Volume 1, 2025, Pages 100-109 *Journal of Smart and Sustainable Farming*
| Regular Article | Open Access

Computational Innovations Driving Bio-Catalyst Development in Green Chemistry

Sung-hyouk Kim
Gachon University, Seongnam-si, Gyeonggi-do, South Korea.
sungkim56@gachon.ac.kr

Article Info
Journal of Smart and Sustainable Farming
https://www.ansispublications.com/journals/jssf/jssf.html

https://doi.org/10. 64026/JSSF/2025010

Received 10 April 2025
Revised from 30 May 2025
Accepted 24 June 2025
Available online 10 July 2025
Published by Ansis Publications.

Corresponding author(s):
Sung-hyouk Kim, Gachon University, Seongnam-si, Gyeonggi-do, South Korea.
Email: sungkim56@gachon.ac.kr

Abstract – The main purpose of this article is to increase the effectiveness of bio-catalysts in technological environments to enhance technical capabilities. With an ambition to explore the developments made in computational techniques, this paper presents a methodology for transforming the characteristics of enzymes and create enhance bio-catalysts. The authors of this study review various approaches of enzyme engineering, such as aided evolution and rational design. The puts more emphasis on the strategic application of intermolecular collaboration, which enhance the attraction between substrates and enzymes. The research also reviews the challenges and development made in approaches employed in immobilizing enzymes. These approaches include a technique for repairing objects by employing polyketone polymer and assesses traditional approaches of permanently attaching objects together. Towards the end of the research, authors introduce a technique for immobilization, such as Huisgen 1, 3-dipolar cycloaddition, and Staudinger ligation. In addition, the research assesses the applications of nanoparticles in nano-biocatalysts and the integration of green chemistry standards and principles in enzyme bio-catalysts.

Keywords – Computational Techniques, Bio-Catalysts, Green Chemistry, Enzyme Enhancement, Bio-Engineering, Enzyme Immobilization.

I. INTRODUCTION

During the 18th century, the world economy gradually developed and the pharmaceutical industry expanded [1]. Increased competition and the decline of green resources have raised concerns about potential future scarcity of renewable resources [2]. Chemical and materials scientists see the creation of durable, recyclable, environmentally benign catalysts as a major task [3]. This field has been growing significantly, and it is becoming increasingly important to comprehend how well metal nanoparticles work in this environment. Reducing waste disposal also heavily depends on the development of reusable green catalysts that are safe for the environment. It is well acknowledged that these catalytic materials are necessary. Catalysts are classified as homogeneous, heterogeneous, and biocatalysts within a broader framework. When the catalyst and reactant are in the same phase or physical state, the reaction is referred to be homogenous. In several industrial processes where the reactants and products are present in the same phase—as gases or liquids—homogeneous catalysts are often used. On the other hand, catalysts that are heterogeneous exist in a different phase from the reactants.

A solid catalyst reacting with liquid or gaseous reactants is an example of an exemplary case. Heterogeneous catalysts are frequently viewed as more active, efficient, and selective in contrast to homogeneous catalysts [3]. This is mostly because there is more metal nanoparticle catalysts dispersed uniformly throughout the reactants, increasing the number of active sites, and accelerating the rate of the reactions. However, the uniform distribution of the catalysts makes it more difficult to separate them after the reactions are finished. In heterogeneous catalysts, it is customary to apply transition compounds and metals onto the catalyst's surface. This creates active sites that effectively decrease the energy by adsorbing reactant molecules on the surface. Heterogeneous catalysts have been pivotal in the chemical industry for many years and are regarded as indispensable for chemical and energy conversions.

Green catalyst refers to the use, retrieval, and reprocessing of enzymes as biocatalysts. Efficient highlighting of chemicals is achieved by decreasing costs, boosting potential efficiency, minimizing environmental exposure, and enhancing overall sustainability method in the advancement of green chemistry. The use of bio-catalysts of enzymes aligns with the Green

| Regular Article | Open Access

Chemistry`s 12 principles and has the potential to be a valuable tool for developing and processing goods [5], as shown in **Fig. 1**. The enzymatic importance relies on the pace at which the bio-catalyst performs in comparison to chemical reactions, as well as the renewable sources like animals, plants, and microorganisms that are required for the production of useful enzymes. The preference of sectors like healthcare, chemical, waste water treatment, food, agriculture, textiles, pharmaceuticals, papers, bio-fuels, etc. for bio-catalysts (either complete cells or) is attributed to the significance of enzymatic properties.

