# Immobilization of lipase onto epoxy resin based bioinspired surface

Project report submitted in fulfillment of requirement for the degree of

**BACHELOR OF TECHNOLOGY**

**IN**

**BIOTECHNOLOGY**

By

**HIMADRI HAUTA (161802)**

**SARA BHINTA (161828)**

**UNDER THE SUPERVISION OF**

**Dr. Ashok Kumar**

JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY, WAKNAGHAT **June,2020**

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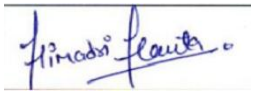
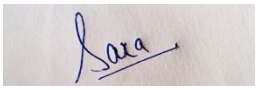
## DECLARATION

We hereby affirm that the work reported in the B. Tech Project Report allowed **"Immobilization of lipase onto epoxy resin based bioinspired surface"** submitted at **Jaypee University of Information Technology, Waknaghat, India** is areliabledocumentation of our work approved under the administration of Dr. **Ashok Kumar.** We have not submitted this work in a different place for any other degree or qualification.

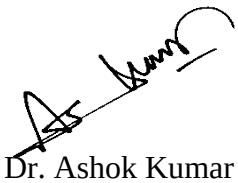




Himadri Hauta — Sara Bhinta

161802 — 161828

This is to certify that the above statement made by the candidates is truthful to the best of my acquaintance.



Dr. Ashok Kumar

Date:

## SUPERVISOR CERTIFICATE

This is to endorse that the work named “Immobilization of lipase on bioinspired surfaces.” Submitted by Himadri Hauta and Sara Bhinta during the mid-semester in December 2019 in fulfilment for the decoration of grade of Bachelor of Technology in Biotechnology of Jaypee University of Information Technology, Solan has been approved out under my command.

**Signature of Supervisor**

Name of supervisor: Dr. Ashok Kumar

Designation: Assistant Professor

**Department of Biotechnology and Bioinformatics**

**Jaypee University of Information Technology**

**Waknaghat, Solan (H.P)**

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Thank you.

**Abstract**

Immobilization of enzymes is imperative in terms of progression of economics by allowing enzyme re-use and by ornamental the largely productivity and strength. Membranes are a bettercarrier and support for enzyme immobilization. Immobilized enzymes are the ones that are attached to inert or insoluble materials. Enzymes are essentiallynatural catalysts that rate up the rate of practically all element reactions that takes place inside the cells. In common immobilization allows the reprocess of biocatalysts and makes the revival of the product easier and increases the enzyme confrontation against inactivation using dissimilar denaturants, in so doingproviding more unwavering catalysts. The modern demands of the world's biotechnological industries square measure sweetening in protein efficiency and expansion of narrative techniques for escalating their time period. These provisions square measure foreseeable to assist large-scale and economic formulation. Protein immobilization provides a astonishing base for growingconvenience of protein to the substrate with larger earnings over a extensive amount of your time. Many accepted and non-natural supports are used for his or her potency for protein immobilization. Nowadays immobilized enzymes square measure most popular over their complimentarycomplement because of their prolonged accessibility that curtails redundant downstream and purification processes. Upcoming investigations ought to attempt towards adopting logistical and smart denial techniques alongside resourcefullyaltered supports to boost the state of protein immobilization and supply new views to the commercial sector. Polypropylene is a thermoplastic polymer resin; its chemical designation is C3H6. Polypropylene is a versatile product and has therefore number of uses. The hydrophobic or deliquescent characteristics of the surface exposed by a enzyme support conditions the number of absorbate super molecule, and doubtless additionally the conformation of the immobilized enzyme. Polypropylene surfaces show the characteristics for immobilization of enzyme accelerator in terms of surface structure and particle size. Even on evaluation of the lyophilized and with the immobilized preparations, it is understandable that immobilization on polypropylene results in preparations with both a higher percentage of titrated active sites and higher activity per active siteprovides biocompatible interface, work at low temperature, low atmospheric pressure and are not usually violent to reactors and accessories**.** The greatest improvement of polymers as support materials is that the monomers that build the compound sequence is designed in keeping with the requirements of the accelerator and also the procedureof purposeful teams determines whether or not the catalyst is anchored to the

matrix via as an example surface adjustment or by the configuration of valence bonds varied chemical compound materials will be used as effective supports and improve properties of the immobilized catalyst like thermal steadiness and reusability. The layers of the compound play a really necessary responsibility in protecting the active sites. The usefulness of the same facilitates effective accelerator binding and conjointly functioning of the chemical compound surface. This typeof the protein formed from negative effects of the ingredients of the reaction mixture with the method conditions included. However, it should be noted that synthesis of a chemical compound with the specified properties and focused teams is typically a long and expensive method. Some of the disadvantages of polypropylene are that they are susceptible to oxidation. Certain limitations of polypropylene surface are enzyme leaching;the solubility of a support in any reaction media may become a serious difficulty.

## Introduction

Enzymes are widely studied biomolecules in various fields of science and technology. Enzymes carry out and normalize a wide range of processes in living organisms and are therefore known as flexible catalysts. Any enzyme that catalyzes the hydrolysis of fats or lipids is known as lipases. Lipase plays main role in digestion, processing and transport of dietary lipids (Svendsen A et al.,2000). Lipases can be of industrial use, diagnostic use or of medical use. Biotechnological advances have brought recombinant lipase enzyme to market for the uses such as baking, laundry (Guo Z et al.,2005). in alternative energy strategies to convert vegetable oil into fuel (Ban K et al.,2001). Lipases has gained efficient consideration for being able to catalyze a wide range of reactions such as alcoholises, hydrolysis, and enantiomer motion (R.A. Sheldon.,2007).

## Article I. Lipase immobilization

Article II. Immobilized lipases are therefore produced on a very large scale mainly under nonaqueous conditions for the efficient production of structured triacylglycerols Quinlan et al. and a variety of fatty acid esters (Macrae et al.,1984). Hydrolases is undeniably the most used enzymes in biotransformation, due to their extensive substrate specificity, profitableaccessibility, lack of any independence on cofactor, and ability of working at high substrate concentrations, not only in their natural environment ,i.e., aqueous solutions but also in organic and neoteric solvents (Bertau et al., 2015; Bornscheuer et al., 2006; Busto et al., 2010; Kazlauskas et al, 2016; Lopez-Iglesias et al., 2015; MendezSanchez et al., 2016; Siirola et al., 2013.)

## Why we should consider polypropylene surface?

Polypropylene is an organic compound and is one of the most adaptable and widely used polymers in the world. There are mainly two types of polypropylene available: homopolymers and co-polymers. Main properties of polypropylene that allows it to be useful for all sorts of applications are high melting point, resistance to chemicals, a good insulator. Few advantages of polypropylene are relatively inexpensive, very resistant to

moisture, good chemical resistance, provides biocompatible interface. Lipase immobilization on polypropylene surfaces for the polyester synthesis is advantageous for obtaining high molecular weight polyesters (Simone Weinberger et al.,2018).

