

Biocatalysis and Enzyme-Mediated Organic Reactions

Pooja

Email - poojadihiya459@gmail.com

ABSTRACT

The use of enzymes as catalysts is almost as old as history of humans. In academic and industrial research, enzymes are utilised extensively, especially to speed up organic syntheses. Under environmentally safe conditions, they can catalyse reactions enantioselectively and regioselectively. Enzymes are recognised to be proteins that only act as catalysts in biological reactions. However in recent years enzymes are proven to be stable in organic solvents at extreme pH and sustain high temperature and pressure. Enzymes are now easily accessible in both pure and whole cell forms.

INTRODUCTION

Enzymes are globular proteins that naturally speed up biological reactions by acting as catalysts. Approximately 4000 biological processes are known to be catalysed by enzymes. Enzymes, like all catalysts, function by significantly boosting the pace of a reaction by lowering its activation energy (E_a or ΔG). One or more polypeptides arranged in a certain three-dimensional structure by interactions between the functional groups on the components of amino acids make up enzymes. Vanderwaal's forces, hydrogen bonds, ionic bonds, and covalent bonds are some of these interactions. High substrate specificity, specificity in encouraging a single biochemical reaction with their substrate that guarantees the synthesis of a particular bimolecular product without the formation of byproducts, stereospecificity, and regiospecificity, which they express in catalysis, are some of the remarkable characteristics of the enzymes. Enzymes are necessary for nearly every biological cell process to proceed at a high rate. The assortment of enzymes produced in a cell dictates which metabolic pathways take place there since enzymes are selective for their substrates and only accelerate a small number of events out of many possible ones. Chemical catalysts and biocatalysis, often known as enzyme catalysis, function according to the same scientific principles. All enzyme function may be explained by logical chemical and physical principles, as demonstrated by the existence of numerous enzyme models, such as oligopeptidic or polypeptidic catalysts. Enzymes, however, have the ability to produce exceptional and exceptional reaction circumstances, such as very low pKa values or a large positive potential for a redox metal ion. It has been discovered that enzymes can catalyse nearly any organic chemical reaction.³ Enzyme catalysis is still gaining traction and has a position alongside chemocatalysis in the toolkit of process chemists.⁴ The use of biocatalysts—both whole-cell systems and isolated enzymes—to aid in the synthesis of complex compounds of economic interest is growing. However, there is currently a lot of interest in using biocatalysis to develop new ways to reduce the price of chemicals.

Over the past ten years, there has been a growth in the use of such "biocatalytic reagents" to carry out organic transformations because of the great properties of enzymes as catalysts. This technology represents a "green chemistry" approach and is becoming more and more popular for industrial applications because the transformations are often carried out at extremely mild reaction conditions and ambient temperature.

This is a summary of bio-catalysis and enzymes.

The breakdown of meat by stomach secretions and the transformation of starch into sugars by saliva and plant extracts were recognised as early as the late 18th and early 19th centuries. But the process by which this happened was still unknown.⁸

The name "enzyme," derived from the Greek word $\epsilon\nu\upsilon\mu\omicron\nu$, "in leaven," was originally used to describe this activity in 1877 by German scientist Wilhelm Kuhne (1837–1900). Later, nonliving materials like pepsin were referred to as enzymes, whereas chemical activity generated by living things was referred to as ferment.

Eduard Buchner published the first study on the fermentation of sugar by yeast extracts devoid of active yeast cells in 1897. He discovered that the sugar fermented even in the absence of active yeast cells in a series of experiments conducted at the University of Berlin. He called the enzyme "zymase" that caused the fermentation of sucrose. He was awarded the 1907 Chemistry Nobel Prize "for his biochemical research and his discovery of cell-free fermentation."
The evolution of biotransformation from antiquity to the twenty-first century is seen here.

Advantages of enzymes:

1. Enzymes are highly effective catalysts:

The speeds of enzyme-mediated processes are 10⁸–10¹⁰ times faster than those of nonenzymatic reactions.

2. Enzymes are safe reagents for the environment.

3. Enzymes function in moderate environments:

Enzymes function between pH 5 and 8 and between 20 and 40 °C. This reduces the likelihood of unfavourable side effects. Some thermostable enzymes, on the other hand, are capable of exhibiting their catalytic behaviours even at elevated temperatures.