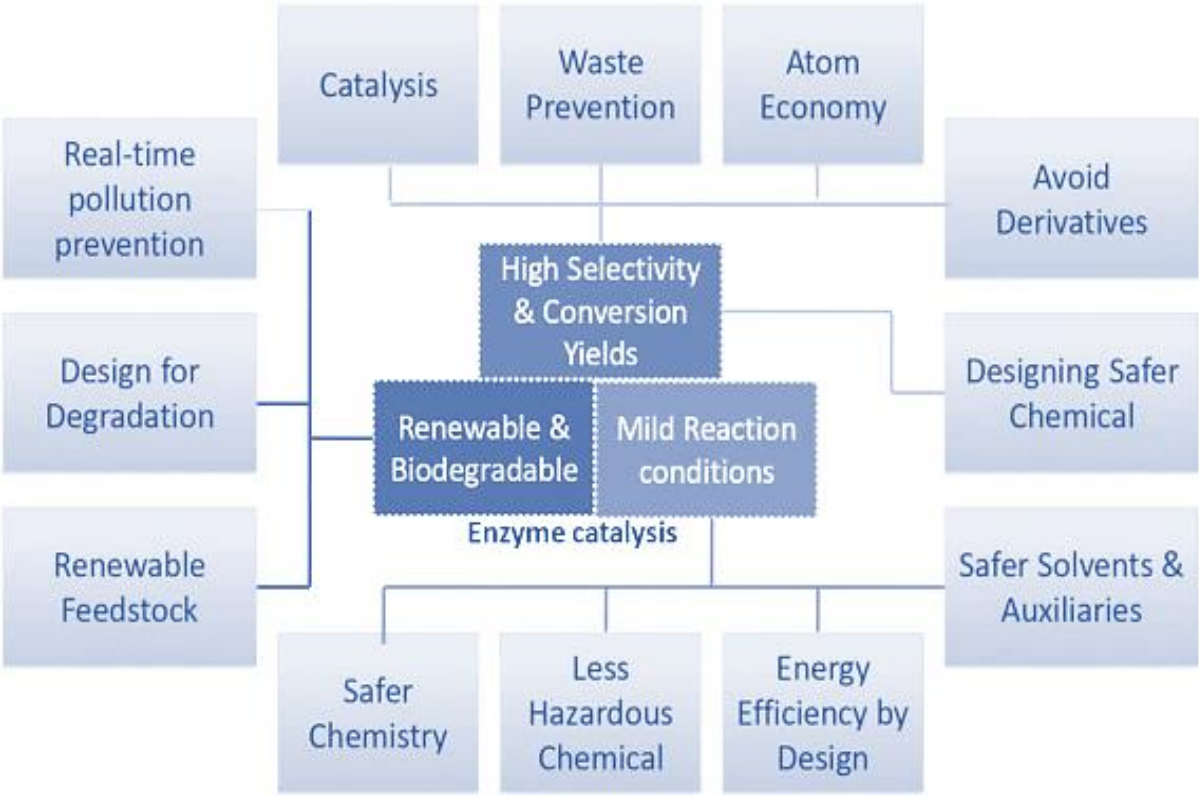

Fig 1. Examining the Relationship Between Enzymatic Bio-Catalysis and The Green Chemistry`S 12 Principles

The enzymatic technique has many advantages, including the use of gentle reaction conditions, minimal energy requirements, and reduced problems associated with isomerization and rearrangement [6]. This strategy also provides excellent specificity, activity, and selectivity, resulting in streams of purer end product. Furthermore, Bio-catalysts have the ability to greatly decrease the activation of functional groups and limit the generation of by-products by displaying chemo, region, and stereo selectivity.

This study aims to investigate how bio-catalysts may be used to improve technology for new surroundings. In order to create improved bio-catalysts as substitutes for chemical processes, the study outlines developments in computational tools for modifying the properties of enzymes. The study highlights the use of enzyme engineering approaches, namely rational design and directed evolution, which have shown promising results in improving enzyme characteristics such as reactivity, stability, and substrate specificity. Furthermore, the research investigates the intentional cultivation of intermolecular bonds and the immobilization of enzymes by various approaches. The main goal is to promote the implementation of green chemistry and sustainable techniques by using bio-catalysts in many industries, such as detergent production, healthcare, and green fuel production.

The rest of the article has been arranged as follows: Section II presents a discussion of bio-engineering and computational advancements for enzyme enhancement. Section III discusses enzyme immobilization to effectively reduce environmental stress. This section discusses key concepts such as modes of immobilization, and the present advancements made in enzymatic bio-catalysts. Section IV discusses the various industrial perspectives of enzymes in the field of green chemistry. Section V presents a summary of the research on the advancements in computational techniques for enhancing bio-catalysts in green chemistry

II. BIO-ENGINEERING & COMPUTATIONAL ADVANCEMENTS FOR ENZYME ENHANCEMENT

The use of bio-catalysts is crucial for optimizing different acknowledged technologies to function well in unfamiliar environments. Recent technological advancements have greatly enhanced the ability to foresee and provide potential approaches for direct improvement [7]. Techniques for computation are effective in manipulating the enzymatic characteristics, including flexibility, reactivity, solubility, transport, pathway, and ligand binding of the protein. This leads to the development of a valuable and an enhanced bio-catalyst that can serve as an alternative to chemical reactions. The

enzyme engineering advancement using computational methodologies and high throughput screening has greatly influenced the production of enzymes with improved characteristics, making them more suitable for green chemistry.

Conventional methods of enzyme engineering via rational design often focus on modifying amino acids that are located in or close to active sites [8]. This is done in order to modify the enzyme affinity for its substrate, enhance its selectivity, or adjust its catalytic reaction rate. Directed evolution has been very effective in producing enzymes that exhibit activity towards novel substrates, improved selectivity, and enhanced stability. This is achieved by deliberate mutations in the active site and other protein molecule regions. These procedures for enzyme engineering are quite advanced, extensively addressed in other sources, and are not within the focus of this course. This section examines recent instances where enzymes were subjected to non-mutation-based techniques to modify the local microscale/nano environment and structure, leading to kinematic parameters enhancements like as K_M and k_{cat}.

Gao et al. [9] have investigated the intermolecular interactions rational design between a nanostructured enzyme and its substrates as one of our approaches. This method drew inspiration from enzymes such as superoxide dismutase (SOD), which is one of the most rapid enzymes known. SOD utilizes electrostatic interaction between oppositely charged residues and a charged substrate on the enzyme's surface. In the SOD case, a cluster of surface residues with a positive charge guides the superoxide substrate towards the entrance of the active site tunnel. The engineering strategy by Kimber and Pai [10] replicates this phenomenon by introducing binding interactions for substrate-enzyme that occur at a distance from the active site. This leads to higher concentrations of substrate in the immediate vicinity, thereby decreasing the apparent Michaelis constant and promoting faster rates of catalysts even when the overall substrate concentration is low (see **Fig. 2**).