**Studies using polypropylene surface**.

Lipase immobilization on polypropylene hollow fiber phospholipids was approved and it was found that adsorption, activity and thermal stability of enzymes that were immobilized onto polypropylene re advancedthan theoriginal enzymes or enzymes that were never immobilized. Phospholipids can be involved without any thing in regulating the enzyme activity. In this experiment three phospholipid polymers were taken and attached to the polypropylene hollow fiber micro filtration membrane.Adsorption capability, motion and thermal stability of enzymes were compared to new or fresh enzymes. Minimally writing in a single line this author has reported the Adsorption capability of lipase was found to be lower than that of the new ones. The Activity of the enzyme increases from 57.5% to 74.1% (first phospholipid polymer), 77.5% (second phospholipid polymer) and 83.20% (third phospholipid polymer). The thermal steadiness of enzyme was kept in 50°C for 2hours. The results showed that thermal stability of enzymes is higher than that of new enzymes (Hong-Tao Deng et al.,2004). Immobilization of lipase onto amine-functionalized polypropylene membrane was carried out to check its application for flavor synthesis. Various parameters were observed in this study, they are:

*Table 1: Parameters measured for free enzyme and immobilized enzyme.*

| Parameters measured | Free enzyme | Immobilized enzyme |
|---|---|---|
| Hydrophobicity/Water contact angle | ≤90° | 78° |
| pH | 7.0 | 7.5 |
| Temperature | 35°C | 40°C |

| | | |
|---|---|---|
| Km | 2.9mM | 8.4mM |
| Vmax | 926 U/mg | 741U/mg |
| Catalytic efficiency (Vmax/Km) | 319.3 | 88.2 |

At 50°C free zed enzymes lost all their activity after 120mins of heat treatment, whereas immobilized enzymes showed conflict towards the thermalactivation. At 60°C lost all its activity after 45mins of heat treatment, whereas immobilized enzymes retained 55% of its initial activity. Free and immobilized lipase store in phosphate buffer at 4°C and activity measured was after 8weeks, the results showed that free enzyme lost all its activity. The results indicated that most of the lipases show higher optimal temperature values than their free counter parts. Km for immobilized enzyme was 2.89 times higher than that of free enzyme. Vmax value decreased about 1.25-fold compared to free enzyme. Catalytic efficiency decreased 3.6-fold upon immobilization. Recovered activity of immobilized lipase was 76% (Gulay Bayramoglu et al.,2011).

Different lipases were additionally defined for super molecule content and specific activity and was immobilized on plastic surface through physical surface assimilation. The work was designed to study microporous polypropylene powder, which is a widely used support for lipase immobilization (Gitlesen et al.,1997) to adapt the immobilized biocatalyst for synthesis of biodiesel. Lipases were used and Polypropylene powder MP1004 along with Bradford reagent, BSA 98%, tributyrin 98% was used.

*Table 2 protein content, tributyrin activity, triolein activity, specific activity of commercial enzymes.*

| Name | Protein content (wt.%) | Tributyrin activity assay (kLU/g commercial lipase) | Triolein activity assay (kLU/g commercial lipase) | Specific activity (tributyrin assay) LU/mg protein | Specific activity (triolein assay) LU/mg protein |
|---|---|---|---|---|---|
| Lipase M | 5.6 wt.% | 7.4 | 5 | 132 | 89 |
| Lipase G | 0.80 wt.% | 0.14 | 0.14 | 17 | 17 |
| Lipase F | 4.3 wt.% | 22 | 28 | 512 | 651 |
| Lipase AY | 1.1 wt.% | 5.9 | 4.7 | 536 | 427 |
| Lipase R | 0.64 wt.% | 6.5 | 0.4 | 1016 | 63 |
| Lipase AK | 2.0 wt.% | 24 | 4 | 1200 | 200 |
| Lipase PS | 0.55 wt.% | 9.4 | 1.8 | 1709 | 327 |
| Lipase A | 3.31 wt.% | 0.69 | 0.05 | 21 | 1.5 |

8 marketable lipases were immobilized onto themicro porous polypropylene through physical adsorption; they were then characterizedfor their protein content and specific activity. The support was highly hydrophobic polypropylene powder, of which the results were beforehand characterized. (Salis A et al.,2003)

Accurel MP1004, porous polypropylene powder was defined for enzyme immobilization. Granulometric analysis of MP1004 was done the results of that area unit as follows:

This table indicates that 80% particles range between 420-177µm. A mesh is measurement of particle size which is usually there to conclude the particle-size distribution of a granular material.

It is evident that internal diffusion becomes more restricting as the diameter of the support particles increases equally too small for the particles that are not useful in packed-bed reactors as their property are to oppose high pressure towards the substrate flow, hence the particle size is important for the characterization of the support. It is commonly known that; the pore diameter should be at least 350Å for lipase penetration into the pores and for casing the accessible surface area (J.A. Bosley et al.,1994). Pore division is quite expansive and fluctuates between macropores and mesopores. The mesopores are large enough to cover the lipase during the immobilization process. Experiments were carried out beforehand and after immobilization to determine the weight of immobilization on catalytic activity of the lipase worn.

Therefore, immobilisation on Accurel MP1004 support certainly improves the catalytic performance either in terms of substrate conversion or enzymatic activity of the lipase. (Salis A et al.,2005) Polypropylene beads as stated are one of the most used supports in the proficient immobilisation of hydrolytic enzymes (Huber et al.,2016) (Manoel et al.,2015) Almeida et al. in this the lipase were immobilised onto polypropylene beads and were then tested for their catalytic effectiveness using polycondensation reactions. Thin-film reaction was used in this study as thin-film reactions have several advantageous. Certain advantages of thin-film reactions are that they provide efficient heat and mass transfer, and also easy removal of the by-product and also helps in preservation of the mechanical stability of the used biocatalyst preparation (Pellis A et al.,2015) (Pellis A el at.,2017).

Immobilization of lipase (CaLB) onto polypropylene (Accurel MP 1000) in different buffer systems showed that lower salt concentrations of 0.1M show the better coupling of CaLB to polypropylene beads. This shows a clear influence of buffer systems. The best results were obtained with CaLB immobilized in 0.1 M Na2HPO4/ NaaH2PO4 buffer at constant pH 8, the reaction preparation produced polyesters with a molecular weight of 4kDa at conversion rates of 96%. The stability of this preparation was also studied at a continuous polymerization process and the results showed that the particular preparation produces lower molecular weights at lower conversion rates, these results were obtained after 4hours of reaction time. However, 24hours reaction time for the meticulousarrangements produces higher molecular weight polyesters (4kDa vs 3.1kDa) (Simone Weinberger et al.,2018)

In this study, the site volumetric analysis and dimensions of the activity were approved to carry out in methane series ontoconserved enzyme arrangements containing different quantities of phosphate buffer with enzyme immobilized onto the porous polypropylene. Lyophilization of Thermomyceslanuginosus enzyme with phosphate salts of large quantities (200 mM) exaggerated the accurate activity fourfold, and also the range of

titratable active sites exaggerated to 50 % from the 13 % determined once smaller amounts of phosphate buffer were used (20 mM) throughout lyophilization. The phosphate buffer worked as an immobilization matrix for the enzyme, and with the increase in specific activity with a minimum part decreased the mass transfer limitations. Once the enzyme was immobilized onto the porous polypropylene, the particular activity was 770 times over. At higher rates, 93% of the enzyme was titrated. This indicates that this adsorption on a hydrophobic surface was a very competentway of dropping mass transport and of immobilizing the catalyst in its active conformation to be used. The divergence in a specific activity with water activity was found to correlate o.k. with the deviation in titratable active sites once lipases from Burkholderia cepacia. The compound process movement per competent site was therefore unvarying over the absolutedifference of water activities.