4. Enzymes work well together:

Because enzymes often operate under the same or comparable conditions, multiple biocatalytic processes can be carried out in a reaction cascade in a single reactor.

5. Enzymes are not restricted to their original function:

A wide range of artificial substrates can be catalysed by enzymes, and they are frequently not needed to function in water.

6. A wide range of processes can be catalysed by enzymes.

7. Enzymes exhibit selectivity:

Chemoselectivity, regioselectivity, and stereoselectivity are the three main categories of selectivity. (enantioselectivity and diastereoselectivity).

8. An important source for environmentally friendly chemicals

Classification of enzymes

Based on the kind of chemical reaction they catalyse, enzymes are categorised into six families by the International Union of Biochemistry and Molecular Biology (IUBMB) Nomenclature Committee (Anonymous 1984). The Enzyme Commission (EC) of the IUBMB assigns a four-digit number to each enzyme: the first represents the family; the second represents the subclass within a family and is related to the type of chemical group upon which it acts; the third represents a subgroup within a subclass and is related to the specific chemical groups involved in the reaction; and the fourth represents the correlative number of identification within a subgroup.¹⁴ Enzymes are divided into six main types based on the kinds of reactions they catalyse.

Oxidoreductases.

enzymes that catalyse oxidation/reduction processes involving the transfer of atoms of hydrogen, oxygen, or electrons. Dehydrogenases, which oxidise a substrate by transferring hydrogen atoms to a coenzyme (NAD⁺, NADP⁺, FAD⁺, FMN) that acts as an acceptor, are among the 22 subclasses of oxido-reductases that are technologically significant. The key metabolic pathways of the cell involve oxidoreductases. They are exclusively intracellular and need coenzymes.

Transferases.

enzymes that facilitate the movement of a functional group from a donor to an appropriate recipient. Depending on the chemical makeup of the group being transferred, transferases can be divided into nine subgroups. These enzymes are essential for cell metabolism; methyltransferases, acyltransferases, transaminases, phosphotransferases, and glycosyltransferases are especially important. Transferases are only found inside cells and need coenzymes.

Hydrolases.

Hydrolysis, or the breakage of a chemical bond by the action of water, is catalysed by enzymes. Depending on the kind of susceptible bond, hydrolases can be divided into 12 categories. Because they provide the cell with assimilable nutrients, these enzymes are important for catabolism. This family includes the majority of technologically significant enzymes, including the well-known esterases, proteases, and glycosidases. Since many hydrolases are extracellular and resilient enough to endure challenging process conditions, the majority do not require coenzymes. The reverse reactions of bond creation with water removal can be catalysed by hydrolases under the right circumstances; these reactions have significant technological promise.

Lyases.

Enzymes catalyse non-oxidative and non-hydrolytic chemical bond cleavage processes. Based on the kind of susceptible bond, they are separated into seven subgroups: C-C, C-O, C-N, C-S, C-X (halides), P-O, and other bonds. This family of enzymes carries out several metabolic tasks linked to both biosynthesis and cell catabolism by working in reverse. Among lyases, hydratases and dehydratases, carboxylases and decarboxylases, and aldolases—which typically function in reverse processes of C-C bond formation—are prominent.

Isomerases.

Enzymes catalyse processes that change a substrate into its isomer. Depending on the kind of isomer generated, isomerases can be divided into six subgroups: racemases and epimerases, cis-trans-isomerases, intramolecular oxidoreductases, intramolecular transferases (mutases), intramolecular lyases, and miscellaneous isomerases. The majority of isomerases are intracellular, and some of them don't need organic coenzymes or cofactors. Technologically, very few isomerases are being used.

Ligases.

Enzymes catalyse the covalent bonding of two molecules. These enzymes, commonly known as synthetases, are in charge of cell anabolism and, as such, play a crucial part in the synthesis reactions that take place inside the cell. Depending on the kind of bond produced, ligases can be divided into six subgroups: C-O, C-S, C-N, C-C, phosphoric esters, and C-metal. The frequency of utilisation of specific enzymes in organic/bio transformations is displayed in Fig. 1.1. This figure shows that lipases have been used increasingly frequently as catalysts to propel the changes.