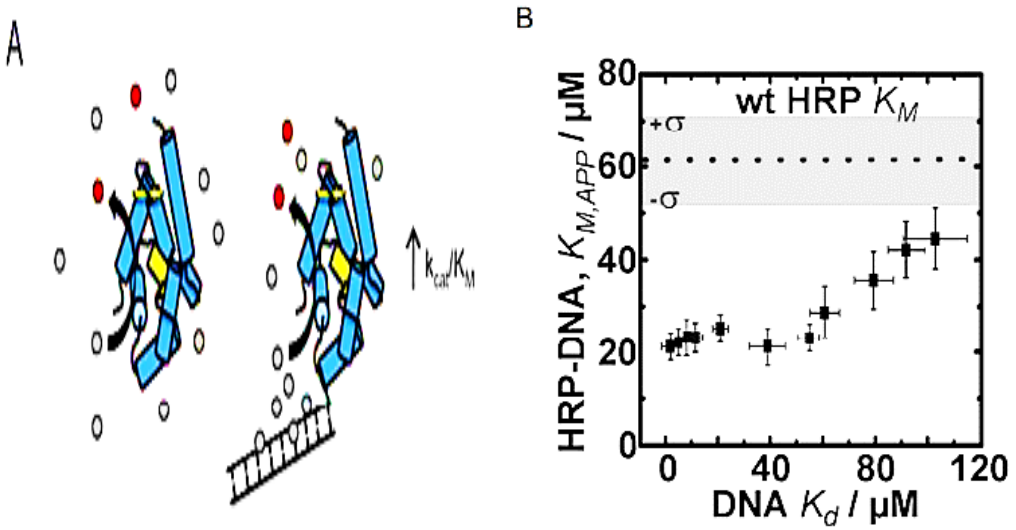

Fig 2. The Process of Enzyme Engineering by using the Deliberate Manipulation of Intermolecular Interactions. (A) an Illustrative Graphic Depicting the Methodology. Conjugating a Fragment of DNA with a Binding Affinity in the Micromolar Range to the Substrate of the Enzyme May Boost the Local Sub Strate Concentration and Improve Catalytic Performance. (B) The Correlation Between the Apparent KM Of HRP-DNA and the HRP Substrate DNA Binding TMB. The Specific Binding of TMB to DNA Sequences Enables the Formation Of HRP-DNA Nanostructures with TMB Binding Affinities That Vary Between About 1 and 100 μm

The research on protein functions, sequences, structure, evolution, and dynamics is progressing rapidly to find more efficient methods for generating novel enzyme characteristics with enhanced usefulness, as shown in [11]. **Table 1** outlines the computational tools and methodologies used to aid in the enzymes production with targeted modifications in their structure and activities. The enhanced enzyme is directly associated with a more effective strategy in developing environmentally friendly goods, taking into account the complications of time and cost.

Although there has been a significant advancement in the approach of computation to enzyme engineering, there are still significant obstacles to overcome in terms of structural modeling, macromolecular stability, and enhancing intermolecular interactions. In recent years, significant focus and effort have been directed towards tackling the issues in protein design. This has resulted in the creation of an improved design of artificial protein, which is bolstered by the interaction between the ligands and protein backbone, leading to more efficient structural design. Significant advancements have been achieved in the fields of prediction and design, despite the potential for further exploration in algorithms and supercomputers. In addition, advancements in the 'Omics' field have created chances to interact with new enzymes, bringing the chemical industry closer to achieving green synthesis.

It is advisable to use the metagenomics technique for enzyme production in order to investigate the diverse range of microorganism communities that cannot be cultured. This is important since only a small fraction (1%) of bacteria are currently targeted for the manufacture of valuable enzymes [12]. Enzymes are synthesized with the assistance of microorganisms and advanced engineering techniques, leading to their commercial viability due to their enhanced efficiency

and beneficial characteristics as bio-catalysts. The process of metagenomics enzyme discovery enables the identification and analysis of novel enzymes obtained from environmental DNA (eDNA).

Table 1. A Compilation of Computational Design Enzymes, Including Information on Their Access Tools and Modification

Enzymes	Tools	Method	Outcome
Kemp eliminase	De nova design via Rosetta	Novel catalysis	>200-fold hyper in kcat/Km
Haloalkane dehalogenase DhaA	FireProt	Phylogenetic analysis and energetic assessment	Tm (thermostability) hypered by 24.6 °C
Limonene epoxide hydrolase	Rosetta Design	Molecular dynamics and docking simulations	enantiomeric preference 93%
Mouse adenosine deaminase	Rosetta Design, Rosetta Match	Hydrolysis of a model organophosphate	Enhanced activity by ~2500-fold
Nitric Oxide reductase	VMD Software (Molecular Modelling	Reconstitute NOR active site in myoglobin	Functional Model of NOR
Transketolase	GROMACS	Simulations of molecular dynamics; coordinated motions analysis	Tm hypered by 3 °C
Adenosine Kinase	BAliPhy	Ancestral reconstruction sequence	Tm hypered by 35 °C
Diels-Alderase Guanine	Design software, Rosetta Match, and MM/QM simulations	Quantum mechanics, de novo, molecular dynamics	Functional performance matches catalytic antibodies, Stereoselective Diels-Alderase
Protozyme esterase	ORBIT	De novo designing	Catalysis advanced 180-fold
Plasma chloin esterase	AMBER and Gaussian	Molecular/mechanics/quantum mechanics, molecular dynamics	catalysis (kcat) advanced 25-fold

III. ENZYME IMMOBILIZATION TO REDUCE ENVIRONMENTAL STRESS

Enzyme immobilization [13] typically involves four methods: (1) bio-conjugation, (2) non-covalent deposition and adsorption, (3) covalent attachment, and (4) physical trapping (see **Fig. 3**).