*Table 3: size of molecular sieves used, size of particles and percentage of particles.*

| Molecular Sieves (mesh) | Particle size µm | % |
|---|---|---|
| >20 | >840 | 0.2 |
| 20-40 | 840-420 | 9.7 |
| 40-80 | 420-177 | 80.6 |
| 80-120 | 177-125 | 8.6 |
| <120 | <125 | 0.9 |

The synthesis of enzymatic biodiesel was carried out in the non-aqueous medium of the mixture of reagents (triglyceride and alcohol). In this media the enzymes are not dissolvable and addition of a small amount of water increases the catalytic activity and special treatment for the grouping of enzyme particles. Due to which only a small number of enzyme molecules are present on the combined surface. Effective spreading of the enzymes on porous materials allows a high increase in the surface area, which further allows a large number of enzyme molecules to communicate their catalytic potentiality (Salis A et al.,2003).

Many different and variant types of supports carry out immobilization of enzymes but usually, porous materials are the best option as these supports allow the active dispersion of enzyme molecules on a large surface, which in turn allows a greater amount of enzyme molecules to address their catalytic potentiality (Salis A et al.,2005).

Accurel EP100 and MP1000 are an example of a number of the already available macro porous, hydrophobic, low-density polypropylene powders that show signs of an outsized extent for adsorption thanks to their very small particle size (Gangoiti J et al.,2010; Al-Duri B et al.,2001) . The following procedures are greatly defiant towards organic solvents (Al-Duri B et al.,2001; Persson M et al.,2002). Features that increases their awareness for lipase immobilization when designed toward oil transesterification (Salis A et al.,2008; Severac E et al.,2011) Celite®545 consists of extremely porous diatomaceous beads composed of silica (SiO2), conjointly containing another inorganic oxide thanks to its chemical inertness and special interconnected pore structure, Celite®545 constitutes very suitable support for physical adsorption (Chang SF et al.,2007) which has been extensively utilized in immobilized-lipase biodiesel production (Lumor SE et al.,2008; Sagiroglu A et al.,2008; Shah S et al.,2007).The answer to the use of immobilized lipase shows some drawbacks towards the synthesis of biodiesel.(Watanabe Y et al.,2002)there are already to catalyze alcoholizes reactions in solvent-free media.

**Advantages of lipase immobilization on polypropylene**

Immobilization of lipase is often done onto many alternatives supports however sometimes, porous materials square measure the easiest opportunity as these supports allow the active spreading of catalyst molecules on the surface too big, which further gives us a large number of enzyme molecules to provide their catalytic potentiality (Salis A et al.,2008)

Accurel EP100 Associate in nursing MP1000 square measure an example of a number of the commercially accessible microporous, hydrophobic, low-density polypropylene powders that exhibit an outsized expanse for surface assimilation as a result of their terribly particle size (Al-Duri B et al.,2001) Some of these provisions are considered extremely opposing towards organic solvents (Al-Duri B et al.,2001; Persson M et al.,2002). A further increase in their interest for lipase immobilization when intended at oil transesterification (Severac E et al.,2011 Salis A et al.,2008) Celite®545 consists of extremely porous diatomaceous beads composed of silicon dioxide (SiO2), additionally containing another inorganic oxide. due to its chemical immobility and special interconnected pore structure, Celite®545 constitutes awfully appropriate support for physical surface assimilation that has been widelyengaged in immobilized-lipase biodiesel fabrication (Sagiroglu A.,2008).

## Chapter 2

## Review of literature

Enzymes are widely studied biomolecules in various fields of science and technology. Enzymes carry out and monitor a extensive field of procedures in alive organisms and are therefore known as resourceful catalysts. Any enzyme catalyzing the hydrolysis of fats or lipids is termed to be lipases. Lipase plays main role in digestion, processing and transport of dietary lipids (Svendsen et al., 2000). Lipases can be of industrial use, diagnostic use or of medical use. Biotechnological advances have brought recombinant enzyme protein to promote for uses like baking, laundry, and biocatalysts (Guo et al., 2005) in energy, methods to convert edible fat into fuel (Ban et al., 2001). Lipases is considered great for being competent of catalyzing a great range of reactions such as alcoholizes, hydrolysis, trans-etherification(Sheldon et al., 2007).

### Lipase immobilization

Immobilized lipases are further fashioned on industrial scale above all under nonaqueous environment for the inventive fabrication of structured triacylglycerols (Quinlan et al., 1993) and a collection of fatty acid esters (Macrae et al., 1984).Hydrolyses are irrefutably the widely used enzymes in biotransformation's, due to their well emerged substrate specificity, marketable ease of use, absence of reliance on cofactor, and capability of working at high substrate concentrations, not only in their ordinary environment ,i.e., aqueous solutions but also in organic and neoteric solvents (Bertau et al., 2015); (Bornscheuer et al., 2006); (Busto et al., 2010); (Kazlauskas et al., 2016); (Lopez-Iglesias et al., 2015); (Sanchez et al.,2016); (Siirola et al., 2013. Immobilization of enzymes will help to augment its sustainability as enzymes are more confrontation to changes in environment and can be reused. Lipases square measure the

foremost wide used enzymes in bio-catalysis, and therefore the most utilized technique for accelerator immobilization is victimization hydrophobic supports at low ionic strength. Methods to prevail over this problem include physical or chemical cross-linking of the immobilized enzyme molecules or using hetero-functional supports. Buffer used in the immobilization of lipase enzyme. There are various buffers that are used in the enzyme assay. Some of them are Tris-HCL, HEPES, phosphate buffer saline etc. These buffers differ from enzyme to enzyme. Some require acidic and some require basic. The best matched buffer for lipase assay according to the researchers is the PBS (phosphate buffer saline) There are various kinds of surfaces that are used on the immobilization of lipase enzyme or otherwise. There is description of these surfaces that determine as to which one must be used for the betterment of the experiment. They should have the following features.Thermally and mechanically stable, insoluble,high capacity of enzyme binding,support should be cheap and environmentally safe.Porous silica is a good substitute for immobilization. The immobilization of two or more enzyme on a single support is promising.

