

Microbial Degradation of Heterocycles- A Review

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Abstract— Heterocycles are organic compounds that are well-known and distributed in nature; they can be used in the pharmaceutical, agrochemical, and chemical industries. Heterocycles composed of sulfur, nitrogen, and oxygen atoms are harmful toxins and can cause cancers; these substances can persist for years in the environment. One attractive alternative to expensive physical and chemical methods is microbial degradations, which present high potential and low cost, causing minimal environmental impacts. The use of these microorganisms makes use of heterocyclic substances as substrates, removing them efficiently and safely. Some strains of wild and genetically modified microorganisms (bacteria and fungi) have already been used to degrade various pesticides and aromatic compounds. Understanding the biodegradation mechanism of microorganisms will benefit future bioremediation studies, which may prove to be one of the alternatives to solving environmental problems. This review will focus on the microbial degradation of heterocyclic compounds, taking into account the most used techniques and their limitations in future research.

Keywords— Biotechnology, Environment pollution, Organic chemistry.

I. INTRODUCTION

Heterocycles are organic compounds naturally distributed in nature; such compounds are involved in the metabolism of all living cells. Some natural and synthetic heterocyclic compounds are considered pharmacologically active and are being used in the clinical area. Various applications exist for these compounds, such as pharmaceutical, agrochemical, and veterinary products. Heterocycles compounds can have antioxidant, revealing, sensitizing, corrosion-inhibiting, and copolymeric dyes necessary for synthesizing critical natural

products [1].

These substances play an essential role in chemistry because they can be applied in several applications such as drug formulations, photochemistry, agrochemicals, dyes, etc. Among them, indole derivatives are examples used to produce synthetic drugs. The structures of the heterocycles can be linked to more than one receptor with high affinity, which can help develop a multitude of compounds considered biologically active. Among them, we have the indole derivatives, recognized for having active ingredients that can treat several diseases, such as dermatitis, peripheral neuropathies, arthritis, airway allergies, glaucoma, and eye inflammation. These compounds can also be used in medicines with antimicrobial activity, such as serotonin antagonists, antidepressants, central nervous system anxiolytic depressants, anticonvulsants, and antihistamines [2].

Some heterocyclic polycyclic aromatic compounds have already shown toxic and mutagenic characteristics in research; these compounds have one or more S, N, or O atoms in their structure. Recently the attention in the study of these chemicals, mainly when referring to Dibenzothiophene (DBT) and its derivatives, which have already been shown to have a cumulative effect (they stay in the environment for about three years) [3]. N heterocycles, such as carbazole and its derivatives, are well-used in many industries and can undergo rapid, radical chemical changes, generating hydroxynitrocarbazoles that are more toxic [3].

Compounds that are widely used as a model to study the biodegradation of N and S-heterocycle are DBT and Carbazole, respectively. Dibenzofuran (DBF), a heterocycle, is well known and used as a pesticide in plantations; the photolysis of chlorinated biphenyl ethers forms this compound. Such compounds are considered a model for the removal of chlorinated dibenzofurans and biaryl ethers, which cause great environmental concern [3].

As we have seen, heterocycles correspond to several kinds of

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compounds; in addition to those mentioned, we also have dyes, which, when placed in the environment, can bring specific problems. Thus, ecologically correct dye production technologies and consolidated ecologically correct bioprocesses that decompose these dyes have been studied. We must emphasize that changes concerning environmental concerns strengthened environmental regulations, which stimulated many types of research and developments regarding these techniques that aim to minimize the impacts caused by certain polluting substances used in several industries, such as heterocycle compounds. For example, we have microbial and enzymatic degradation that act in the degradation of some products [4].

The search for techniques that aim to present activities of a biodegradative character, which use microorganisms to remove environmental pollutants, such as heterocycles, has become an alternative to physical and chemical methods since biodegradative techniques have potential and low cost, causing environmental impacts when compared to conventional methods. Much research has already sought to study the isolation and identification of strains with specific applications, such as the use of bacteria capable of removing pollutants from the sulfur in the oil, as well as the number of sulfur oxides released, avoiding harmful to the environment [3].

This review aimed to address the removal of heterocycle compounds by microorganisms in general, considering the most used techniques and their limitations in future research to provide helpful information for treatment that can decrease the accumulation of heterocycle compounds in the environment that can cause adverse environmental impacts.