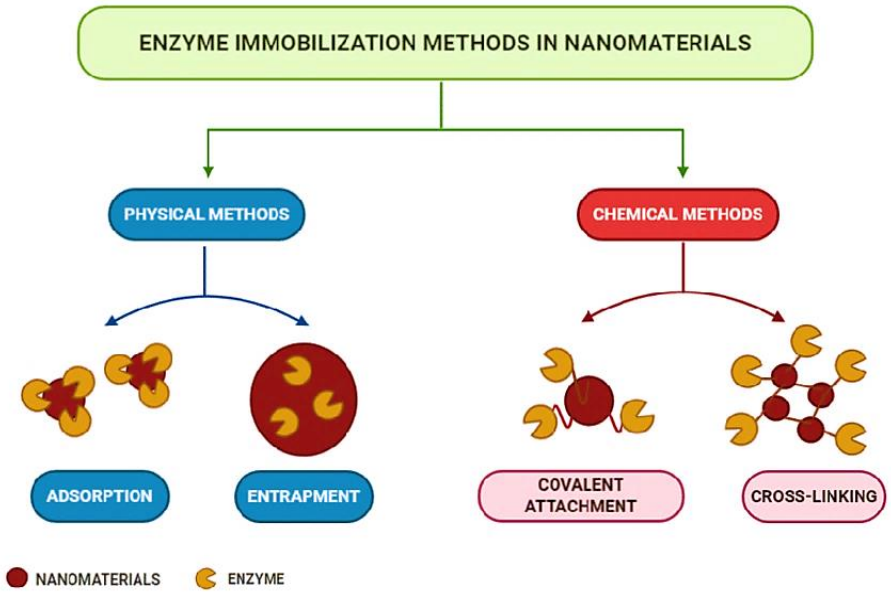

Fig 3. Techniques used for Enzyme Immobilization

Support binding may occur via chemical or physical means, which can include either covalent or weak connections. Typically, physical bonding is quite weak and lacks the ability to securely get the carrier attached with the enzyme in industrial settings. The support may consist of an inorganic polymer like silica and zeolite or a biopolymer. Entrapment refers to the process of including an enzyme into a network of polymers, like a silica sol-gel or an organic polymer, or within a membrane gadget like a microcapsule or a hollow fiber. Entrapment necessitates the polymeric network formation while the enzyme is present. The last category pertains to the enzyme crystals or aggregates cross-linking by using a bifunctional reagent to create large particles that are devoid of carriers.

Several other techniques, including novel amalgamations of the aforementioned approaches, sometimes tailored for a particular substrate or enzyme, have been devised. Nevertheless, there is no universally optimal approach or support for all

enzymes and their many uses. The reason for this is the significant variations in the enzymes composition and chemical features, the distinct qualities of products and substrates, and the many applications of the result. Nevertheless, each of the strategies may have various benefits and limitations. Adsorption is a cost-effective and efficient process, but it often has the drawback of being reversible. On the other hand, cross-linking and covalent attachment methods are very successful and long-lasting, but they may be costly and have the potential to negatively impact enzyme function. Diffusional issues are micro-encapsulation, micro, and inherent in membrane reactor-confinement techniques.

Modes of Immobilization

Protein Adsorption

Protein immobilization techniques that do not entail the sharing of electrons (non-covalent methods) are often used [14]. These approaches include either the protein passively sticking to hydrophobic surfaces or interacting with charged surfaces via electrostatic forces. Electrostatic binding using nitrocellulose membranes or polylysine-coated slides is well recognized and often used. As previously mentioned, the primary benefit of this kind of immobilization is that it does not need the use of extra coupling chemicals or alteration of the protein being studied. Nevertheless, non-covalent immobilization often entails interactions that are quite weak and may be easily reversed. Consequently, proteins might seep out from the support, leading to a decline in surrounding medium contamination and activity over time. These consequences affect the overall durability and ability to be reused of systems, especially when used in sensing gadgets and analytical assays. It is well recognized that when proteins adhere to surfaces, they often undergo structural alterations and denaturation, which may cause significant reductions in protein function. Moreover, the lack of control over the immobilized proteins packing density might lead to a further decrease in their activity due to huge crowding.

Protein Immobilization by Absorption on Mesoporous Silicates

Over the last ten years, significant efforts have been made to create mesoporous silicates and to attach enzymes to these materials. Avcı [15] identified a subclass of mesoporous silicates characterized by a limited range of pore diameters, amorphous silica surfaces, and pore sizes ranging from 20 to 300 Å. The introduction of these novel, periodic mesoporous structures presented the potential to adsorb or encapsulate sizable molecules inside their pores. Since their inception, these materials have offered the potential for enhancing catalytic studies using immobilized enzymes. The expectation was that mesoporous silicates would offer a secure and isolated ecosystem that would facilitate reactions with certain substrates. Mesoporous silicates have been created utilizing block copolymer, neutral, and cationic surfactants after their first discovery. These copolymers consist of organic functional groups and metals that are either embedded in their structure or attached to their surface. They have been used as a framework to create mesoporous materials of carbon.

Mesoporous silicates exhibit many further characteristics that provide them appealing options for the immobilization of proteins. One may chemically alter the surfaces of the mesoporous silicate support by attaching different functional groups, which allows for electrostatic repulsion or attraction between the support and the desired biological molecule. Mesoporous silicates possess a silicate inorganic foundation, which grants them both chemical and mechanical stability, making them very resistant to microbial assault.

Silica sol-gels, which are materials with comparable stability to mesoporous silicates, have been used in the protein's encapsulation for the purpose of biosensor advancement. Nevertheless, sol-gels are hindered by the drawback of exhibiting a significantly fluctuating range of pore sizes, ranging from 10 to 400 Å [16]. Significantly, their production may include the use of reagents or severe temperatures, which might induce protein denaturation and adversely affect enzyme function. The use of mesoporous silicates enables protein encapsulation to take place subsequent to the manufacture of the support, so circumventing this challenge.

Protein Immobilization on Polyketone Polymer by Hydrogen Bonds

A novel immobilization technique has recently been suggested, using a polyketone polymer as a fully original substrate. The polyketone polymer, formed by the copolymerization of carbon monoxide and ethane, has been used to immobilize three distinct enzymes [17]. These enzymes include a peroxidase from horseradish, as well as two amine oxidases from lentil seedlings and bovine serum. The straightforward immobilization method was conducted in a diluted aqueous solution, delicately blending the proteins with the polymer. The immobilization process does not involve spacer arms or bi-functional agents. Instead, it relies only on hydrogen bonds multitude formed between the polymer`s carbonyl chains and the polypeptidic chains –NH units (see **Fig. 4**).