**Bio-inspired materials**

The use of bio-inspired material is not new to the industry. Evolution of new materials has been derived from natural selection. There are many bio-materials that have been used previously by humans for defense purposes. But what exactly are bio-inspired materials? These are unnaturally processed materials mimicked from natural materials or living matter. Living organisms show confirmation of acapacity to change structure as a means to acclimatize to their environment. Nature, along four and a half billion years of evolution and natural selection, has evolved to take advantage of minimum resources to achieve maximal function by (Meyere et al., 2008).

Nature has a lot of examples of these materials they are organic or inorganic composite materials. Global challenges like food, water, homeland safekeeping, public health and clean energy can be solved by directly

applying the design principle of materials. In the natural world, numerous materials and systems are determined by molecular reformation and self-assembly. Learning from nature, the bio mimetic materials with smart structures are stimulated to be fabricated by (Walther et al., 2010).. In the past ten years noteworthy progress has been made in using the building block to realize various complex structures at the nano scale. The designed and fabrication of bio-mimetic material is greatly benefited by understanding the equivalent concept of formation and self-assembly. A decisive need for bio-mimetic materials in cellular biology in functional system to control and enumerate the bio physics and differentiation of cells. Various applications in bio-inspiration and bio- mimetic approach are headed to a number of techniques like chemical and biological sensors, actuators and flexible electronic devices. Not only has bio-inspired materials been processed by these examples mentioned above but by their materials and are also being used in bio- catalysis and enzyme immobilization. Increasing consideration is being applied to bio- catalysis due to its numerous economic and environmental benefits. Bio-catalysis generally refers to the use of enzymes, or enzyme-containing cells, in chemical renovation. Many active and promising bio-assisted processes benefit from the immobilization of enzymes in inorganic supports are nature's sustainable catalyses. The application of bio-catalysis and enzyme immobilization is to increase even further in future. Hence there is an unvarying need for more and advanced materials to be produced, that would outdo the existing materials. Compared to traditional chemical synthesis, the bio-mimetic or bio-inspired synthesis reveals a few advantages. The reviews in this special issue focus on some of the topics related to bio-inspired and bio-mimetic materials. Molecular recognition and self-assembly is vital for the structure, properties, and function of numerous materials and systems in the natural world.

Hence this review would commence the readers to the emerging and active bio-inspired materials and their use in the industry and to how humans are being benefited from the budding field.

## Bio-inspired materials

Bio-inspired materials as the name suggests, are materials that are plagiaristic from biological processes. The term ‘inspired’ means that of astonishing quality, as if arising from some external artistic impulse. Hence materials inspired by natural aspirations are bio-inspired materials. The properties of materials over millions of years have been optimized through natural selection. There are numbers of natural materials on hand for the production of bio-materials. The ambition and objective of producing bio-inspired materials or bio-inspiration is to resolve the aspect or modelling of the biological systems and to have a deep or detailed understanding of the natural materials and to increase the familiarity and understanding of physics-based processes in biological systems. Mimicking natural materials not only helps us to build better synthetic materials but provides us with the basic of the properties of the natural materials. It is due to ‘nature’ or natural process that the ideas of science and technology come to pass. There has always been a false impression between the term’s bio-inspiration and bio-mimicry. They may sound comparable but the latter is based on replicating the designs and processes of the biological materials. Multi-scale modelling has been hopeful transformation techniques. Bio-inspiration has been defined as ‘product or processes’ that is influenced or ‘informed by biology’. Observing the function, properties or trait of any product of biological evolution is what helps us to process bio-inspired materials. It is not based on the complexity of molecular structure of the product but simply on the function. For example, the researchers do not have to know how a bird flies to make an airplane.Someexamples of bio-inspired materials are discussed below.

## 2.1Naturally derived and bio-inspired materials.

There are hundreds and thousands of natural materials that are synthetically derived from biological materials. These materials have helped govern the basic principle behind naturally made bio-inspired materials. Some of the examples of naturally derived bio-materials are discussed further.

## 2.1 Materials inspired by tissues

The ECM (extra cellular matrix) has properties of bio chemical and mechanical properties that provide protection or a barrier to tissue. As we know the importance of ECM many important and useful strategies are being developed to reproduce its properties. The composition may consist of proteins, peptide and polysaccharide. With these new technologies and new methods in hand we will be able to produce better tissue development in injury and diseases, and better tackling of medical problems.

*Table 4: some of the materials inspired by tissues*

| Serial no. | Composition | Structure | Physiochemical properties | Bioactive factor presentation | References |
|---|---|---|---|---|---|
| 1. | DERIVED NATURALLY: | 2D VS 3D structures | Electrical conductivity | transport via | Cosgriff-Hernandez et al. (2015) |
| 2. | Peptides, polysaccharides. | patterned shapes and structures | hydrophobic / hydrophilic | degeneration rate of a carrier | Kilian et al. (2015) |
| 3. | Intact proteins | fibers | porosity | Binding domains | Gerecht et al. (2015) |

| | | | |
|---|---|---|---|
| 4. | -Denatured proteins | - Mechanical properties | Stabenfeldt et al. (2015) |

## 2.2 Bio-inspired functional materials with specific wettability

Wettability refers to the interaction between fluid and solid phase. A hierarchical structure has a great impact on their bulk property. This example deals with advances of bio-inspired materials with specific Wettability. Investigation of bio-inspired surface with special wettability has display great potential in forms like material science, medical, engineering fields and so on by (L. Wen et al., 2015). Investigation on bio-inspired surfaces with special wettability is owing to wettability related with other charming functions. Wettability determines the functional material surface and liquid droplets. The grouping of wettability with other super functions allows the invention of incorporated biological surfaces with enhanced performances. Here are some more examples. For example, super hydro phobic cicada wings display good antibacterial ability as well as outstanding anti relativity by (Sun et al., 2009). Super hydro phobic Guto's foot also holds self-cleaning capacity and high adhesion character by (Autumn et al., 2002).

