilis, Staphylococcus aureus, S. epidermidis, Listeria monocytogenes* and *Candida* strains (İşcan et al., 2020). The antimicrobial activity of *O. munzurense* has been reported by studies on *Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa* and *S. aureus* (Yabalak et al., 2020). The antimicrobial activity of *O. saccatum* has been reported by studies on *S. aureus, S. epidermidis, Enterococcus faecalis, Streptococcus pyogenes, E. coli, K. pneumoniae, Enterobacter cloacae, Serratia marcescens* and *Proteus vulgaris* (Sozmen et al., 2011). Antimicrobial activities of *O. bilgeri* and *O. solymicum E. coli, S. aureus, K. pneumoniae, P. aeruginosa, Proteus vulgaris, B. cereus, Mycobacterium smegmatis, L. Monocytogenes, Micrococcus luteus, Candida albicans, Rhodotorula rubra* It has been reported by studies on *Kluyveromyces fragilis* (Dulger, 2006). There are antimicrobial activity of *O. hypericifolium* on some microorganism (Çelik et al., 2010; Ocak et al., 2014). It has been reported that the antimicrobial activity of *O. sipyleum* on *Enterobacter aerogenes, E. coli, E. faecalis, M. smegmatis, K. pneumoniae, P. vulgaris, P. aeruginosa, Pseudomonas fluorescens, Salmonella enteritidis, Salmonella typhimurium* and *S. aurecica* (Özkan et al., 2007). The antimicrobial activity of O. acutidens has been reported by studies on *E. coli, P. aeruginosa, Salmonella typhi*, *K. pneumoniae, P. vulgaris, B. subtilis, Corynebacterium diphtheriae*, and *C. albicans* (Goze et al., 2010). The antimicrobial activity of O. haussknechtii has been reported by studies on E. coli and P. aeruginosa (Ayaz et al., 2021). Antimicrobial activity of *O. bargyli Bacillus megaterium, B. cereus, B. subtilis, E. coli, P. aeruginosa, S.aureus, L. monocytogenes, M. luteus, K. pneumoniae, M. smegmatis, P.vulgaris*, It has been reported by studies on *Torulopsis holmii, Saccharomyces cerevisiae, Candida tropicalis* and *C. albicans* (Karaman et al., 2004). The antimicrobial activity of O. husnucanbaseri has been

reported by studies on *S. pyogenes* and *S. aureus* (Uysal et al., 2010). The antimicrobial activity *of O. minutiflorum* has been reported by studies on *S. aureus, S. faecalis, B. subtilis, E. coli, C. albicans*, and *C. tropicalis* (Şarer et al., 1996) The antimicrobial activity of *O. onites* has been reported by studies on *L. monocytogenes*, *S. typhimurium*, *E. coli, Shigella dysenteria, B. cereus* and *S. aureus* (Burt, 2004; Ultee et al., 1997). Antimicrobial activity of *O. syriacum, Botrytis cinerea* and *Clavibacter michiganensis* subsp. *michiganensis* has been reported in studies (Letchamo et al., 1995;Yanar et al., 2016). The antimicrobial activity of *O. vulgare* has been reported by studies on *E. coli* and *S. cerevisiae* (Mastelic et al., 2008; Dorman and Deans, 2000). The antimicrobial activity of *O. virens* has been reported by studies on *S. aureus*, *S. epidermidis, E. faecalis, E. coli, Morganella morganii, Proteus mirabilis, S. enteritidis, S. typhimurium* and *P. aeruginosa* (Arantes et al., 2019). Antimicrobial activity of *O. compactum S. aureus*, *L. monocytogenes*, *Listeria innocua, E. faecium, E. coli, P. mirabilis, P. aeruginosa* and *Salmonella* sp. It has been reported by studies on (Bellakhdar et al., 1998; Chebli et al., 2003).

Anticholinesterase activity

It has been reported that *O.boisseri, O. saccatum, O. solymicum, O. amanum* and *O. ehrenbergii* species have anticholinesterase activity as a result of analyzes (Loizzo et al., 2009;Özer et al., 2020)

Antiproliferative activity

It has been reported as a result of studies on *Artemia salina* that *O. sipyleum* has a cytotoxic effect (Kaska, 2018). The cytotoxic effect of *O. minutiflorum* has been reported on tumor cells by MTT test (Anlas et al., 2017). Studies on the cytotoxic effect of *O. haussknechtii* have also been reported (Ayaz et al., 2021).

Other effects

It has been reported that *O. munzurense* has DNA damage protection activity (Yabalak et al., 2020). The antispasmodic effect of *O. saccatum* and *O. hypericifolium* has been reported by studies (Çam et al., 2020). The anticancer effect of *O. rotundifolium* has been reported as a result of studies (Erenler et al., 2017). It has been reported that *O. bilgeri* has the effect of acaricidal activity (Başer et al., 1996). It has been reported that *O. majorana* has antiasthmatic, antiparalytic, antidiuretic and anticancer effects (Johannes et al., 2002; Leung and Steven, 2003; Yadava and Khare, 1995). *O. vulgare* has been reported to have antihyperglycemic and antithrombin activity (Cervato et al., 2000; Goun et al., 2002; Lemhadri et al., 2004). It has been reported that *O. syriacum* has antiseptic, antibacterial and antimycotic effects (Gardner, 1989; Mastelic et al., 2008; Yanar et al.,

2016). It has been reported as a result of studies that *O. ehrenbergii* has an anti-inflammatory effect (Loizzo et al., 2009).

Conclusion

In this study, the biological activities of species belonging to the genus Origanum were compiled. According to the obtained studies, it has been determined that many species of plant species mainly exhibit important activities such as antioxidant and antimicrobial. In this context, Origanum species are considered to be an important natural resource.

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