II. THE CHEMISTRY OF HETEROCYCLES

The discussion of saturated and aromatic heterocycles brings together many concepts and applications. Heterocycles are organic compounds essential to the biochemical processes because the side groups of living cells' most typical and essential constituents, DNA and RNA, are based on these molecules [5]. In general, these compounds have something in common in their structures, whether due to the presence of carbon and hydrogen (hydrocarbons) also the presence of more common heteroatoms, such as nitrogen (N), oxygen (O), and sulfur (S). In addition, organic compounds generally have a ring (aromatic or not), so they are considered cyclical. The rings present in organic structures directly influence molecular properties, from molecular conformation to the reaction process - these properties make the class of heterocyclic organic compounds extraordinary.

Most physiologically active compounds owe their biological potential to the presence of heteroatoms, mainly in the form of heterocycles [6]; most natural products are heterocycles [7]. Figure 1, is presented some of the heterocycle compounds common in organic chemistry, such as nicotine, present in cigarettes [8]; cocaine, an illicit product that affects the central nervous system [9]; and morphine, an essential painkiller used against severe pain [10].

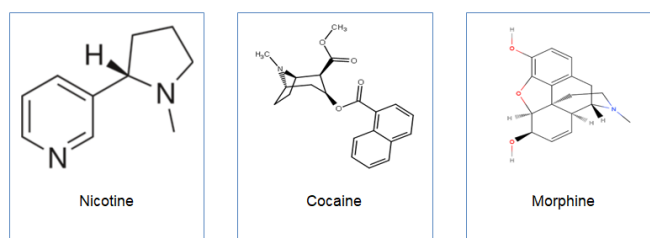


Figure 1. Heterocyclic compounds common in organic chemistry. Adapted from [8]–[10].

A. Nitrogen heterocycle

The main heterocycle compounds with nitrogen are amines, which primarily function as bases (nucleophiles). They are mainly involved in uni and bimolecular electrophilic substitution reactions (SN1 and SN2). Morpholine, for example, is acylated by 3,4,5-trimethoxybenzoyl chloride to form the tranquilizer and muscle relaxant [11]; already N-methyl piperazine can be alkylated in an SN1 reaction with diphenyl methyl chloride to obtain cyclizine as a product [12], seasickness medicine. The addition of pyrrolidine to an aldehyde or ketone forms a product that belongs to the class of enamines, which is very important in the chemistry of heterocycles because it has broad applicability as precursors in the synthesis of both synthetic and natural organic compounds [13], [14].

B. Oxygen and sulfur heterocycles

The heterocycle class that contains oxygen as a heteroatom has advanced in many studies - as the six-membered heterocycles present in natural products of plants and microorganisms present themselves as good natural bioactive and continue to stimulate the development and synthesis of new molecules with similar characteristics, especially for the pharmaceutical industry that constantly seeks the implementation of new drugs to combat various diseases that affect the planet [15].

Significant groups of heterocycle compounds are the phenolic derivatives belonging to the class of flavonoids, these present an oxygen heterocycle called ring C, and the variation of this ring is decisive in the classification of subgroups such as flavonols, flavones, flavanones, isoflavones among others. In addition, the addition and position of unsaturation in this ring, as well as the hydroxylation of position 3, confer effective biological potentials; it is worth mentioning antioxidants, antibacterials, anti-inflammatories, etc. [16], [17]. In summary, oxygen heterocycles are generally compounds that contain lactones, furans, and pyrans in their structure - those of natural origin follow the pathway of HMG-CoA reductase and shikimic acid; syntheses are produced by a general cyclization method where the target molecule is produced from a γ -carbonylated compound, previously treated with a P_2O_5 derivative in an acidic medium.

In addition to oxygen heterocycles, those containing sulfur as a heteroatom are considered unique. In general, the compounds containing sulfur as heteroatoms correspond to products

derived from oxygen heterocycles that undergo various oxidation reactions in the geological cycle, and this involves all balance and availability of minerals in a natural environment. In summary, this group of compounds is used more frequently in disinfectants, flavorings, and other industries. Almost some of them are harmful to living beings and the environment; despite this, they are of paramount importance to the biochemistry of life, but they can be accumulated in excess [16].

III. APPLICATION OF HETEROCYCLES IN THE CHEMICAL, FOOD, AND PHARMACEUTICAL INDUSTRY

Many heterocycle compounds are found in substances applied in the food, chemical, and pharmaceutical industries. In chemical industry routes, agriculture, and domestic areas, we see the global use of many pesticides. Over the years, such consumption has caused several environmental problems, as they are highly toxic compounds for the environment and human health. However, the pesticides guarantee the protection of plantations, being quite helpful and vital, despite presenting substances considered severe threats to natural resources and poisoning animals and plants. Living beings are affected indirectly through the food chain due to these toxic compounds' chemical complexity and persistent nature [18].