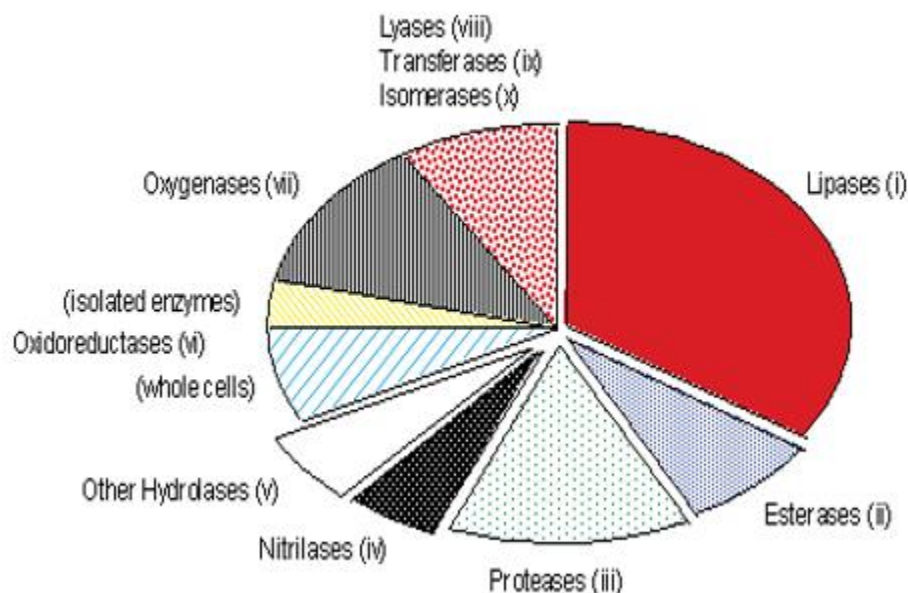


Fig.1. Frequency of use of enzymes in biotransformations; (i) Ester formation, -aminolysis,

-hydrolysis; (ii) ester hydrolysis; (iii) ester and amide hydrolysis, peptide synthesis; (iv) nitrile hydrolysis; (v) hydrolysis of epoxides, halogens, phosphates, glycosylation; (vi) reduction of aldehydes, ketones and enoates; (vii) biohydroxylation, sulfoxidation, epoxidation, Baeyer-Villiger oxidation, dihydroxylation; (viii) cyanohydrin formation, acyloin and aldol reaction; (ix) glycosyl transfer; (x) Claisen-type rearrangement, isomerization of carbohydrates, racemization and epimerization.

Sources and production of enzymes

It is possible to extract biologically active enzymes from any living thing. Commercial enzyme manufacturing uses a highly diverse spectrum of sources, from spinach to snake venom and from Actinoplanes to Zymomonas. More than half of the roughly 100 enzymes used in industry come from fungi and yeast, more than a third from bacteria, while the remaining 4% come from plants and 8% from animals.⁴ Plant, animal, and microbial enzymes are the three main sources of enzymes.

Plant Enzymes: These are essentially plant-derived enzymes. These include the well-known proteases such ficin, papain, and bromelain as well as the cereal amylolytic enzymes, soybean lipoxygenase, and specialised citrus fruit enzymes. The

majority of plant enzymes are sold as relatively unrefined powder extracts, with the exception of papain, which was just recently made available as a stabilised and refined liquid.

Animal Enzymes: Animal glands are the primary source of these enzymes. These include lipases, rennets, pancreatic trypsins, and other enzymes like pepsin that are produced in both ultra pure and industrial bulk quantities.

Microbial Enzymes: Microorganisms manufacture about 90% of all enzymes. A variety of bacteria, yeast, and fungi have been tested for their ability to produce enzymes.

Microorganisms are the source of the majority of enzymes utilised in industry.

Enzyme production

Fermentation is the process that produces enzymes. This is one of the most popular techniques for making enzymes from microorganisms. Both solid and liquid fermentation methods have been applied. Solid fermentation is an antiquated method that is still utilised in Japan to make proteases, acylases, and other enzymes, while liquid fermentation generates extracellular and intercellular enzymes. The fermentation process produces close to 3000 enzymes, which are then purified using sophisticated chromatographic methods. They have well-characterized structures.

