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A short review on biocatalysis: Sustainable protocol in synthetic chemistry

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Amazing advancement and development of molecular biology and biotechnology has opened up new avenues in synthetic chemistry where biological systems can be efficiently mimicked in laboratory and intra as well as extra cellular enzymatic catalysis can be utilized in chemical transformations. Apart from known natural biological protocols modern day protein engineering enabled chemists to design artificial enzymatic reactions to efficiently suit predefined features of a reaction. Use of preferential solvents, substrate, reaction-container and reaction condition can further enhance the width and depth of biocatalysis application. Utilization of nanotechnology in tandem with catalyst immobilization helps in improving catalyst efficacy and reusability of such catalysts. More attempts are being successfully carried out to apply this greener technology in industrial procedures towards sustainable growth.

Keywords: Green chemistry, atom economy, biocatalysis, non-conventional solvent, substrate engineering, cascade reaction.

Introduction

The span of biocatalytic conversion[1] in chemical synthesis is very broad, and it is usually classified between two different patterns.

The primarily available category is incubation mediated whole-cell biotransformation where the starting material acts as the reactant source as well as the multiplication medium of the micro-organism. As a consequence the synthetic utility is obviously dependent on the multiplication rate of the micro-organisms.

The second type of biocatalytic processes are those in which the synthesis of the biocatalyst (major contribution is enzyme) is carried out prior to the synthetic application.

Fermentation protocol had been historically used as a bio-catalyst for facilitating functional material[2] synthesis by natural conversion. Such materials' list includes industrially important materials like ethanol, glycerol, hydracrylic acid[3], lactic acid, pyruvic acid and many others. In recent times successful attempts are being reported for utilization of fermentation for itaconic acid and adipic acid[4] synthetic protocols.

The latest protocols are usually being designed to differentiate the cell growth and cell application steps as often the reaction medium capable of bringing the organic reactant molecules together stands not so favorable for smooth and rapid biotic growth of the desired micro-organism. According to the basic concept of catalysis the cells are supposed to remain alive after the extraction of the target product so that the cells can be reused to make the process cost-effective but it is scarcely achievable due to toxicity in the medium due to presence of different chemical constituents[5]. Moreover, all living cells contain multiple enzymes among which a few can promote unwanted parallel reactions causing decomposition of reactants as well as products to result in lower yield of the targeted molecule. It is also observed that the whole cell biocatalysis loses efficacy in case of bulkier substrates due to poor permeability of the sterically demanding substrate through cell LPL layer[6].

Isolated enzyme biocatalysis is the synthetic protocol where the optimum enzyme is extracted from the biotic organism and purified before the catalytic application. As the catalyst is isolated before the catalytic application the substrate scope increases many fold. Those reactions where the enzymatic catalysis was initially avoided due to lower permeability of the substrates can now be brought under the purview of biocatalysis. But as the cost of catalyst is effec-

tively increased due to extraction and purification procedure the recyclability[7] of the enzyme becomes an essential requirement and is achieved most efficiently using solid phase catalyst immobilization[8].

Importance of catalyst

Inventors and developers of pharmaceutical, chemical and food industry are always in search of new catalytic systems and cycles which can effectively facilitate synthesis of more complex functional organic molecules as well as can satisfy ecological demand of environment-friendly sustainable chemical procedures.

However, environmental concerns and challenge to achieve eco-friendly organic synthetic protocols are increasingly motivating the movement towards efficient catalytic alternatives to stoichiometric organic transformations. Anastas and Warner introduced the idea of sustainable chemistry and outlined some basic directive protocols which act as the visionary goal while research and improvisation towards minimizing and eliminating chemicals as well as chemical protocols with adverse ecological impacts are fabricated. Therefore from a Green chemist's point of view catalytic reagents are always preferred as compared to the stoichiometric reagents as the atom economy is improved by using catalysts during a chemical reaction.

The World Commission on Environment and Development[9] lead by Brundtland in the year 1987 came out with a detailed documentation entitled "Our Common Future" where the requirement demanding extensive increase in agricultural and industrial production was accepted to cater the basic human needs of the global population but simultaneous caution was also raised about the depletion and deterioration of natural parameter which must be addressed properly to sustain the improvement of quality of life achieved by scientific endeavors.