Another example of N-heterocyclic applied in the chemical industry is nicotine, which can be found in many solid, liquid, and airborne wastes produced in the tobacco industries. The degradation intermediates of this compound, such as 6-hydroxynicotin and 6-hydroxy-3-succinoyl-pyridine (HSP), are considered precursors during the synthesis of insecticides and pharmaceutical products [3].

According to [19], quinoline is a heterocyclic compound for industrial use (chemical, pharmaceutical, textile, etc.) that has become harmful to human health and the environment, as it has carcinogenic, teratogenic, and mutagenic effects that can be accumulated in food. Since this compound presents a steric impediment of its fused bicyclic structure and a long half-life of photooxidation, it takes work to decompose naturally. It is the target of many studies for this purpose.

We know that heterocycles are of industrial importance, although these compounds are harmful if exposed to the environment for a long time. Studies to obtain these compounds from lignocellulosic biomass can make these compounds "greener" and more sustainable [20].

The growing energy demand, the decrease in fossil fuel reserves, and climate change are the main problems facing humanity today [20], [21], with the need to transition to an economy more sustainable and a carbon neutral society. In this context, this challenge can be addressed from:

- a) Transform biomass (a renewable energy source) into products that can be applied to different industries and fuels, such as bioethanol,
- b) To develop new heterogeneous catalysts with superior performance in the target reactions and,
- c) Exploring the potential of some technologies, such as a microwave-assisted process, mechanochemical and flow chemistry, among others, that allow greener transformations to be carried out [22].

The simple hydrolysis of lignocellulosic biomass can give rise to a range of products that can be applied and of interest to industries, among which we have levulinic acid (LA), which is among the ten platform molecules considered promising in the production of compound heterocycles nitrogen, or N-heterocycles [23]. It has become very promising in recent years due to the remarkable ability of N-heterocycles to serve as active biomimetics and pharmacophores, being inputs widely used in the pharmaceutical industry for polymers, agrochemicals, dyes [24]–[26] and compounds used in the food industry.

The heterogeneous catalysts most used in synthesizing N-heterocycles are noble metals such as Au, Pd, Pt, Ru, In, and Ir, supported on carbon, or metallic oxides, with high cost being a significant disadvantage. However, numerous catalysts of non-noble metals (Cu-, Ni-, Co-, Zr) have been proposed for several valorization routes of LA. However, they present low activity and little stability [23], [27]–[29]. The design of construction of noble metal catalysts that are efficient and have high stability using the technique of immobilization on functional supports, such as porous nanomaterials, is still a challenging and reasonably necessary process.

A way to obtain a cleaner alternative to carrying out conventional transformations is mechanochemistry (chemical transformations supported by mechanical force); this method is promising because it is fast, efficient, and sustainable [30].

In this context, the benefits of mechanochemical procedures for the preparation of catalytic nanomaterials using residues derived from biomass as sacrificial models are considered promising [31], in which an Nb/ZnO nanocatalyst modeled by biomass, it is synthesized using a mechanochemical and sacrificial assisted mold method, promoting the catalytic conversion of levulinic acid into N-heterocycles under solvent-free conditions [22].

Mechanochemistry as a synthetic, simple, solvent-free method and short reaction time proves to be advantageous for the preparation of nanocatalysts, with the recovery of underutilized biomass residues (in the particular orange peel) as sustainable and renewable resources compared to the oil-based industry, for its use as a sacrificial mold, favoring the appearing of nanostructured materials with defined morphologies [22].

The chemistry of N-heterocycles is an exciting area for the research of synthetic and medicinal chemistry, constituting more than 60% of organic used, in which the position and the number of nitrogen atoms in the rings differentiate them as pyrroles, pyrazoles classes, imidazoles, triazoles, pyridines and pyrimidines [32]; presenting applications in many detectors, including medicinal and agricultural fields, with antibacterial, antifungal, herbicidal, antiviral activities, medicines such as antifungal and anti-inflammatory, antidepressant, antioxidant [32]–[35], among others.

Among the most elaborate heterocyclic compounds, sulfur-containing heterocycles (S-heterocycles) are of particular interest due to their structure in introductory organic chemistry, medicine, and biochemistry [36]. Sulfur-containing heterocycles in various natural compounds and drugs serve as biologically active molecules in various pathophysiological

conditions, exhibiting many pharmacological actions such as anticancer, antiviral, anti-inflammatory, antimicrobial, antitubercular, etc., being the best known in the conception of the drug, thiazole, isothiazole, thiophene, thiopyran, thiazolidine and thiazepine [37].

Due to Sulfur's high volatility and reactivity, many sulfur-containing compounds also play a role in the aroma of food products, such as vegetables, meat, and roasted products [38].