Classical Covalent Immobilization Methods

To obtain a long-lasting fixation, it is crucial to form covalent connections, which are typically formed by interacting with functional groups present on the protein's surface. These methods, which rely only on naturally existing functional groups, may be employed for unmodified proteins, much as non-covalent adsorption. As an example, the lysine residues` amine groups that are exposed react easily with help that have active esters. The most often used active ester is N-hydroxy succinimide (NHS) esters [18]. This reaction leads to strong amide bonds formation that are stable. Nevertheless, a drawback of using NHS esters is their susceptibility to instability in aqueous environments. Consequently, proteins bonding in aqueous buffers will contend with hydrolysis of ester, potentially leading to a relatively low immobilization yield. On the other hand,

a strong primary amine bond could be formed by connecting aldehyde groups to amino groups that are susceptible to reduction with sodium cyanoborohydride or a comparable reagent.

The amine group nucleophilicity enables it to react with materials that have been functionalized with epoxides, such as ethers of diglyceryl. These epoxides possess the benefit of exhibiting comparatively high resistance to hydrolysis under neutral pH conditions, facilitating convenient material manipulation. However, this characteristic may lead to sluggish or incomplete coupling. Additionally, it is crucial to utilize supports that possess a high concentration of epoxy groups. These supports should be highly stable to facilitate prolonged enzyme-support reactions. Furthermore, it is advantageous to have spacer arms that are as short as possible, particularly in the epoxy supports third generation, in order to effectively immobilize the structure of the enzyme. The use of novel heterofunctional supports or the exploration of alternative applications for the aforementioned supports might accelerate the adoption of several industrial bioprocesses, which are now hindered by the absence of appropriate biocatalysts.

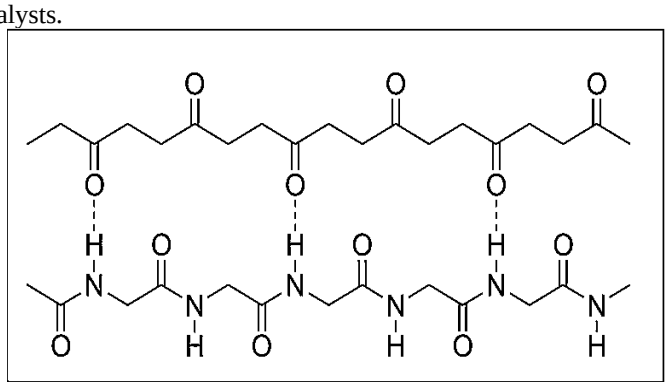

Fig 4. Connection Between the-NH Groups and Carbonyl Groups of the Polypeptide Chain Via Hydrogen Bonding

Experiments confirm that amine oxidases possess a very high affinity for binding and display extraordinary durability when immobilized. Although there is a little increase in the KM value, activity measurements confirm that immobilized enzymes retain all of their catalytic activities. A packed bed was activated a peroxidase-activated polymer in an enzymatic reactor to assess hydrogen peroxide solutions by flow injection and continuous flow conversion.

Cysteine residues, which contain the thiol group, are often used for immobilization of protein [19]. They quickly react with unsaturated carbonyls (such as maleimides) by conjugate addition to create stable thioether linkages. Evidence demonstrates that maleimide groups have a considerable preference for conjugate summation with thiols at physiological pH (6.5–7.5). This preference arises because, under these circumstances, amines are mostly protonated and hence unreactive (see **Fig. 5**). Due to the limited number of cysteine residues on the protein surface, it is feasible to achieve specific immobilization at a particular site. This is particularly achievable if the interest protein can be modified to eliminate all but one cysteine sediment on the surface or introduce a single cysteine in a location where there was none before. The thiol group's nucleophilicity enables it to undergo reactions with epoxides and NHS esters. However, it should be noted that the reaction with NHS esters is somewhat sluggish in practical terms. Additionally, the resulting thioester moiety is prone to hydrolysis.

The standard strategy for immobilizing aspartic and glutamic acid residues includes turning them into active esters using an auxiliary nucleophile and a carbodiimide coupling agent. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide is a frequently used example of the former, whereas NHS is frequently used to create the NHS ester on the protein. Afterwards, this reactive ester might react with supports that contain amines. An advantage of using this particular mix of chemicals is that they possess solubility in water and may be effectively used in aqueous solutions. It is worth mentioning that carbodiimides and the resultant active esters are not very stable in such conditions, leading to low reaction yields. Moreover, there is a possible risk that the esters of NHS produced on the molecule of protein may later attach to other molecules of protein, leading to the creation of uncertain polymers.

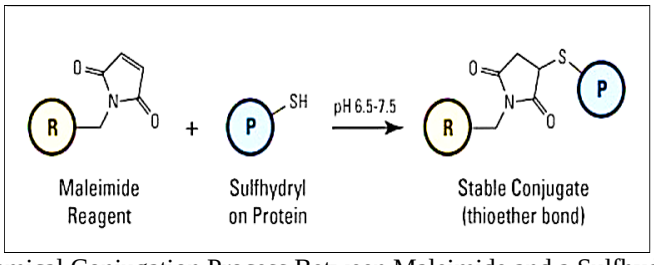

Fig 5. Chemical Conjugation Process Between Maleimide and a Sulfhydryl Group

Oxidation, often with periodate, is used to break down the 1,2-diols on the oligosaccharides, resulting in the formation of aldehyde groups. The aldehyde groups may be specifically linked to supports that have been functionalized with hydrazine or hydroxylamine, forming hydrazone or oxime connections, respectively. This methodology has been expanded to include several proteins that undertake post-translational glycosylation, like oxidase and protease enzymes, in addition to antibodies.