Although the principles of green chemistry aimed at reducing the environmental hazard associated with chemical synthesis and functionalization the associated cost hindered its wider application in industry as economic competition is essential for a scientific methodology becoming sustainable.

As natural resources are getting diminished rapidly a sustainable scientific protocol must emphasize on reducing use of natural resource along with reuse and recycling of different components of the protocol[10].

Biocatalysis in green chemistry

Researches revealed[11] in the year 1984 that in contrary to the common assumption of familiarity of enzymes with aqueous medium, lipase remains active up to a higher temperature in aprotic non-polar solvents like toluene and xylene as compared to water. This discovery initiated the broader application of bio-catalysis in reactions involving non-aqueous organic synthesis.

Likewise, the search for economically efficient routes towards pure enantiomers of chiral drugs[12] offered a break for the expansive utility of highly enantioselective biocatalytic protocols[13]. Today's technological growth has made it exceedingly viable to manipulate enzymes to fit a designed synthetic protocol. Effective and efficient immobilization of enzyme on solid support has also paved the way for longer shelf life, lesser denaturation loss during course of the reaction and most significantly greater scope of recyclability[14].

As a result of this scientific advancement biocatalysis is now considered as an economically viable greener alternative protocol for regular industrial synthesis[15] of functional organic molecules[16] with a special emphasis on the Stereoselective synthesis of Active Pharmaceutical Intermediates (API)[17].

As biocatalysts i.e. enzymes are selected on the basis of extrapolation of their natural role in living system, the reaction conditions involving biocatalysis usually comprise of moderate temperature and atmospheric pressure using aqueous media. This reaction condition helps us arriving in procedures which are more step-economical[18] and generating lesser amount of hazardous waste materials. Due to these benefits biocatalytic methods are more ecological as well as economical and therefore offer greater sustainability.

Choice of media in biocatalyzed reaction

It is a fact that almost all enzymes function optimally in water but the immiscibility of most of the organic reactants in water makes the condition complex. Additionally the nucleophilic character of water molecule renders it invalid in case of reactions involving electron deficient carbon atoms due to obvious chance of hydrolysis even at room temperature for reactive substrates. Hence there is growing demand of non-aqueous catalysis[19] to overcome these pertinent issues in application of bio-catalysis in organic synthesis. But apart from air pollution associated with volatile organic solvents

there is often disruption of characteristic three dimensional protein folding of the enzymatic proteins caused by H-bond rupture when highly polar solvents like DMF or DMSO is used as the medium for enzyme catalyzed transformations. To overcome this problem with most of the broad spectrum organic solvents non-conventional alternative solvents like ionic liquids (ILs)[20,21] and deep eutectic solvents (DESs)[22,23] are the latest area of exploration.

It should be relevant to note, if water is eventually used as the solvent, there should be solvent extraction using immiscible organic solvent to trap organic impurities[24] so that the water can be released in nature. But in spite of these procedural challenges owing to biological acceptance water still remains the favorite choice of solvent for most of the biocatalytic reactions.

Biocatalysis in organic solvents has bunch of operational benefits, most importantly wider solubility and easier product recovery. Application of suitably designed organic solvent cannot only enhance the catalytic efficacy of enzymes and if suitably designed it can even act as a chiral auxiliary to increase the enantioselectivity[25] and synthetic application[26] of the biocatalytic process. In contrary, ecological hazards involved with different classes (Volatile and High Boiling) of organic solvents as well as slower reaction rate indicate a stern problem of biocatalysis in organic media[27].

As lyophilization enhances the activity of enzymes[28] it can be presumed that considerable rate enhancements would be expected by carrying out the reaction at room temperature in ionic liquid (IL), due to its close resemblance with aqueous solution of ionic salts. Due to the non-volatility and high dielectric constant ILs are being widely promoted as superior alternatives to volatile organic solvents for biocatalytic processes[29].