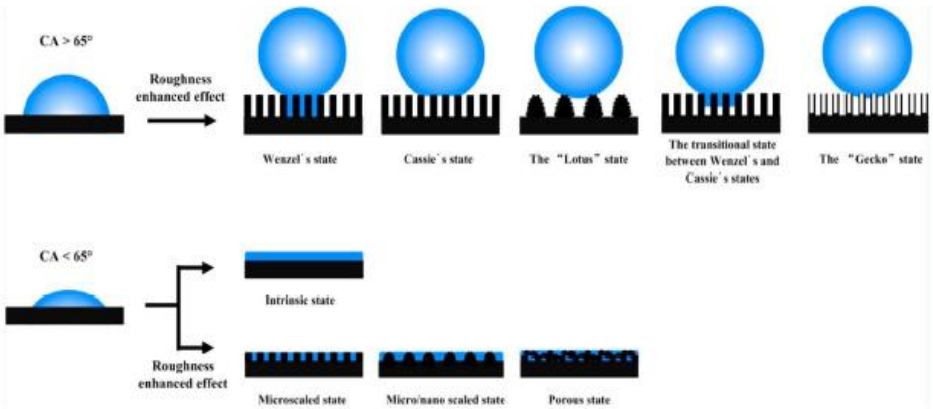

**Figure: 1. various types of super hydrophobic and super hydrophilic models (Guo et al., 2012)**

### 2.3 Bio-inspired materials and surfaces

Bio-inspired surface with advanced properties has been developed which show promising characteristics that are must be advanced than the traditional surfaces. By taking the concept and principle behind these naturally developed materials researchers have been able to open wide ranges of materials that provide us with the physicochemical choices like element selection, temperature, building block etc. These ranges can help improve the materials characteristics and bring about better functions like better robustness, stimuli-responsive or facture resistant. Some examples of these bio-inspired surfaces and materials are mentioned below. (Heinzimann et al., 2014) breathe life into the field of supra molecular polymer adhesives. The non- covalent binding nature's mechanism is what the materials are inspired from. Artificial materials were developed that depicted better performance. In the above, the materials showed enhanced surface selectivity. (Philips et al., 2015) mentioned about colloid based porous materials. These were inspired by material self-assembling hierarchical structures. These designs were inspired for the usage in optics, wetting, sensing, catalysis etc. **(**Wu et al., 2105)contributed an article about micro and nano fabricated structures. This was done to spatially coalesce and impound bacteria. Such structures are expected to lead to synthetic microbial societies. (Palivan et al., 2015) described about the polymer vehicles and membranes. These provide stable and functional compartments with preserving the cell structure of the living organism. Not only this they also support singling and selective transport.

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### 2.4 Bio-inspired materials that self-shape through programmed microstructure

Materials that change their shape in rejoinder to exterior environment have displayed a numerous in nature. These self-shaping bio-inspired materials have shown great applications in bio medicine, aerospace and material science. We consider self-shaping when the changes occur internally rather than externally. Here are some examples of bio-inspired materials that self-shape. For example, joining of two layers of metal on temperature change. This would create thermostat. This concept has been produced micro-scale multilayered objects with self- folding property by (Gracias et al., 2002). There are some natural systems

that take up CMF to resist swelling and growth of some direction in change provides self-shaping by (Burget et al., 2009).

### 2.5Bio-inspired material for regenerative medicine

According to (Barthelat et al., 2015) home of the best concepts that will make the translation from nature to material technologies are bio moralization and self-governing action. For example, the stimulation from the sticky toepads of frogs for improving the gripping surface of surgical grasps by (Chen et al., 2015).


**Figure: 2. Process chart showing how new bio mimetic products are produced**

### 2.6 Bio-inspirations for energy and environmental applications

To adapt the changes in environment biological systems optimized their complex structure and properties by billions of years of evolution and natural selection by (Liu et al., 2010). For instance. The microscopic studies on some organic matter like the Tokay geek foot (Hamen et al., 2005) lotus leave (Liu et al., 2008), morpho butterflies by (Watanabe et al., 2005) cicada wings by (Zhang et al., 2006). These bio-inspired materials provide milder conditions, the shape, their size, chemistry or even their crystal structure can be controlled. Not only this but these materials are highly specific and show multi-functions.

### 2.7Miscellaneous examples

1. Structural materials likes armor is used as a bio-inspired material .

2. High-performance fibers like Silk are another example of bio-inspired materials. The construction of recombinant silks based on parts of these sequences has been confirmed in bacteria.

3. Functional material like soft electronics is another example.

## 3. Role of biomaterials in bio-catalysis

Bio-catalysis plays a major role in a wide selection of biological and industrial processes ranging from environmental applications to the synthesis of chemicals. Mounting concentration is being applied to bio catalysis due to its numerous economic and environmental benefits. Bio-catalysis in the main refers to the use of enzyme or enzyme-containing cells in chemical transformation. Enzymes are nature's sustainable catalyzes. The applications of bio catalysis are to increase further in the outlook. Hence there is a constant need for more and advanced materials to be produced, that would outshine the existing materials. However, these catalysts are prone to inactivation during processing due to their biological nature and their detailed compassion towards physicochemical properties like ph, temperature or osmotic stress. Hence conception of long-lasting materials capable of creating keen environmental conditions around the biocatalyst is of most importance.

There are numerous roles of bio-inspired materials in the field of bio catalysis. Some of them will be described below.

**3.1 Bio inspired peptides**

These are one of the most sophisticated materials used in the industry. The bio functions of the peptides can be enriched by binding the inorganic nanomaterial's through their functional groups. Various bio materials are being constructed from peptides with specific sequences due to their properties like litheness, environmental compatibility, biodegradability etc. (Zou et al., 2015). A deep understanding of amino acid structure, self-assembly, mechanisms and dynamic behavior of peptide provides a better scheming of peptide (Nowinski et al., 2012). There are various advantages of bio molecules guides though self-assembly such as: 1 milder condition and simple/easy maneuver systems both in air-liquid interface. 2 high yields of products. 3 no usage of toxic reagents and no lasting by-products (Zang et al., 2014). As a result, capable applications in bio catalysis are obtained (Dehnorkhi et al., 2013). A spacious awareness has been drawn by peptide-based bio catalysis due to their inimitable cell penetration characteristic and the activity that is derived from in organic nano materials. Moreover, by absorbing enzyme on the surface or by exploiting the catalytic activity can peptide-based bio-catalysis accelerates biological reactions. For example, Wu and Algar analyzed the kinetics of proteolysis by using multivalent QD peptide substrate conjugate. The GSH-coated. QDs exhibited the highest level of proteolysis activity among other thiol ligands due to easier absorption of protease at the interface of the QD, which confirmed that QD surface chemistry played a noteworthy responsibility in mediating the physicochemical characteristics.

### 3.2 Bio inspired silica

Marvelous advancement has been made in biological and bio-inspired silica configuration. A range of applications has arisen in the area of bio-catalysis. Silica manufacture industry covers a great range of applications in catalysis, division, and food and drug technology. (Pagliaro et al., 2009). It appears that biology has recognized ‘clever’ and green ways to manufacture metaphorical structures of non-materials. Bio silica in organisms, for the most part, serves as a mechanical/structural support that provides fortification from predators and acts as sensor. This method was used to immobilize butyryl-cholinesterase onto silica where superior performance, up to 90% immobilization capability and high loading was achieved.