However, it is crucial to recognize excluding the many capable attachment sites on a polysaccharide chain, random orientations could happen if the target protein has several glycosylation sites on its surface.

There is now increasing interest in several methods of selective immobilization that may be performed under mild physiological conditions. Typically, these methods rely on covalently bonding an azide moiety to the target protein. The Staudinger ligation is a chemical process where an azide and a phosphine combine to form an intermediate iminophosphorane (aza-ylide). Subsequently, this intermediate may react with electrophiles to generate a diverse range of compounds. The generation of an iminophosphorane may lead to its subsequent interaction with an ester, eventually leading in the production of a durable amide bond. In the first phase of this procedure, the electrophilic ester undergoes a reaction with the phosphine, leading to the formation of a final product in which the phosphine oxide is connected to the bond.

The Staudinger ligation has been widely used in several applications, including as protein and cell-surface glycan labeling, as well as chemical protein synthesis [20]. Over the past few years, the Staudinger ligation has also been used for the proteins and peptides immobilization. In each case, a sequential process was needed: first, the protein of interest was tagged with the azide, followed by the immobilization reactions. Hence, to accomplish immobilization that is unique to a certain location, it is essential to use a method that selectively introduces the appropriate azide functional group, like an enzymatic approach that precisely identifies certain protein sequences. Therefore, the first scheme is to include the sequence of DNA that encodes the tag next to the gene responsible for producing the desired protein. Eventually, the genetically engineered synthetic gene is activated, leading to the synthesis of a fusion protein. The fusion protein is composed of the primary interest protein connected to a tagging peptide or protein that includes the site of attachment. Afterwards, this hybrid protein is used for the procedure of immobilization.

An additional approach for selective immobilization involves the use of azides and the Huisgen 1,3-dipolar in addition to an alkyne [21]. The reaction leads to the creation of a covalent bond by the production of a 1,4-substituted 1,2,3-lithium molecule. The reaction, referred to as "click" chemistry, employs Cu(I) catalysts to achieve near-complete conversion of the terminal alkyne and azide into a triazole molecule. The occurrence of the alkyne unit in biological pathways is infrequent, which enhances the adaptability of this reaction since the alkyne has the potential to be incorporated into the biomolecule instead of the azide. The use of this kind of "click" chemistry has been applied in diverse scenarios to facilitate the bonding of biomolecules that include either alkyne or azide functional units to various polymers, fluorophores, or biological identifiers that have been altered with their corresponding chemical groups. Similar to Staudinger chemistry, the precise binding of proteins also needs the use of an enzyme approach that specifically labels the desired location.

Another category of reactions is referred to as "photoclick chemistry," which employs photoirradiation to induce the production of pyrazolines from alkenes and tetrazoles. An additional benefit of this approach is that the freshly formed heterocycle exhibits fluorescence, allowing for the monitoring of the progress of the reactions. Even though these developments are fascinating, they are currently not used for immobilization of protein (**Figs. 6** and **7**).

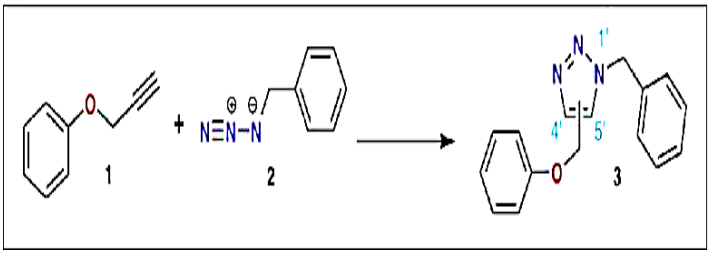

Fig 6. The Alkyne (1) Undergoes a “Click” Chemical Reaction with Azide (2) to Produce a 1,4-Disubstituted 1,2,3-Triazole Compound

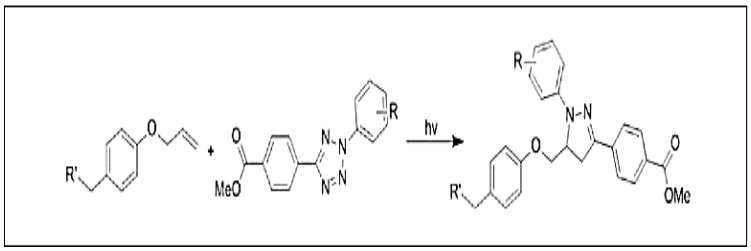

Fig 7. The Reaction between Alkenes and Tetrazoles, Known as “Photoclick Chemistry,” Results in the Formation of Pyrazoline

Advancements in Enzymatic Bio-Catalysts

In [22], the enzymatic bio-catalyst is essential for the advancement of reliable processes, like green catalysts. These catalysts enable reactions to occur at lower temperatures and pressures, leading to more productive, efficient, selective, and specific process flow. In addition, immobilizing the enzymes reduces the risk of contamination of product by bio-catalyst filtrates and allows for the use of small reaction volumes. Nevertheless, it is crucial to comprehend the material qualities as they serve as the foundation for immobilization of enzyme. These features should include insolubility in the reaction media, as well as porosity, stability, and chemical resistance. The carrier must be compatible with the enzyme's features in order to

optimize the production of bio-catalysts and ensure their requisite efficiency in every reaction media. Various scientific literature has several suggested methods that showcase immobilization approaches for biological, inert, and cross materials, resulting in the production of more dynamic and stable bio-catalysts.

Currently, there is a field called nano-biocatalysis that focuses on using nanoparticles to immobilize enzymes. Research has explored the use of nanocarriers, nano capsules, and nanoparticles to create unique nano-biocatalysts, leading to the development of promising structures using various techniques. Although bio-catalysts of this kind possess high specificity and align properties, they are significantly limited by their extensive exposure to the reaction liquid and the difficulty in creating a suitable microenvironment inside the porous structure. However, several carriers consisting of core-shell polymer were generated. Particles of polymer generated from the emulsion polymerizations and concurrent suspension provide very effective characteristics for synthesizing materials on a small scale. This area focuses on generating specialized materials that may be effectively linked to the examined enzymes in order to act for the bio-catalysts with possible results.