Deep Eutectic Solvents (DESs) are the latest persuasive variety of reaction media employed for smooth execution of enzyme catalyzed organic synthesis. The most important advantages of application of DESs[30] as solvent in many reactions as compared to Ionic Liquids are their cost-effectiveness and less hazardous preparative procedure. Most interestingly a new set of DESs which are called Natural Deep Eutectic Solvents (NADES) have been synthesized from bioorganic metabolites like amino acids, sugars, choline and organic acids[31] to offer an optimum biochemical environment for the enzymes to act upon the not so regular substrates.

Biocatalysis mobilization

Smith and co-workers[32] invented the genetic engineering tool by site-directed mutagenesis (SDM) where exclusively local artificial genetic mutations are carried out on a specific protein chain of an enzyme by replacing a predetermined amino acid residue at the substrate encoding region. Preceding the breakthrough of sequential Polymerase Chain Reaction (epPCR) random arbitrary mutagenesis was carried out to generate a wide range of mutant enzymes amongst which a very few would be effective for the desired catalysis. The first application of sequential epPCR was carried out by Chen and Arnold[33] to arrive at a genetically modified protease subtilisin E, hundred fold active as compared to the naturally occurring variety extracted from *Bacillus subtilis*. However more scientific as well as accurate catalytic efficacy can be achieved in a most cost-effective fashion when instead of isolating a specific mutant enzyme from a large variety of derived enzymes the specific one is exclusively synthesized by altering the DNA sequence of the organism[34].

Catalysts found their novel role in organic synthesis due to the highly stereoselective as well as stereospecific nature of the reactions catalysed. However, the specificity and selectivity are often largely hampered by even minute differences between the regular and planted substrate. Studies revealed[35] that sequential random mutagenesis of (*S*)-selective transaminase and catalytic screening can offer significant increase in the enantiomeric excess from 65% to 94% during synthesis of amine derivative of β-tetralone. Recent researches in mutation study has made it feasible to predefine the optimum parameters required for commercial viability accordingly modify the biocatalyst to arrive at those parameters[36].

Substrate engineering can be useful for the optimization of existing biocatalytic reactions as well as for the contraption of completely new transformations, which is referred as enzyme promiscuity[37–40]. The most easily presumable application of substrate engineering is applying an enzyme for its original designated reaction on a different substrate. Let us take the example of lipases. This group of enzymes is found to catalyze triglyceride hydrolysis to generate a mixture of glycerol and fatty acid(s) according to Serine Protease Mechanism (Fig. 1). Hence, initial attempts were taken to use the enzyme to establish a protocol for ester hydrolysis

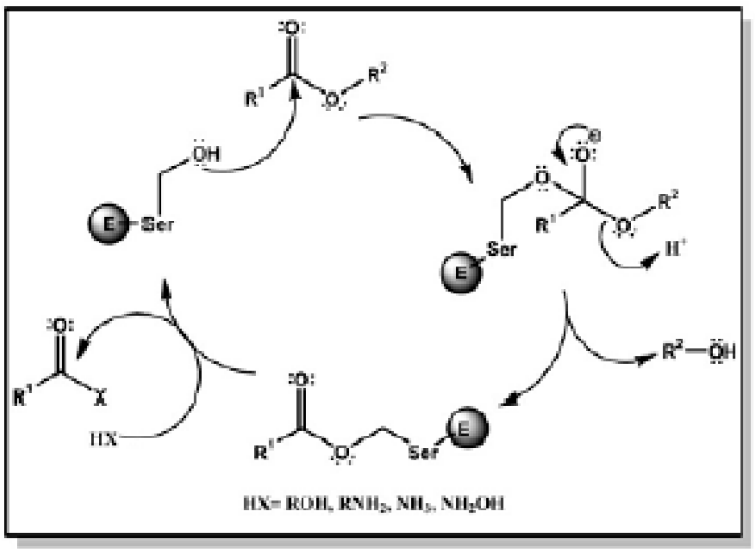

Fig. 1. Serine protease mechanism.

reaction in neutral condition. This reaction protocol was of immense demand as all conventional ester hydrolysis protocols are carried out in either acidic or alkaline reaction medium. Scientists were not only successful in this attempt further extrapolation of this protocol was achieved by substituting water with other nucleophiles, to arrive at a vast series of pH sensitive carboxylic acid derivatives.