IV. MICROBIAL DEGRADATION OF HETEROCYCLES

The microbial degradation of aromatic compounds involves the breakdown of these compounds by producing intermediate metabolites through microorganisms. The enzymes involved in the microbial degradation of aromatic compounds are hydrolases, dehalogenase, oxygenase, dehydrogenases, and oxidases. Microorganisms can degrade several pollutants in anaerobic conditions, that is, in the absence of oxygen, where the compound is converted by a state of superior oxidation, which becomes soluble in water and more accessible to be degraded by the microbes they produce intermediates and non-toxic products that are part of the carbon cycle [39].

Heterocycles are toxic when disposed of in the environment. Research about microbial techniques for their removal is urgent nowadays, and it is necessary for the complete bioremediation of these compounds. Microorganisms such as bacteria, fungi, algae, cyanobacteria, and genetically modified microbes can rapidly degrade a large number of pesticides and xenobiotics [18].

According to [18], microbial bioremediation can safely and efficiently remove some toxic pollutants to the environment and human health; such a technique can use pollutants or contaminants as a substrate. Regarding heterocycles, we have the removal of carbofuran, which has already been studied, and powerful carbofuran-degrading bacteria have already been isolated from soil contaminated with carbofuran, sludge, and water samples through enrichment culture techniques. These bacteria include *Achromobacter sp.* WM111, *Rhodococcus* TE1, *Pseudomonas sp.*, *Sphingomonas sp.*, *Cepa* SB5, *Enterobacter sp.*, *Burkholderia sp.* PLC3, *Bacillus sp.* and *Cupriavidus sp.* ISTL7.

Many pharmaceutical products have heterocycle compounds in their structure, and as we have already discussed, if these compounds are in the environment, they can be harmful. However, only a few studies have examined the role of different microbial populations in applying the biotransformation of organic pollutants and pharmaceutical products in activated sludge systems [40], [41].

Fernandez-Fontaina et al. [41] carried out under heterotrophic conditions study of the effect of nitrification on the biotransformation of water treatment for the removal of various drugs; in such a study, the focus was on using ammonia-oxidizing bacteria (AOB), nitrite-oxidizing bacteria (NOB) and heterotrophic bacteria. As a result, the authors noted that nitrifiers could process ibuprofen at the expense of hydroxylation by ammonia monooxygenase (AMO), producing 2-hydroxy-ibuprofen. The biotransformation of naproxen under nitrification conditions was also studied. In this case, the

heterotrophic bacteria present in the nitrifying activated sludge (NAS) could biotransform sulfamethoxazole. However, nitrifying and heterotrophic activities were inefficient against diclofenac, diazepam, carbamazepine, and trimethoprim. Similar rates of biotransformation of erythromycin, roxithromycin, and fluoxetine were observed in all conditions tested. Thus, further evidence on the role of the different microbial communities present in activated sludge reactors for the biological removal of pharmaceutical products is required.

A. Bacterial degradation

There are several types of research about the bacterial degradation of heterocycles; among them, the degradation of carbazole is quite punctuated, as it can be degraded by several bacterial strains that have already been isolated, as an example we have: *Pseudomonas*, *Sphingomonas*, *Ralstonia*, *Bacillus*, *Gordonia*, *Mycobacterium*, *Nocardioideis*, *Xanthomonas*, and *Janthinobacterium* [3], [42].

Pseudomonas spp. and *Sphingomonas spp.* were studied in more detail among the main strains for removing carbazole; such action has been reported to occur by the angular deoxygenation pathway. This way, the carbazole pyrrole ring is first opened by an angular deoxygenation reaction to produce an unstable hemiaminal compound of 1hydro1, nine α -dihydroxy carbazole. After some more complex reactions, this can cause the resulting compound to be hydrolyzed to anthranilate and 2-hydroxypenta-2, 4-dienoic acid, and the anthranilate to be converted to catechol by a deoxygenation reaction. Therefore, the catechol turns into a tricarboxylic acid cycle, and then carbazole degradation occurs, which is used by such bacteria as a substrate [35].

According to [43], nicotine catabolism through the pyrrolidine route was initially identified in 1953 using *Pseudomonas* strains. This initial degradation step occurs with the pyrrolidine ring that undergoes dehydrogenation and produces N-methylmimosin (NMM), which spontaneously is hydroxylated to form pseudooxynicotin (P.N.), which is then transformed into three succinyl semialdehyde pyridine (SAP), 3-succinyl pyridine (S.P.), six hydroxy-3-succinoyl-pyridine (HSP) and 2,5-dihydropyridine (2,5DHP). After this step, 2,5DHP will be converted into fumaric acid (F.A.) by the maleamate route: 2,5DHP in N-formylmaleamic acid (NFM), maleamic acid (MAA), maleic acid (M.A.) and F.A. Finally, the F.A. product enters the Krebs cycle (TCA) cycle.