IV. INDUSTRIAL PERSPECTIVES OF ENZYMES IN GREEN CHEMISTRY

The adoption and implementation of green chemistry concepts have become a global strategy for achieving sustainable growth [23]. Considerable effort must be dedicated to developing novel processes that include non-polluting materials, therefore preventing the emission of pollutants into the atmosphere. Developing novel, more secure techniques and discovering less detrimental goods may provide a formidable task. The motivation for exploring other energy sources, such as biomasses, stems from the rise in oil costs. These alternatives provide both cost-effectiveness and environmental sustainability. Additionally, the push from the public to favor green technology is growing. People grow increasingly mindful of the environment and a want of substituting chemical processes with eco-friendly biocatalytic reactions may be recognized.

Enzymes have immense promise for scientists in the realm of green chemistry. Enzymes are intrinsic biological catalysts found in all living organisms [24]. Enzymes, acting as catalysts, need gentle conditions, making them an excellent means of conserving resources like energy and water. Enzyme-based processes are economically viable due to their cost-effectiveness and environmentally favorable nature. Enzymes are increasingly pervasive in many facets of civilization. They are used in textile, paper, and detergent manufacturing, as well as in food processing, healthcare, and pharmaceutical, where they are subjected to exploitation. They may be employed for synthesis in non-aqueous media. The use of enzymes or whole cells in the manufacture of biological and chemical substances has been shown to result in reduced process time, little waste generation, and fewer reaction steps. Considering the green chemistry advantages, it is evident that enzyme biocatalysis would swiftly supplant conventional chemical processes in several domains. The expansion of this replacement will include more sectors due to the emergence of new technologies in enzyme engineering.

The growing apprehensions about chemical consumption and the industrialization of green technologies have resulted in a significant demand for goods made using bio-catalysts to ensure a sustainable and environmentally friendly future. **Table 2** below illustrates a range of commercial products that have been produced using enzymatic bio-catalysis, specifically for use in green chemistry.

Table 2. Displays Sustainable Items That Have Been Produced Using a Green Approach with the Assistance of a Bio-Catalyst

Product	**Green Strategy**	**Outcome**
Bio-detergent	Using enzymes such as proteases, lipases, cellulases, and amylases	• An eco-friendly substitute for detergents that lack biodegradability • Enhances efficiency and cleaning of the final product because of the hydrolytic degradation
Bio-Insecticides	The enzyme chitanase may be used as a biological pest control agent. Protease enzyme act as insect reproductivity natural inhibitor Lipases are accountable for lipids fractionation on the insect surface resulting into control of pest	• Easy production • Being safe for non-target organisms • Cost effectiveness • Ecosystem friendly
Bio-lubricants	Alternative to derivatives of petroleum	• Decreases the acid wastewater • Reaction temperature is lowered • Greater selectivity • Usually does not produce the unwanted byproduct that corrodes equipment
Green fuels	Lipases enzyme for transesterification and esterification reaction for production of bio-fuel forming high-purity products	• Conditions for milder reaction • Prevention of oxidation of oil • Low cost of energy

The products shown above are the result of enzymatic catalysis, which highlights the importance of biotechnology in promoting environmentally-friendly practices and creating sustainable products and processes. The usage of bio-catalysts in

the chemical revolution enables the creation of a habitat friendly ecosystem and improves processes by implementing environmentally benign chemical practices, in accordance with global requirements and the tenets of green chemistry.

V. CONCLUSION

The research highlights the importance of bio-catalysts in improving technology for unexplored domains. The use of computational tools has greatly enhanced the ability to modify enzyme characteristics, leading to the development of enhanced bio-catalysts that may serve as alternatives to chemical processes. Enzyme engineering, accomplished via the methods of rational design and controlled evolution, has shown to be effective in improving the characteristics of enzymes for the aim of eco-friendly chemical applications. Encouraging outcomes have been seen via the intentional manipulation of intermolecular interactions and the use of metagenomics techniques in the development of enzymes. Various immobilization techniques, including as non-covalent adsorption, physical trapping, covalent attachment, and bio-conjugation, have specific benefits and limitations. Selective immobilization techniques, such as Staudinger ligation and Huisgen 1,3-dipolar cycloaddition, may be used under mild physiological conditions. Studies have also explored the use of nanoparticles as transporters for enzymes in the realm of nano-biocatalysis. Overall, the implementation of green chemistry concepts and the use of bio-catalysts have the potential to replace conventional chemical methods in several sectors, leading to more sustainable and environmentally aware practices.

CRediT Author Statement
The author reviewed the results and approved the final version of the manuscript.

Data Availability
The datasets generated during the current study are available from the corresponding author upon reasonable request.

Conflicts of Interests
The authors declare that they have no conflicts of interest regarding the publication of this paper.

Funding
No funding was received for conducting this research.

Competing Interests
The authors declare no competing interests.