Another intelligent application of biocatalytic reaction is the Halohydrin Dehalogenase (HHDH) catalyzed ring opening reaction of three member heterocycles by suitable and desired nucleophiles. The beauty of this enzymatic catalysis is that this catalyst can induce the otherwise strained cyclization of vicinal chlorohydrins as well as other α,β-disubstituted substrates to generate epoxides as well and due to reversibility of the reaction stereoselective synthesis is favored (Fig. 2).

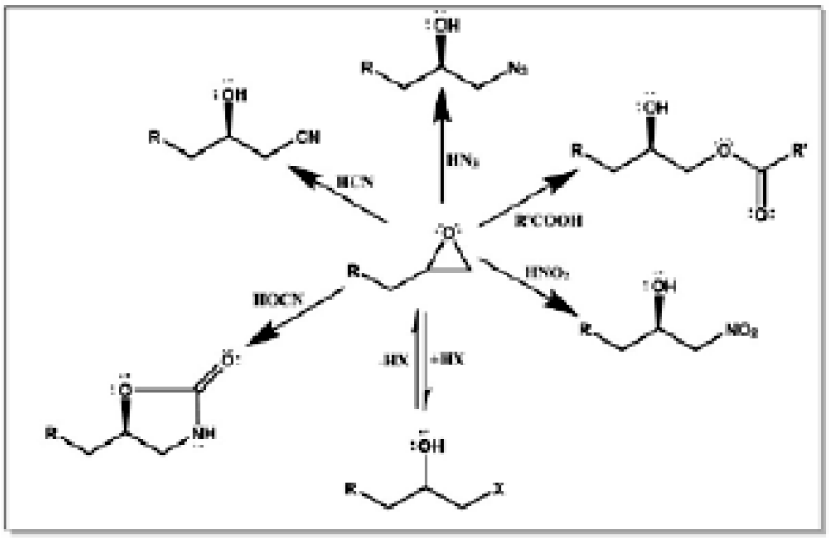

Fig. 2. Bio-catalyst promoted ring opening of cyclic ethers.

Another application of enzyme promiscuity is the chemomimetic biocatalysis approach.

Various transition metals take a crucial role in catalyzing a wide variety of biological redox reactions which would be otherwise very sluggish if attempted with the oxidizing agent oxygen or H_2O_2 as the sole oxidizing agent alone. Therefore a suitably designed transition metal embedded hydrolase enzyme could result in a designed metalloenzyme competent of facilitating enantioselective oxidations.

In recent time Cytochrome P450s has received enormous attention as a useful bio-catalyst[41–45] in course of mimicking biological reactions in laboratory set up. In P450s the active oxidizing species comprises of a super positive iron (+4) oxo porphyrin high spin cation generated by aerial oxidation of iron(II) center linked to a cysteine thiolate residue in presence of NAD(P)H co-factor as the hydrogen donor (Fig. 3).

As enzymes are operating in biological systems almost all have reasonably high solubility in aqueous solution and therefore the recycling becomes costly when water is the used solvent and as a consequence a large portion of the enzyme is present in the solvent phase along with unreacted starting materials which becomes tedious to recover for recyclability. Therefore, a shift from homogenous catalysis to heterogeneous catalysis was planned where the water soluble enzymes were immobilized on an inert solid surface to enable smoother and faster recovery and reuse. The two most significant examples of this catalyst immobilization are Penicillin G. Amidase and Glucose Isomerase. The second one is till date the most widely[46] used bio-catalyst in industry and immobilized catalyst shows 90% catalytic efficacy in ethanol[47].

Consecutive reaction

Conventional organic synthesis usually follows a multistep protocol where the products of earlier steps are used as the reactants in the succeeding steps. Although the individual steps are simple almost all of the steps require isolation and purification of the products which eventually causes a lower atom efficiency, larger reaction time, tedious catalyst recycling and disappointing waste content. Hence the chemists always wanted to substitute the linear continuous reaction strategy with a converging synthetic approach[48] which reduces reaction time as well as byproduct formation. Enzymatic catalysis is expected to be best suited for this purpose as the biochemical processes taking place in living organisms in presence of enzyme are usually convergent and concerted.