According to [19], quinolone degradation has been studied recently, and many technologies have been applied for its degradation. Luo et al. (2020) [19] conducted a rigorous review study that focused on providing a comprehensive view of the degradation status of quinoline to improve its degradation efficiency without causing any harm to the environment. The same authors collected in their database strains, research progress, and the mechanisms of several methods to degrade quinoline; here in this chapter, we primarily address the removal of quinoline.

Ali et al. (2019) [44] conducted a study testing the *Pseudomonas aeruginosa*. These FA-HZ1 strains showed a high

degrading activity of DBF under optimal cell growth conditions of a temperature of 30°C, pH 5.0, rate 200 rpm, and 0.1 mM DBF, using DBF as a substrate. These same authors also studied the biochemical and physiological characteristics of the microorganism in question, in addition to using different carbon sources by *P. aeruginosa* FA-HZ1. The genomic study of the strains that were able to identify 158 genes involved in the catabolism of heterocyclic compounds such as DBF through a proteomic study; these authors also showed that the degradation pathway of DBF by *P. aeruginosa* FA-HZ1 was promising, which can be applied for industrial use in waste treatment.

Li et al. (2005) [42] carried out a study about the desulfurization of DBT in 2-hydroxyphenyl (2-HBP) using a thermophilic bacterium called *Mycobacterium goodii* X7B, which also converts 2-HBP into 2-methoxyphenyl (2-MBP). Also responsible for the formation of the methoxylated product 2-MBP by *Mycobacterium* sp. The G3.

B. Fungal degradation

Fungi are the largest microbial class that makes up the biomass of the soil. However, mechanisms of its catabolism when acting on biaryl ether compounds are still limited, conditioned only to hydroxylation processes. The *Trichosporon mucoides* SBUG 801 could metabolize dibenzothiophene (DBF) [3]. The mechanism starts with the accumulation of a high concentration of monohydroxylated DBF followed by the formation of hydroxylated derivatives of 2-hydroxy dibenzofuran and 3-hydroxy dibenzofuran, indicating that the action of a monooxygenase reaction occurs at the beginning of the degradation stages. *T. mucoides* SBUG 801 is capable of degrading DBF to 2,3-dihydroxy dibenzofuran and cleaving the compound ring leading to the formation of 2-(1-carboxymethylidene)-2,3-dihydrogen-[b]-furanilidenoglycolic. Thus, this cleavage of the dihydroxylation ring uses a mechanism similar to the microorganism *Trichosporon* sp. SBUG 752 in the degradation of biphenyl ether. In both species, a third hydroxylated group must be introduced in the hydroxylated intermediates before the ortho-cleavage of the aromatic structure occurs [3].

Bioremediation is still the most viable option for removing environmentally hazardous compounds. Studies involving these techniques applied to the degradation of heterocyclic compounds described in the literature represent an important milestone in this field, demonstrating the possibility of aerobic biotransformation of persistent organic pollutant (POP) substrates. A more precise understanding of the biodegradation mechanism of wild-type strains or genetically modified bacteria should be studied in detail and is crucial for future bioremediation studies [3].

V. GENETIC ENGINEERING FOR THE DEGRADATION OF HETEROCYCLE COMPOUNDS

Genetic engineering can be used to make some modifications to improve the efficiency of removing heterocyclic substances by microorganisms, such as the removal of sulfur compounds from petroleum. The use of genetically modified microorganisms (GEMs) has become necessary in several

aspects of science, as they may be able to act in reactions that conventional microorganisms cannot act. In removing sulfur heterocycles, some cultures with improved substrate bands were tested in a mixture of chemicals present in petroleum [3].

Another example is the biological removal of nitrogen from the oil, known as denitrogenation, which conventional microbes cannot biodegrade. There are few reports in this case, and the reported nitrogen reduction could have been better. This leads to the conclusion that a type of nitrogen heterocycle degradation strain alone cannot degrade complex nitrogen compounds and the various compound structures present in the oil, and no biocatalyst is yet able to remove this [3] selectively. Bioremediation by these heterocycle degraders will likely become a commercial prospect shortly [3].

We must understand the metabolism of microorganisms in the degradation of heterocycles since approximately 0.1-1% of them can be used through current techniques. Therefore, metagenomics is crucial when considering the study of new genes that have specific capacities for the degradation of toxic components, with techniques that aim at mutating and reorganizing genes of microorganisms with efficient enzymatic systems in a less complicated way. Such microorganisms can be used as biocatalysts, as they will have an affinity with a wide range of substrates (their carbon source) in biphasic reactions that may present toxic or heterocyclic solvents and be used for environmental bioremediation, oil treatment, or production of derivate compounds. These possibilities represent a future challenge to the researchers to minimize heterocycles compounds in the environment [3].