Enzyme mechanism

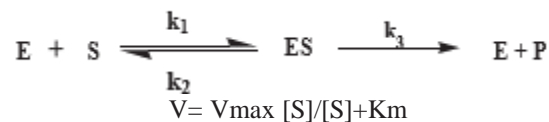
Three-dimensional proteins with "active sites" are called enzymes. The substrates and active sites containing amino acid residues interact to speed up the conversions. Several mechanistic models have been put out to help comprehend the molecular paths of enzyme catalysis.

Lock-and-Key' model

Emil Fischer originally proposed a general mechanism of enzymatic action in 1894. According to his theory, an enzyme and its substrate form a complex that resembles a "lock and key." As a result, each enzyme has a very unique substrate and a hard structure. This approach, however, is unable to account for the fact that many enzymes are inactive on smaller substrates but active on larger ones. Furthermore, this theory is unable to account for the fact that numerous enzymes are capable of converting a wide range of artificial substances in addition to their natural substrates. As a result, a different model had to be created.

Enzyme kinetics

An enzyme's "activity" is the pace at which it transforms substrates into products. An enzyme is considered more "active" when a smaller amount of it can convert a larger amount of substrate. Numerous enzymes have had their reaction kinetics described. The productivity of the enzyme as determined under rigorous standard circumstances is known as enzymatic activity. Michaelis and Menten extracted relationships that are used to estimate the kinetic properties of enzymes using a straightforward unimolecular process (Equation 1). The reaction is represented by the symbols E=Enzyme and S=Substrate. According to Michaelis and Menten, the reaction goes through three stages. Since the substrate and enzyme come into close contact with the enzyme's active site during the beginning or stationary phase, this phase is crucial to the reaction. The amount of enzyme (E) or enzyme-substrate complex (ES) determines the rate of the second phase of the enzyme reaction, known as the steady state, where the enzyme is thought to be fully saturated with substrate. The conversion from ES to the product (P) is the rate-limiting phase, according to Michaelis Menten (M-M) kinetics. Equation 1 expresses the Michaelis Menten connection.



Equation 1. The Michaelis-Menten Equation (V=reaction velocity; V_{max}= Maximum/maximal reaction velocity; [S] = substrate concentration; K_m = Michaelis-Menten constant; E= enzyme; S = substrate, P = product).

K₃ is the turnover constant, while K_m is the M-M constant. These elements are crucial for determining how effective an enzyme-substrate system is. The concentration of substrate needed for an enzyme to reach half of its maximum velocity, or V_{max}, is known as K_m. In essence, K_m is a measure of an enzyme's sensitivity or affinity for a specific substrate.

Trends in biocatalysis

Here is a quick summary of current biocatalysis trends.

a) Whole cell biocatalysis

For many years, organic molecules have been biologically transformed using microorganisms. Since these reactions have shown their value in the asymmetric synthesis of molecules of biological significance, the use of whole cells and their enzymatic systems to perform stereospecific and stereoselective reactions has gained more importance recently. Bioconversions with living microbial cells, which can regenerate their own cofactors and display a broad spectrum of enzymatic activity, are a very useful tool in organic synthesis.²¹ Additionally, they provide an opportunity to use cells for biocatalysis in reaction mediums other than water.²² This is crucial when substrates or products cannot be introduced to an aqueous media because they are unstable or insoluble in water. The utilisation of biphasic media, biocatalysis in emulsions, or anhydrous conditions (organic solvents, supercritical fluids, or ionic liquids) are some of the procedural solutions to this issue that have been discovered.²⁵ It permits expanding the substrate spectrum with water-insoluble chemicals, but it necessitates certain biocatalyst preparations like lyophilization and/or immobilisation to stabilise the biocatalysts in such reaction conditions and shield the cells from the harmful effects of organic solvents.²⁶

Without a doubt, the benefit of microbial biotransformations is that, with the right preincubation techniques, it is possible to induce enzymes with specific, desired activity (which are not constitutively presented inside the microbial cells). Temperature pretreatment, anaerobic or starving cultivation, and the use of various growth media with various sources of supporting materials are all part of this technique.²⁷

In certain research, microbiological media have unique chemical additions that affect the activity of specific enzymes or serve as an exogenous supply of hydrogen for dehydrogenase cofactor regeneration systems.²⁸

b) Isolated enzyme based biocatalysis Vs. whole-cell biocatalysis 29,30

Biocatalysts might be entire cells or isolated enzymes. Compared to complete cells, separated enzymes offer various benefits, including simpler reaction apparatus, increased productivity owing to higher catalyst concentration and simpler product purification. Until recently, the only enzymes suitable for industrial usage were those that were produced in large quantities by cells.