Let us check an amazing practical application of this idea involving a biocatalytic pathway of epimerization reaction resulting in attempted inversion of the D variety of methionine into its enantiomer through an enzyme quadruple[49] in the following fashion (Fig. 4).

The initial step involves D-amino acid oxidase-catalase pair which oxidizes the amine group at the chiral center into carbonyl functionality and thus destroying the chirality of the molecule. In the second and final step the pair involved is L-phenylalanine dehydrogenase and FDH which converts the intermediate keto compound into L-methionine in presence of ammonium bicarbonate as the ammonia source.

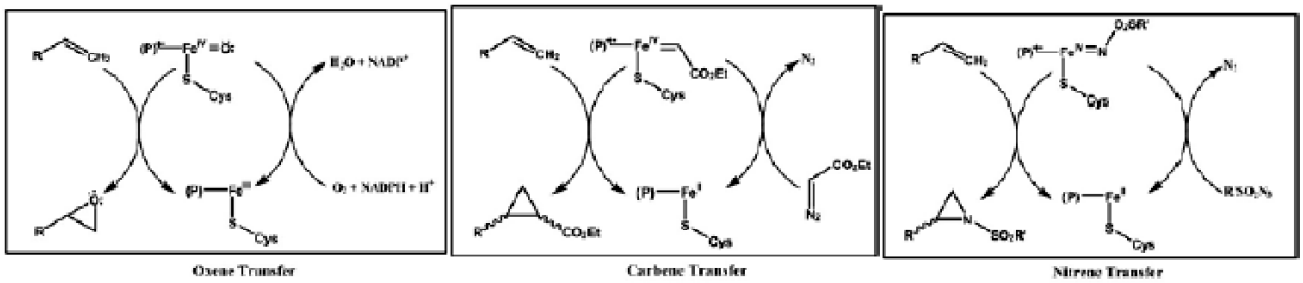

Fig. 3. (a) Enzymatic epoxidation; (b) cyclopropanation; (c) aziridination.

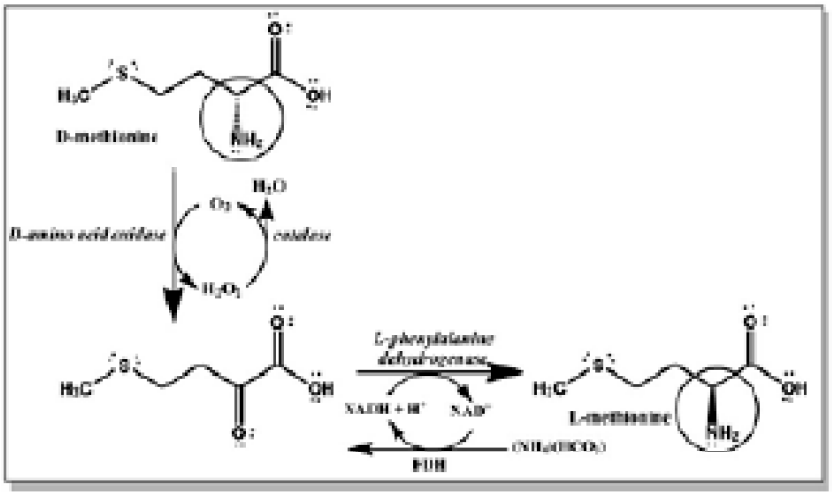

Fig. 4. Enzyme mediated epimerization of methionine.

Conclusion

It has always been a mammoth task to mimic natural biochemical reactions in laboratory or industrial setup and extrapolate those protocols in chemical synthetic route towards functionally important substrates. Although the initial hurdle of applying the delicate methodology in place of hazardous conventional chemical synthesis[50,51] had been overcome innumerable research works are taking place throughout the world to deploy this sustainable idea in achieving a greener, cleaner and more sustainable biobased economy.

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