VI. CONCLUSION

Heterocycle compounds, whether chemical, pharmaceutical, or food, are essential for the industry. Thus, its accumulation in the environment has become an environmental concern in recent years. However, it is noted that much research has been carried out to minimize these problems, such as the application of biotechnology for the degradation of heterocycles, as there is a range of microorganisms capable of degrading these compounds for using it as a substrate, thus reducing the environmental impacts and toxicity caused by these compounds. Using some microorganisms has already become promising, and the cost-benefit is interesting for properly treating heterocycles.

REFERENCES

- [1] R. R. Gupta, M. Kumar, and V. Gupta, *Heterocyclic Chemistry: Volume II: Five-Membered Heterocycles*. Springer Science & Business Media, 2013.
- [2] R. B. Toche and R. A. Janrao, "Synthesis, characterization and antimicrobial evaluation of novel urea, sulfonamide and acetamide 3,4-dihydropyrazino[1,2-a]indol-1(2H)-one derivatives," *Arabian Journal of Chemistry*, vol. 12, no. 8, pp. 3406–3416, Dec. 2019, doi: 10.1016/j.arabjc.2015.08.034.
- [3] P. Xu, B. Yu, F. L. Li, X. F. Cai, and C. Q. Ma, "Microbial degradation of sulfur, nitrogen and oxygen heterocycles," *Trends Microbiol.*, vol. 14, no. 9, pp. 398–405, Sep. 2006, doi: 10.1016/j.tim.2006.07.002.
- [4] K.-Y. Choi, "Discoloration of indigo dyes by eco-friendly biocatalysts," *Dyes and Pigments*, vol. 184, p. 108749, Jan. 2021, doi: 10.1016/j.dyepig.2020.108749.