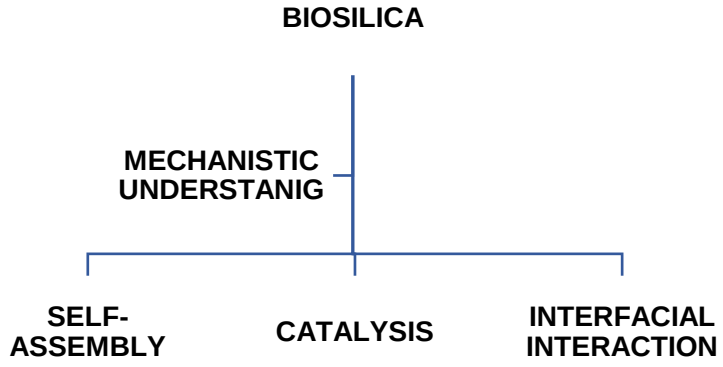


**Table 2: Schematic representation of the journey from research on bio-silica**

### 3.3 Bio-inspired drug liberation systems.

These have also shown magnitude in the enlargement of materials synthesis with nano scale exactness is well long-awaited by (Park et al., 2010). Fabrication of materials can create modernization of functionality where improvement of fabrication precision might result in efficient creation of new function. Precision in materials fabrication resembles the nanometer scale with the association technology becoming known as nanotechnology. A new hypothesis shelf in nanomaterial’s science has become necessary. However, nano-architectonics is not as simple as the already well-established micro fabrication techniques.

### 3.4 Enzyme encapsulation for bio-catalysis

Bio-catalysis adapts to the resourcefulness of enzyme to catalyze an assortment of production of narrative compounds. Bio-catalysis capitalizes on the metabolic diversity of an enzyme for commercial understanding of specially produced products. The development of successful strategies to immobilize enzyme while retaining catalytic activity is compulsory by the development of enzyme in bio-catalysis. Immobilization of silica has so far been inhibited to enzymes but the loom for the encapsulation and stabilization of other bio molecule is level-headed but requires future studies. A wealth of diverse nano scaffolds has been provided through nanotechnology that could support enzyme immobilization by (Kim et al., 2006). To weaken the surface area and to increase enzyme loading, along with eliminating the limitations is the prime target for using nano scale structures for immobilization. The catalytic activity of the attached enzyme can have an impact from the physical attribute of nano particles by (H. et al., 2003). When silica is the product reaction is termed as bio-silicification and the process has been comprehensively studied epically in the field of diatoms and marine sponges by (C.C et al., 2003) .Silica configuration catalyzed by the R5 peptide seems to be versatile in its ability to encapsulate a wide verity of monomeric and multi meric enzyme. The biological fusion of the inorganic oxides has shown leads to a variety of novel matrices for enzyme immobilization.

### 3.5Genetic engineering of supramolecular assembled FDH using bio-catalysis

The genetic engineering of supramolecular assembled FDH using bio-catalysis is another role of bio-catalysis in the fabrication of bio-inspired materials. A bio-inspired approach for the production of supramolecular and bio-catalytically the genetic engineering and protein-protein interaction techniques were used to develop the materials. Formats dehydrogenase and its useful fragments were fused distinctly so that is forms a multi-function sphere. Characterization was done on the basis of morphology and the scanning-electron microscopy was used to show the proteins that were fused and the assembled functional fragments that formed a 3D and 2D layered structure. Furthermore, the biocatalysts of the oligomers showed much higher structural stability and NAH(H) salvaging efficiency compared to the structures that were unassembled when applied along with the co-enzyme rejuvenation system. These results shows that the bio-inspired procedureoffers a improved approach for the supramolecular fabrication of FDH materials through the genetic engineering and the self-assembly approach. The major improvisation on the bio-catalytically

Bustle exposes the important roles of the interface design of the supramolecule in their bio-catalysis application.

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## 4. Applications of bio-inspired material in enzyme immobilization

Many standing and evolvingdevelopments that are bio-assisted are benefitted from the enzymes immobilization onto the inorganic supports. Enzymes are the biological catalysts for any chemical reaction which has a high specificity and productivity under normal to mild conditions. To increase the stability and reusability, enzymes are frequently immobilized with the help of certain carriers (Klibanov. 1983).During enzymatic reactions the materials that follow the typical protocol face some serious negative aspect like the poor enzyme loading and high enzyme discharge by (Pandya et al., 2005). The same existing applications includes the industrial processes, waste management treatment, the pharmaceuticals products and the biosensors by (Klibanov et al., 1983).Immobilization of the enzymes issignificant because of the process economics which allows the re-using of the enzymes and by improving the overall productivity and strength of the procedure and the enzyme. Membranes are well thought-out to be an appropriate support for immobilization of the enzyme. The enzymes that are immobilized are the ones that are to be attached to the inert material. This section will be discussingthe applications of the enzymes that are immobilized for bio inspired materials. The development of the biomaterials for immobilization systems requires the contribution of biochemicals, kinetics and reactor designs principles. One of the examples of the commercial scales are the production of fructose rich syrup from starch. Other examples of application of bio inspired material in enzyme immobilization are discussed below.

### 4.1 Immobilized biomaterials used in surgery

Application of immobilized biomaterials used in surgery. In the years as the expansion of technique has developed in case of the binding enzymes to the insoluble supports, the immobilized enzymes are to be utilized freshly in numerous types of therapeutic applications, specifically in clinical analysis. For the enzyme immobilization, the synthetic polymer composite materials of collagen are applied to the support so as to set up the biological function of the same on the surface due to which the enzyme is effectively bound

to the membranesof the collagen by the carboxyl group activation. To the surface properties of the composite materials Trypsin and Urokinase are chosen with the purpose of adding proteolysis activities .For the purpose of producing bacteriolytic and antibacterial bio materials, the lysozymes and an antibiotic of peptide form are bound to the material, which will prevent serious problem caused by bacterial infection from arising when the artificial organs and the biomaterials are rooted into the body.

### 4.2Production of the l-amino acids

Production of the l-amino acids is another example of bio-inspired material used in enzyme immobilization. Due to their consumption for medical and food purpose, the production of the L-amino acid is one of the big businesses in the world. Approximately $300-million worth was produced in Japan alone(Yamada et al., 1977). Amino acid can be formed by the isolation from a protein hydrolyses by the fermentation or by using the chemical synthesis. The technology of immobilization of the bio-inspired has helped in making the chemical synthesis a feasible option. In an automatically controlled process which is continuous, the mixture of the amino acids is first of all acylated then this mixture is exposed to hydrolysis with the help of immobilized amino acylase. A blend of acryl-D and the L-form of the amino acids are attained, and these can be separated with the means of the solubility differences. The aCy1-D formation of the amino-acid is racemized and then reused Snamprogetti S.P.A. In Italy was able to achieve pilot-scale application of the amino acylase after immobilization for the production of tryptophan (Bartoli et al., 1976).

### 4.3 Production of the organic complexes

The production of the organic complexes is one other application of immobilized enzyme using bio-inspired materials. Manufacturing by the exploitation of the biomaterial that was immobilizedand its technology such as the L-malic and the urocanic has been practiced out (Chibata et al., 1977). Immobilization of the whole-cell often have bioactivity other than the one that is desired of it. It was also studied that interrupting the polyacrylamide gel that is entrapped in the cells in the solution of the substrate that contains the detergents such as deoxycholic acid or bile acid, not only suppresses the formation of the succinic acid but it also enhances the activity of the fumarase 6-7times. Biomaterial immobilization technology give the impressiondignified to deal with the dairy industry which is a new product opportunityout of the problem of the maximum utilization.