One benefit of using isolated and purified enzymes is that they prevent the creation of unwanted byproducts caused by contaminating enzymes. However, the process of extracting and purifying the enzyme is expensive, and in biocatalytic reactions, the refined version of the enzyme is often less stable than the crude preparation or when it is present in intact cells. Therefore, the biocatalyst used in many commercial biotransformation processes is entire cells for increased cost effectiveness.

Because other enzymes are present, whole cell biotransformations have issues with the production of unwanted byproducts. In these cases, environmental reaction conditions have to be manipulated to minimize byproduct formation or product degradation, or alternatively the activity of the undesirable enzyme may be reduced or eliminated by repression of enzyme synthesis, mutation of the cells or denaturation of the undesired enzymes. When enzyme cofactors are involved in the biotransformation reaction and must be regenerated, cellular biotransformation systems are particularly beneficial. The issues with cofactor recycling and regeneration that arise in single enzyme type biotransformations can frequently be avoided by creating metabolic circumstances that encourage cofactor regeneration.

c) Nonaqueous biocatalysis

Enzymes have been widely employed in aqueous media throughout history. Although biotransformations in industrial synthesis frequently include organic compounds that are insoluble in water, enzymes are well adapted to their natural aquatic environment. More significantly, water is typically the least preferred solvent for the majority of organic processes due to its high boiling point and high heat of vaporisation. It is therefore very desirable to move enzymatic processes from an aqueous to an organic medium.

Research conducted in the last fifteen years has demonstrated that enzymes can function in organic solvents. In contrast to water, the enzymatic activity in an organic solvent is relatively modest. Enzymes with enhanced activity in organic solvents have been developed thanks to recent developments in directed evolution and protein engineering. Additionally, advancements have been achieved in the development of straightforward, affordable, and scalable methods for creating highly active biocatalyst preparations for use in organic solvents. By lyophilising (freeze-drying) an aqueous biocatalyst solution in the presence of organic and inorganic compounds known as excipients, one such technique increases enzyme activity in organic solvents. These excipients consist of cyclodextrins, crown ethers, and nonbuffer salts. Using ionic liquids as solvents in biocatalytic reactions has also produced some amazing outcomes.

Need of enzyme catalysis for sustainable organic syntheses

The ongoing process is the synthesis of biologically significant molecules.³⁷ Synthetic organic and medicinal chemists have been particularly interested in developing reliable, affordable, and environmentally friendly synthetic protocols for the cyclocondensations that result in biodynamic heterocycles because nearly all of the compounds that we know as synthetic drugs, including diazepam, semotiadil, isoniazid, reluzole, celcoxib, glitazone, antipyrine, captopril, mefloquine, and others, are heterocycles. Heterocyclic compounds can also be found in some dyes (like mauveine), luminophores (like acridine orange), insecticides (like diazinon), and herbicides (like paraquat). Additionally, every biological process is chemical in nature. Chemical reactions involving the involvement of numerous heterocyclic compounds, including vitamins, enzymes, coenzymes, ATP, DNA, RNA, and serotonin, are the basis for such basic manifestations of life as energy provision, nerve impulse transmission, sight, metabolism, and the transfer of genetic information.

These heterocyclic substances, both manufactured and natural, can and do take part in chemical reactions within the human body.