- [5] A. T. Balaban, D. C. Oniciu, and A. R. Katritzky, "Aromaticity as a Cornerstone of Heterocyclic Chemistry," *Chem Rev*, vol. 104, no. 5, pp. 2777–2812, May 2004, doi: 10.1021/cr0306790.
- [6] A. F. Pozharskii, A. R. Katritzky, and A. T. Soldatenkov, *Heterocycles in life and society*. Wiley Chichester, 2011.
- [7] K. C. Majumdar and S. K. Chattopadhyay, *Heterocycles in natural product synthesis*. John Wiley & Sons, 2011.
- [8] S. Taghavi *et al.*, "Nicotine content of domestic cigarettes, imported cigarettes and pipe tobacco in Iran," *Addiction & health*, vol. 4, no. 1–2, p. 28, 2012.
- [9] L. Avois, "Central nervous system stimulants and sport practice," *Br J Sports Med*, vol. 40, no. Supplement 1, pp. i16–i20, Jul. 2006, doi: 10.1136/bjsm.2006.027557.
- [10] B. C. Nunes, J. B. dos Santos Garcia, and R. K. Sakata, "Morphine as first medication for treatment of cancer pain," *Brazilian Journal of Anesthesiology (English Edition)*, vol. 64, no. 4, pp. 236–240, Jul. 2014, doi: 10.1016/j.bjane.2013.06.016.
- [11] H. Hai-Ying, Q. Yan-Ling, and Z. Cheng-Xue, "Reactions of enamines with fluorinated acyl chlorides - Synthesis of fluorinated 1,3-diketones and 1,3-keto-aldehydes," *Chin J Chem*, vol. 16, no. 6, pp. 549–556, Aug. 2010, doi: 10.1002/cjoc.19980160611.
- [12] J. Clayden, N. Greeves, and S. Warren, *Organic chemistry*. Oxford University Press, USA, 2012.
- [13] A. R. Chamberlin and D. J. Sall, "Reduction of Ketones to Alkenes," in *Comprehensive Organic Synthesis*, Elsevier, 1991, pp. 923–953. doi: 10.1016/B978-0-08-052349-1.00251-1.
- [14] A. F. Abdel-Magid, "8.01 Reduction of C=O to CHOH by Metal Hydrides," in *Comprehensive Organic Synthesis II*, Elsevier, 2014, pp. 1–84. doi: 10.1016/B978-0-08-097742-3.00801-6.
- [15] H. M. Merken and G. R. Beecher, "Liquid chromatographic method for the separation and quantification of prominent flavonoid aglycones," *J Chromatogr A*, vol. 897, no. 1–2, pp. 177–184, Nov. 2000, doi: 10.1016/S0021-9673(00)00826-8.
- [16] B. L. Tan, M. E. Norhaizan, W.-P.-P. Liew, and H. Sulaiman Rahman, "Antioxidant and Oxidative Stress: A Mutual Interplay in Age-Related Diseases," *Front Pharmacol*, vol. 9, Oct. 2018, doi: 10.3389/fphar.2018.01162.
- [17] P. M. Kris-Etherton and C. L. Keen, "Evidence that the antioxidant flavonoids in tea and cocoa are beneficial for cardiovascular health," *Curr Opin Lipidol*, vol. 13, no. 1, pp. 41–49, 2002.
- [18] S. Mishra *et al.*, "Carbofuran toxicity and its microbial degradation in contaminated environments," *Chemosphere*, vol. 259, p. 127419, Nov. 2020, doi: 10.1016/j.chemosphere.2020.127419.
- [19] Y. Luo, X. Yue, P. Wei, A. Zhou, X. Kong, and S. Alimzhanova, "A state-of-the-art review of quinoline degradation and technical bottlenecks," *Science of The Total Environment*, vol. 747, p. 141136, Dec. 2020, doi: 10.1016/j.scitotenv.2020.141136.
- [20] P. Sudarsanam, R. Zhong, S. Van den Bosch, S. M. Coman, V. I. Parvulescu, and B. F. Sels, "Functionalised heterogeneous catalysts for sustainable biomass valorisation," *Chem Soc Rev*, vol. 47, no. 22, pp. 8349–8402, 2018, doi: 10.1039/C8CS00410B.
- [21] A. F. Lee, J. A. Bennett, J. C. Manayil, and K. Wilson, "Heterogeneous catalysis for sustainable biodiesel production via esterification and transesterification," *Chem. Soc. Rev.*, vol. 43, no. 22, pp. 7887–7916, 2014, doi: 10.1039/C4CS00189C.
- [22] D. Rodríguez-Padrón *et al.*, "Exploring the potential of biomass-templated Nb/ZnO nanocatalysts for the sustainable synthesis of N-heterocycles," *Catal Today*, vol. 368, pp. 243–249, May 2021, doi: 10.1016/j.cattod.2020.06.076.
- [23] Z. Xue, D. Yu, X. Zhao, and T. Mu, "Upgrading of levulinic acid into diverse N-containing functional chemicals," *Green Chemistry*, vol. 21, no. 20, pp. 5449–5468, 2019, doi: 10.1039/C9GC02415H.
- [24] M. Hong, X. Tang, L. Falivene, L. Caporaso, L. Cavallo, and E. Y.-X. Chen, "Proton-Transfer Polymerization by N-Heterocyclic Carbenes: Monomer and Catalyst Scopes and Mechanism for Converting Dimethacrylates into Unsaturated Polyesters," *J Am Chem Soc*, vol. 138, no. 6, pp. 2021–2035, Feb. 2016, doi: 10.1021/jacs.5b13019.
- [25] Y. Qi *et al.*, "Synthesis of an aromatic N-heterocycle derived from biomass and its use as a polymer feedstock," *Nat Commun*, vol. 10, no. 1, p. 2107, May 2019, doi: 10.1038/s41467-019-10178-0.