Hence there are so many examples for the industrial applications in which the enzyme in the native of immobilized enzymes are being used everywhere like in the food industries, in material processing industries, in textiles industries, in making of the detergents, biochemical, biotechnology and in pharmaceuticals industries. Due to the relative instability under certain condition of the enzymes, they are still limited to the extent of their applications. To increase the steadiness of the enzymes and to encounter these drawbacks techniques like addition or additives, chemical; adaptation, protein engineering and enzyme immobilization have been used.

**Limitations of enzyme immobilization**

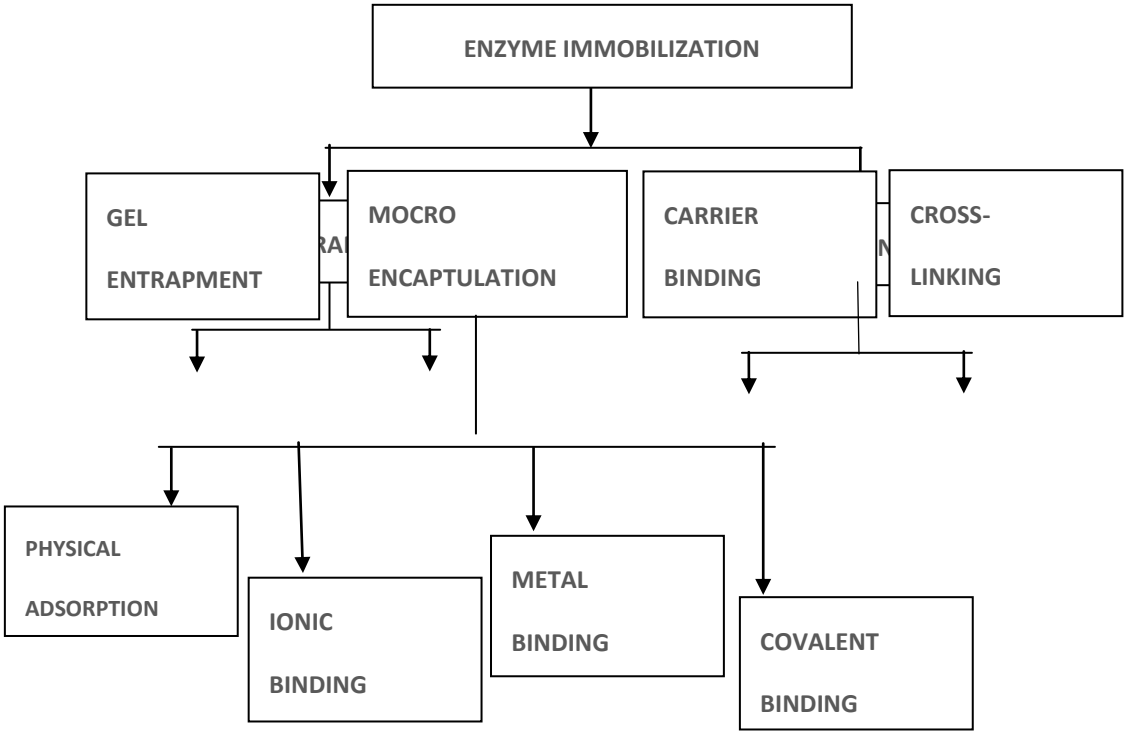
Despite the fact that immobilized enzymes with all aforementioned advantages have tremendous potential, their industrial applications have been limited so far. The cost-effectiveness that an immobilized enzyme is supposed to provide is often not there in true sense. Many sensitive enzymes which are isolated in small quantities at high cost either become inactive or exhibit truncated activities on immobilization. The yield of the scarce enzyme is reduced further, as the immobilization process is not quantitative and the amount of immobilized enzyme is generally restricted to less than 0.02gramme per gramme of the matrix. Immobilization generally results in orientating the enzyme with respect to the matrix surface. This may end up with the blockage of some active sites so that they are not accessible to the substrate. This phenomenon is very broad-based and is associated with practically every immobilization strategy. Although immobilization by milder physical effects like adsorption or encapsulation generally do not affect the conformation of the enzyme, covalent bonding between the enzyme and the matrix support may distort the enzyme structure, and thus, affect the enzymatic activity.

## Chapter 3

## 3. METHODOLOGY

The term immobilized means unable to move or stationary and is truly used in the context of enzymes as well. Enzymes are literally tied to a solid support or imprisoned within physical boundaries that make them immobilized such that they are not able to roam around like a free molecule anymore. They become part of the physical structure that immobilizes them and are aptly called immobilized enzymes. Once immobilized, enzymes undergo several behavioral changes. The ultimate dream of any person engaged in purification and utilization is to get an enzyme that has a long shelf life, and like any other tool, can be reused. Enzymes are very sensitive and generally highly unstable at elevated temperatures. Unfortunately, none of these requirements are ordinarily met in catalytic reactions used in most industries. Fortunately, immobilized enzymes have the potential to overcome most of these negativities associated with soluble free enzymes.

### 3.1 Strategies of enzyme immobilization



### 3.2 Experimental demonstration

- Preparation of PBS buffer: PBS has many uses because it is isotonic and non-toxic to cells. It is used to rinse containers containing cells. PBS can beused as a diluent in methods to dry biomolecules, as water molecules within it will be structured around the substance (protein, for example) to be 'dried' and immobilized to a solid surface.

  A final volume of 1l of PBS buffer was prepared. The pH was set to 7.2 which is according to the requirements for lipase assays and immobilisation. The solution was kept at 4°C for further use.

- Preparation of substrate: the substrate of lipase is para-nitro-phenyl palmitate (pnpp). During the determination of lipase enzymatic assay, the substrate of lipase, i.e. para-nitro-phenyl palmitate (pnpp) breaks down into para-nitro-phenol which gives a yellowish colour. The change in colour of solution was used as an indicator for lipase activity as the dye turns yellow. Mol weight of pnpp is 263.05g/mol. 0.263g of pnpp was dissolved in 10ml of isopropanol.
- Preparation of lipase stock solution: A stock of 10mg/ml was prepared by adding 10mg of commercial lipase to 10ml of PBS buffer. This was then stored at 4°C for future use.
- Enzymatic assay and enzymatic activity: Enzymatic assay are laboratory methods for measuring enzymatic activity, whereas enzymatic activity is the measure of catalytic ability of the enzyme. For enzymatic assay, readings (OD) were taken in spectrophotometer at 410nm.