The need for the production of bioactive molecules cannot be addressed by conventional organic synthesis techniques, which are orders of magnitude too slow. There has been more pressure on the synthetic chemical community to quickly and environmentally create the wide range of compounds that society needs. A number of environmentally friendly methods, such as the use of non-volatile organic solvents (such as water, ionic liquids, PEG, glycerol, supercritical fluids, etc.), microwave irradiation, solvent-free environments, heterogeneous catalysts, ultrasonication, organocatalysis, etc., have emerged to address this societal requirement. Enzyme catalysis is the best way to speed up synthetic processes because the aforementioned methods have also failed to reduce environmental risks.^{52–59} The use of enzymes in organic synthesis is well-established in both academic and industrial research in the twenty-first century. An efficient and desirable substitute for the conventional synthesis of fine chemicals and optically active molecules is biocatalysis. Because they can take non-natural molecules as substrates and carry out regio- and stereo-specific reactions under mild circumstances (at neutral pH, ambient temperature, and atmospheric pressure), biocatalysts are great catalysts. These days, nonaqueous solvents can be employed with the enzyme. In organic synthesis, a variety of enzymes have been employed to catalyse several transformations and the production of beneficial medications.

Pure lipases and whole cell enzyme sources, such as baker's yeast, are frequently utilised in the production of valuable compounds with significant medical applications. All organic chemistry reactions can be catalysed by enzymes, either in pure form or in complete cells, however the use of enzymes to speed up cyclocondensations that result in bioactive heterocycles receives the least attention. The use of baker's yeast and lipases in cyclocondensations is ongoing. Here are the specifics of lipase and baker's yeast, two of the most popular biocatalysts used by chemists.

Lipases

The family of hydrolases that act on carboxylic ester bonds includes lipases. Their physiological function is to catalyse the breakdown of triglycerides into fatty acids, glycerols, diglycerides, and monoglycerides. Additionally, they can catalyse the conversion of glycerol and free fatty acids into acylglycerols.

Animals, plants, and microbes all have lipases. There are currently a number of lipases on the market. Due to their ease of large-scale cultivation, fungus, yeast, and bacteria are the primary sources of commercial lipases. Lipases have molecular sizes ranging from 20 to 60 kDa.⁶² An α/β -hydrolase fold and a nucleophilic elbow, which contains the catalytic serine, are structural features. Furthermore, the majority of lipases have a helical oligopeptide called a "lid" that protects the active site. When the enzyme comes into contact with a hydrophobic interface, such as a lipid droplet, this lid will open to give the substrate unrestricted access. Thus, at the lipid-water interface, substrate activation transforms lipase into an activated form. This distinctive structural feature of this class of enzymes is known as interfacial activation.

The majority of microbial lipases demonstrate maximum stability in the neutral pH range, but the majority of animal lipases often display pH optima on the alkaline side, pH 8.0–9.0. The majority of lipases function best at temperatures between 30 and 40 °C. Microbial extracellular lipases are often more thermostable than animal and plant lipases.

Lipase's promiscuity in organic synthesis

Recent years have seen the emergence of a new field called biocatalytic promiscuity, which has significantly expanded the use of enzymes. Specifically, it is an enzyme's capacity to catalyse synthetic processes that may differ from its normal function. Biocatalytic promiscuity, one of the fastest-growing fields in enzymology, may offer new and useful synthetic pathways that aren't now accessible in addition to highlighting the catalysts that are already in use. The significance and extensive uses of biocatalytic promiscuity in organic syntheses are discussed in a few excellent papers.

Numerous studies have been published demonstrating the promiscuous function that lipases play in catalysing a variety of other chemical transformations for which they are not anticipated.

The Michael addition reaction, 1,4-addition of a nucleophile to an α,β -unsaturated carbonyl molecule, aldol reaction, Knoevenagel condensation, and cyclocondensations leading to heterocycles have all been found to be catalysed by lipases. According to reports, the His-Asp pair acts as a proton shutter and the stabilisation of the negative charge of the transition states in the active site's oxyanion hole is the suggested explanation for lipase's promiscuity.

CONCLUSION

The current work has been undertaken on the theme of "Enzyme catalysed organic transformations," taking into account the effectiveness, specificity, adaptability, and environmental friendliness of the enzymes in accelerating the rates of condensations, redox reactions, hydrolytic cleavages, some cyclocondensations, and the significance of heterocycles as biodynamic agents. In order to synthesise some clinically significant bioactive heterocycles, such as 2-aryl benzothiazoles, 1,4-benzothiazines, 2,3-diaryl-4-thiazolidinones, 2-amino-3-cyano-4-aryl-4H-dihydropyrans, and 3,5-diaryl pyrazolines, the plan was created and successfully carried out using pure isolated enzymes from active dry baker's yeast (*Saccharomyces Cerevisiae*). Using appropriate biocatalysts, efforts are also made to create quick and safe methods for significant organic transformations such as Knoevenagel, Aldol, and Henry. In the upcoming Chapters II, III, and IV of this thesis, the specifics of the work have been described.