- [26] E. Vitaku, D. T. Smith, and J. T. Njardarson, "Analysis of the Structural Diversity, Substitution Patterns, and Frequency of Nitrogen Heterocycles among U.S. FDA Approved Pharmaceuticals," *J Med Chem*, vol. 57, no. 24, pp. 10257–10274, Dec. 2014, doi: 10.1021/jm501100b.
- [27] G. Gao *et al.*, "Highly Stable Porous-Carbon-Coated Ni Catalysts for the Reductive Amination of Levulinic Acid via an Unconventional Pathway," *ACS Catal*, vol. 7, no. 8, pp. 4927–4935, Aug. 2017, doi: 10.1021/acscatal.7b01786.
- [28] Z. Xue, Q. Liu, J. Wang, and T. Mu, "Valorization of levulinic acid over non-noble metal catalysts: challenges and opportunities," *Green Chemistry*, vol. 20, no. 19, pp. 4391–4408, 2018, doi: 10.1039/C8GC02001A.
- [29] J. Zhang *et al.*, "Zirconium Oxide Supported Palladium Nanoparticles as a Highly Efficient Catalyst in the Hydrogenation-Amination of Levulinic Acid to Pyrrolidones," *ChemCatChem*, vol. 9, no. 14, pp. 2661–2667, Jul. 2017, doi: 10.1002/cctc.201600739.
- [30] C. M. Cova and R. Luque, "Advances in mechanochemical processes for biomass valorization," *BMC Chemical Engineering*, vol. 1, no. 1, p. 16, Dec. 2019, doi: 10.1186/s42480-019-0015-7.
- [31] D. Rodríguez-Padrón, A. R. Puente-Santiago, A. M. Balu, A. A. Romero, M. J. Muñoz-Batista, and R. Luque, "Benign-by-Design Orange Peel-Templated Nanocatalysts for Continuous Flow Conversion of Levulinic Acid to N-Heterocycles," *ACS Sustain Chem Eng*, vol. 6, no. 12, pp. 16637–16644, Dec. 2018, doi: 10.1021/acssuschemeng.8b03896.
- [32] S. V. H. S. Bhaskaruni, S. Maddila, K. K. Gangu, and S. B. Jonnalagadda, "A review on multi-component green synthesis of N-containing heterocycles using mixed oxides as heterogeneous catalysts," *Arabian Journal of Chemistry*, vol. 13, no. 1, pp. 1142–1178, Jan. 2020, doi: 10.1016/j.arabjoc.2017.09.016.
- [33] L. M. De Coen, T. S. A. Heugebaert, D. García, and C. V. Stevens, "Synthetic Entries to and Biological Activity of Pyrrolopyrimidines," *Chem Rev*, vol. 116, no. 1, pp. 80–139, Jan. 2016, doi: 10.1021/acs.chemrev.5b00483.
- [34] M. D. Hill, "Recent Strategies for the Synthesis of Pyridine Derivatives," *Chemistry - A European Journal*, vol. 16, no. 40, pp. 12052–12062, Oct. 2010, doi: 10.1002/chem.201001100.
- [35] C. Liu *et al.*, "Efficient Approach To Discover Novel Agrochemical Candidates: Intermediate Derivatization Method," *J Agric Food Chem*, vol. 64, no. 1, pp. 45–51, Jan. 2016, doi: 10.1021/jf5054707.
- [36] B. F. Abdel-Wahab, S. Shaaban, and G. A. El-Hiti, "Synthesis of sulfur-containing heterocycles via ring enlargement," *Mol Divers*, vol. 22, no. 2, pp. 517–542, May 2018, doi: 10.1007/s11030-017-9810-3.
- [37] M. Feng, B. Tang, S. H. Liang, and X. Jiang, "Sulfur Containing Scaffolds in Drugs: Synthesis and Application in Medicinal Chemistry," *Curr Top Med Chem*, vol. 16, no. 11, pp. 1200–1216, Mar. 2016, doi: 10.2174/1568026615666150915111741.
- [38] L. Schutte and R. Teranishi, "Precursors of sulfur-containing flavor compounds," *CRC Critical Reviews in Food Technology*, vol. 4, no. 4, pp. 457–505, Mar. 1974, doi: 10.1080/10408397409527166.
- [39] P. S. Phale, A. Sharma, and K. Gautam, "Microbial degradation of xenobiotics like aromatic pollutants from the terrestrial environments," in *Pharmaceuticals and Personal Care Products: Waste Management and Treatment Technology*, Elsevier, 2019, pp. 259–278. doi: 10.1016/B978-0-12-816189-0.00011-1.
- [40] N. H. Tran, T. Urase, and O. Kusakabe, "The characteristics of enriched nitrifier culture in the degradation of selected pharmaceutically active compounds," *J Hazard Mater*, vol. 171, no. 1–3, pp. 1051–1057, Nov. 2009, doi: 10.1016/j.jhazmat.2009.06.114.
- [41] E. Fernandez-Fontaina, I. B. Gomes, D. S. Aga, F. Omil, J. M. Lema, and M. Carballa, "Biotransformation of pharmaceuticals under nitrification, nitratation and heterotrophic conditions," *Science of The Total Environment*, vol. 541, pp. 1439–1447, Jan. 2016, doi: 10.1016/j.scitotenv.2015.10.010.
- [42] F. Li *et al.*, "Microbial Desulfurization of Gasoline in a *Mycobacterium goodii* X7B Immobilized-Cell System," *Appl Environ Microbiol*, vol. 71, no. 1, pp. 276–281, Jan. 2005, doi: 10.1128/AEM.71.1.276-281.2005.
- [43] Y. Mu *et al.*, "Bacterial catabolism of nicotine: Catabolic strains, pathways and modules," *Environ Res*, vol. 183, p. 109258, Apr. 2020, doi: 10.1016/j.envres.2020.109258.
- [44] F. Ali *et al.*, "Characterization of a Dibenzofuran-degrading strain of *Pseudomonas aeruginosa*, FA-HZ1," *Environmental Pollution*, vol. 250, pp. 262–273, Jul. 2019, doi: 10.1016/j.envpol.2019.04.026.