*Table 5: enzymatic assay*

| **Blank** | **Control** | **Test** |
|---|---|---|
| 1000µL buffer | 825 µL buffer + 175 µL substrate | 800 µL buffer + 175 µL substrate + 25ml enzyme |

**Incubation at 40°C for 5mins**

- Bradford Protein assay: the assay is used to measure the concentration of total protein in a sample. Bradford is used to measure the concentration of *protein* in a solution. The reaction is dependent on the amino acid composition of the measured *proteins*.

This was then vortexed for proper mixing of the chemicals.

Readings (OD) were taken in spectrophotometer at 595n

*Table 66: Results for enzymatic activity, protein concentration and specific activity (c: without modification and cc: with modification)*

| **Assays** | **Without immobilization** | **With supernatant (after immobilization)** | | **with surface (after immobilization)** | |
|---|---|---|---|---|---|
| | | **c** | **cc** | **c** | **cc** |
| Activity (U/ml) | 72.001 | 16.09 | 26.53 | 63.74 | 118.76 |
| Protein Conc. (mg/ml) | 0.378 | 0.19 | 0.17 | 0.60 | 0.60 |
| Specific activity (U/mg) | 190.47 | 84.73 | 94.7 | 105.54 | 196.62 |

*Table 7: Immobilization yield and Immobilization efficiency*

| **Parameters** | **c (without modification)** | **cc (with modification)** |
|---|---|---|
| Immobilization yield% | 50.26 | 44.97 |
| Immobilization efficiency% | 55.41 | 103.22 |

To further verify and compare the results of our previous procedure, the assays were again performed on the surfaces.

*Table 8: For C (without modification)*

| **Control** | **T1** | **T2** |
|---|---|---|
| 0.701nm | 0.705nm | 1.108nm |

*Table 9: for cc (with modification)*

| **Control** | **T1** | **T2** |
|---|---|---|
| 0.445nm | 0.877nm | 1.221nm |

*Table 10: Activity and Specific activity*

| **Activity** | | **Specific Activity** | |
|---|---|---|---|
| **For c** | **for cc** | **For c** | **for cc** |
| 33.08 U/ml | 97.72 U/ml | 54.76 U/mg | 161.78 U/mg |

For further optimization and comparison of results we are going to perform immobilization with buffers ranging from different pH.

- For acidic pH buffer used will be: Phosphate buffer and acetate buffer
- For basic pH buffer used will be: Tris buffer

**Preparation of Tris Buffer (1M)**

**Components used**:

Tris base = 60.57g

**Preparation**

Prepare 400 mL of distilled water in a suitable container.

Add 60.57g of Tris base to the solution.

Adjust solution to desired pH using HCl (pH ≈ 8.0).

Add distilled water until volume is 500ml.

**Preparation of Acetate Buffer**

**Components used:**

Sodium acetate = 2.886g

Acetic acid= 0.889g

**Preparation**

Prepare 400mL of distilled water in a suitable container.

Add 2.886g of Sodium Acetate to the solution.

Add 0.889g of Acetic Acid to the solution.

Adjust solution to desired pH using HCl (pH ≈ 5.0).

Add distilled water until volume is 500mL.

**RESULTS**

**Without immobilization**

*Table 11: Bradford results*

| **Ph** | **4** | **5** | **6** | **7** | **8** | **9** |
|---|---|---|---|---|---|---|
| **ΔOD (nm)** | 0.101 | 0.108 | 0.114 | 0.134 | 0.133 | 0.134 |

*Table 12: Protein Concentration*

| pH | 4 | 8 |
|---|---|---|
| **Protein conc. (mg/ml)** | 0.323 | 0.476 |

*Table 13: Activity*

| pH | 4 | 8 |
|---|---|---|
| **Protein Concentration (mg/ml)** | 13.105 | 567.89 |

*Table 14: Specific Activity*

| pH | 4 | 8 |
|---|---|---|
| **Specific Activity (U/mg)** | 40.57 | 106.99 |

**With Immobilization**

*Table 15: With Supernatant for pH 4*

| Surfaces | Without modification (c) | With modification (cc) |
|---|---|---|
| **Activity (U/ml)** | -27.02 | -51.93 |
| **Total protein (mg/ml)** | 0.3781 | 0.3785 |
| **Specific activity (U/mg)** | -71.46 | -137.199 |

*Table 16: With supernatant for pH 8*

| **Surfaces** | **Without modification (c)** | **With modification (cc)** |
|---|---|---|
| **Activity (U/ml)** | 252.16 | -186.23 |
| **Total protein (mg/ml)** | 0.480 | 0.486 |
| **Specific activity (U/mg)** | 515.66 | 383.18 |

*Table 17: With Surface for pH 4*

| **Surfaces** | **Without modification (c)** | **With modification (cc)** |
|---|---|---|
| **Activity (U/ml)** | 57.11 | -16.50 |
| **Total protein (mg/ml)** | 0.053 | 0.53 |
| **Specific activity (U/mg)** | 1077.54 | -311.32 |

*Table 18: With surface for pH 8*

| **Surfaces** | **Without modification (c)** | **With modification (cc)** |
|---|---|---|
| **Activity (U/ml)** | 151.52 | 162.20 |
| **Total protein (mg/ml)** | 0.013 | 0.01 |
| **Specific activity (U/mg)** | 11655.38 | 16220.0 |

## Conclusion

In this review, we concluded about lipase immobilization onto Polypropylene surfaces and tried to study the advantages of polypropylene surface. Polypropylene was efficient support as the advantages of polypropylene are provides a biocompatible interface, work at low temperature, low atmospheric pressure and are not usually violent to reactors and accessories. Immobilization is very engaging for lipases as a result of, additionally to the traditional edges of accelerator immobilization, it can even result in a substantial increase in chemical process activity, in all probability caused by conformational changes within the enzyme molecules. Activation is often achieved, for instance, exploitation hydrophobic support materials or surfactants throughout the immobilization procedure. We have also concluded about what bio-inspired materials are and there uses in bio-catalysis and its applications in enzyme immobilization. It is concluded that the basic principle and role of bio-inspired materials in various aspects such as materials properties and their novel application, multi-scale approaches provide development of new generation intelligent materials from nano to macro scale. As new implements are made-up and acquaintance gained, we will be able to better emulate tissues in development, injury and disease, and subsequently provide better solutions to challenging medical problems. Although current research is still far from reproducing the functionality of natural systems, momentous progress has been made in the speedily growing field during the past couple of decades. Enzyme immobilization methods have been widely investigated for many years, but current developments in stabilizing enzymes within bio-inspired inorganic matrices have substantially absolute the range of operational stabilities. The recent successes in some self-assembling protein systems indicate a great potential for improving the performance of functional materials, ranging from bio-catalysis, bio-detection, vaccine design to targeted and controlled drug delivery. The noteworthy growth in this field will continue to be fueled by the development of interdisciplinary collaborative efforts between nano science, materials science, and chemical biology, which are expected to bring new breakthroughs in engineering bio-inspired materials Hence bio-inspired materials are the new technology of the new generation and in the approaching years there will be greater advances in this field than seen today.

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