REFERENCES

- [1] Umesh R. Pratap, Jyotirling R. Mali, Dhanaji V. Jawale, Ramrao A. Mane, Baker's yeast catalyzed synthesis of benzothiazoles in an organic medium, *Tetrahedron Lett.*, 2009, 50, 1352-1354.
- [2] Umesh R. Pratap, Dhanaji V. Jawale, Balaji S. Londhe, Ramrao A. Mane. Baker's yeast catalyzed synthesis of 1, 4-benzothiazines, performed under ultrasonication, *J. Mol. Cat. B: Enzym.*, 2011, 68, 94-97.
- [3] Umesh R. Pratap, Dhanaji V. Jawale, Rahul A. Waghmare, Dinesh L. Lingampalle and Ramrao A. Mane, Synthesis of 5-arylidene-2,4-thiazolidinediones by Knoevenagel condensation catalyzed by baker's yeast, *New J. Chem.*, 2011, 35, 49-51.
- [4] Umesh R. Pratap, Dhanaji V. Jawale, Manisha R. Bhosle and Ramrao A. Mane, *Saccharomyces cerevisiae* catalyzed one-pot three component synthesis of 2, 3-diaryl-4-thiazolidinones, *Tetrahedron Lett.*, 2011, 52, 1689-1691.
- [5] Jyotirling R. Mali, Umesh R. Pratap, Prashant D. Netankar, Ramrao A. Mane, An efficient synthetic route for quinazolinyl 4-thiazolidinones. *Tetrahedron Lett.*, 2009, 50, 5025-5027.
- [6] Jyotirling R. Mali, Umesh R. Pratap, Dhanaji V. Jawale and Ramrao A. Mane, Water-mediated one-pot synthetic route for pyrazolo[3,4-*b*]quinolines. *Tetrahedron Lett.*, 2010, 51, 3980-3982.
- [7] Dhanaji V. Jawale, Dinesh L. Lingampalle, Umesh R. Pratap, Ramrao A. Mane, One-pot synthesis of 2-aminothiazoles in PEG-400, *Chin. Chem. Lett.* 2010, 21, 412-416.
- [8] Balaji S Londhe, Umesh R. Pratap, Jyotirling R. Mali, Ramrao A. Mane. Synthesis of 2-arylbenzothiazoles catalyzed by biomimetic catalyst, β cyclodextrin, *Bul. Korean Chem. Soc.*, 2010, 31, 2329-2333.
- [9] Dhanaji V. Jawale, Umesh R. Pratap, Dinesh L. Lingampalle, Ramrao A. Mane, Dicationic ionic liquid mediated synthesis of 5-arylidene- 2,4-thiazolidinediones, *Chin. J. Chem.* 2011, 29, 2421-2427.
- [10] Dhanaji V. Jawale, Umesh R. Pratap, Manisha R. Bhosale, Ramrao A. Mane, One-pot three component synthesis of 2-amino pyrimidines in aqueous PEG-400 at ambient temperature, *J. Het. Chem.*, 2010 (In Press).
- [11] Dhanaji V. Jawale, Umesh R. Pratap, Ramrao A. Mane, Novel synthetic route for new 5-((4-((2-chloroquinolin-3-yl) methoxy) phenyl) methylene) thiazolidine-2, 4-diones. *Bull. Korean Chem. Soc.*, 2011, 32, 1-7.
- [12] Dhanaji V. Jawale, Umesh R. Pratap, Neha Rahuja, Arvind K. Srivastava, Ramrao A. Mane, Synthesis and Antihyperglycemic Evaluation of New 2, 4-Thiazolidinediones having biodynamic Aryl sulfonylurea moieties. *Bioorg. Med. Chem. Lett.*, 2011 (Revised).