References

[1]. "Technology and industrial progress: the foundations of economic growth," Choice Reviews Online, vol. 33, no. 07, pp. 33–4028, Mar. 1996, doi: 10.5860/choice.33-4028.
[2]. J. M. Reilly, "Green growth and the efficient use of natural resources," Energy Economics, vol. 34, pp. S85–S93, Nov. 2012, doi: 10.1016/j.eneco.2012.08.033.
[3]. F. Qureshi et al., "Latest eco-friendly avenues on hydrogen production towards a circular bioeconomy: Currents challenges, innovative insights, and future perspectives," Renewable & Sustainable Energy Reviews, vol. 168, p. 112916, Oct. 2022, doi: 10.1016/j.rser.2022.112916.
[4]. D. Astruc, F. Lu, and J. R. Aranzaes, "Nanoparticles as Recyclable Catalysts: The Frontier between Homogeneous and Heterogeneous Catalysis," Angewandte Chemie International Edition, vol. 44, no. 48, pp. 7852–7872, Dec. 2005, doi: 10.1002/anie.200500766.
[5]. P. T. Anastas and N. Eghbali, "Green Chemistry: Principles and practice," Chemical Society Reviews, vol. 39, no. 1, pp. 301–312, Jan. 2010, doi: 10.1039/b918763b.
[6]. J. Gao, S. Ma, D. T. Major, K. Nam, J. Pu, and D. G. Truhlar, "Mechanisms and free energies of enzymatic reactions," Chemical Reviews, vol. 106, no. 8, pp. 3188–3209, Jul. 2006, doi: 10.1021/cr050293k.
[7]. C. D. Anobom et al., "From structure to catalysis: Recent developments in the biotechnological applications of Lipases," BioMed Research International, vol. 2014, pp. 1–11, Jan. 2014, doi: 10.1155/2014/684506.
[8]. S. Lutz, "Beyond directed evolution—semi-rational protein engineering and design," Current Opinion in Biotechnology, vol. 21, no. 6, pp. 734–743, Dec. 2010, doi: 10.1016/j.copbio.2010.08.011.
[9]. Y. Gao, C. A. Roberts, J. Zhu, J.-L. Lin, C. A. Chang, and I. Wheeldon, "Tuning Enzyme Kinetics through Designed Intermolecular Interactions Far from the Active Site," ACS Catalysis, vol. 5, no. 4, pp. 2149–2153, Mar. 2015, doi: 10.1021/acscatal.5b00130.
[10]. M. S. Kimber and E. F. Pai, "The active site architecture of Pisum sativum beta -carbonic anhydrase is a mirror image of that of alpha -carbonic anhydrases," The EMBO Journal, vol. 19, no. 7, pp. 1407–1418, Apr. 2000, doi: 10.1093/emboj/19.7.1407.
[11]. D. Petrović, V. A. Risso, S. C. L. Kamerlin, and J. M. Sanchez-Ruiz, "Conformational dynamics and enzyme evolution," Journal of the Royal Society Interface, vol. 15, no. 144, p. 20180330, Jul. 2018, doi: 10.1098/rsif.2018.0330.
[12]. P. Turner, G. Mamo, and E. N. Karlsson, "Potential and utilization of thermophiles and thermostable enzymes in biorefining," Microbial Cell Factories, vol. 6, no. 1, Mar. 2007, doi: 10.1186/1475-2859-6-9.
[13]. Homaei, R. Sariri, F. Vianello, and R. Stevanato, "Enzyme immobilization: an update," Journal of Chemical Biology, vol. 6, no. 4, pp. 185–205, Aug. 2013, doi: 10.1007/s12154-013-0102-9.
[14]. L. S. Wong, F. N. Khan, and J. Micklefield, "Selective covalent protein Immobilization: Strategies and applications," Chemical Reviews, vol. 109, no. 9, pp. 4025–4053, Jul. 2009, doi: 10.1021/cr8004668.
[15]. Avcı, "Synthesis, characterization and pore size control of mesoporous li4ti5o12, cotio3 and mntio3 thin films," 2014. [Online]. Available: http://repository.bilkent.edu.tr/handle/11693/28920
[16]. Stein and R. C. Schroden, "Colloidal crystal templating of three-dimensionally ordered macroporous solids: materials for photonics and beyond," Current Opinion in Solid State & Materials Science, vol. 5, no. 6, pp. 553–564, Dec. 2001, doi: 10.1016/s1359-0286(01)00022-5.

[17]. E. Agostinelli et al., "Polyketone polymer: A new support for direct enzyme immobilization," Journal of Biotechnology, vol. 127, no. 4, pp. 670–678, Jan. 2007, doi: 10.1016/j.jbiotec.2006.08.011.

[18]. E. J. F. Demant, P. B. Jensen, and M. Sehested, "Characterization of the cooperative cross-linking of doxorubicin N-hydroxysuccinimide ester derivatives to water soluble proteins," Biochimica Et Biophysica Acta (BBA) - Proteins and Proteomics, vol. 1118, no. 1, pp. 83–90, Dec. 1991, doi: 10.1016/0167-4838(91)90444-5.

[19]. M. Frasconi, F. Mazzei, and T. Ferri, "Protein immobilization at gold–thiol surfaces and potential for biosensing," Analytical and Bioanalytical Chemistry, vol. 398, no. 4, pp. 1545–1564, Apr. 2010, doi: 10.1007/s00216-010-3708-6.

[20]. R. S. Loka, C. M. Sadek, N. A. Romaniuk, and C. W. Cairo, "Conjugation of synthetic N-Acetyl-Lactosamine to Azide-Containing proteins using the Staudinger ligation," Bioconjugate Chemistry, vol. 21, no. 10, pp. 1842–1849, Sep. 2010, doi: 10.1021/bc100209r.

[21]. M. S. Singh, S. Chowdhury, and S. Koley, "Advances of azide-alkyne cycloaddition-click chemistry over the recent decade," Tetrahedron, vol. 72, no. 35, pp. 5257–5283, Sep. 2016, doi: 10.1016/j.tet.2016.07.044.

[22]. Sharma and S. K. Arya, "Bio-catalysis as a green approach for industrial waste treatment," in Nanotechnology in the life sciences, 2020, pp. 359–405. doi: 10.1007/978-3-030-44176-0_14.

[23]. T.-L. Chen, H. Kim, S.-Y. Pan, P.-C. Tseng, Y.-P. Lin, and P. Chiang, "Implementation of green chemistry principles in circular economy system towards sustainable development goals: Challenges and perspectives," Science of the Total Environment, vol. 716, p. 136998, May 2020, doi: 10.1016/j.scitotenv.2020.136998.

[24]. N. S. Punekar, ENZYMES: catalysis, kinetics and mechanisms. 2018. doi: 10.1007/978-981-13-0785-0.