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Immobilization as a powerful Bioremediation tool for abatement of dye pollution: A review

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Abstract: Dyes are xenobiotic compounds widely used by textile, leather, paper, printing, food, pharmaceutical, and cosmetic industries. Decolorization and dye degradation in the effluents is a prime hurdle in its treatment, and there is still a shortage of economically attractive and easy-to-operate treatments that can eliminate dye pollution. In recent years, chemical-based treatments are being replaced by greener technologies at the lab and industrial scale to combat dye pollution. It is noteworthy that immobilization is a biotechnological tool that greatly enhances bioremediation's potential. The present review has covered the basic concepts of immobilization, including the different immobilization techniques and the various carriers used for immobilization. The efficient immobilization of a biocatalyst depends on the proper choice of a carrier combined with a suitable immobilization technique. Hence, this review provides a comparative analysis of the different immobilization techniques and carriers used. Further, there is an in-depth discussion on the potential of immobilized enzymes and cells as bioremediation agents for dye degradation. Nearly all the studies indicated that immobilization enhanced the biodecolorization of colored wastewater compared to free systems. Further, the potential of immobilized systems for large scale industrial implementation was also examined. The article ends with a note on the loopholes of research on immobilization and future scopes of this technique.

Keywords: Biocatalyst; Biodecolorization; Bioremediation; Carriers; Dye degradation; Immobilization

Introduction

Industrialization is an essential pillar of socioeconomic growth. However, it has consequences in disrupting environmental health (Bilal and Asgher 2015a; Lade et al. 2015). Considerable amounts of wastewater are generated year-round through different industrial processes. It was estimated that more than 80% of industrial effluent is released globally without proper treatment (Connor et al. 2017). The dyeing industry is a crucial generator of environmental pollution in terms of the large volume of water usage and the chemical composition of the wastewater. A significant concern is the release of industrial dyes or dye-containing toxic industrial effluent without or with ineffective treatment (Verlicchi et al. 2012; Ba et al. 2014; Hayat et al. 2015; Zucca et al. 2016). Synthetic dyes have a broad application in diverse industries viz., textile, leather, printing ink, paper, pulp, plastic, photographic, cosmetics, food, and medicine (Benkhaya et al. 2020). Generally, color indexing

enlists about 8000 chemically differing synthetic dyes registered with 40,000 different trade names (Yuan et al. 2020). Among these synthetic dyes, azo dyes are the commercially dominant class of dyestuffs. Chrysoidine is among the earliest commercial azo dyes and is used for dyeing cotton. Mordant Black 17 is widely used by the textile and dyeing industry. Direct Red dye is used for dyeing leather. Reactive Blue 171 is used for dyeing cellulosic fibers. Azo dyes as Tartrazine, Yellow 2G, Sunset Yellow, Ponceau 4R are frequently applied in the food industry (Benkhaya et al. 2020). Anthraquinones, phthalocyanines, aryl-carboniums, and polymethines are the other dye classes (Gordon and Gregory 1987). The textile industry alone utilizes more than 3600 different types of chemical dyes (Kant 2012). During the dyeing process, not all of the dyes are fixed and what remains is discharged along with wastewater. The amount of dye lost varies among the different dye classes. The amount of unfixed dye ranges from 20-50% for reactive dyes, 30-40% for sulfur dyes, 5-10% for azoic dyes, 5-20% for vat and direct dyes, 7-20% for acid and disperse dyes, 2-3% for basic dyes, and 1-2% for pigment dyes (Ghaly et al. 2014). Azo dyes, in particular, are associated with several hazardous environmental impacts. For example, untreated wastewater from the industries pollute water bodies by rendering them unsuitable for other purposes, reducing sunlight penetration thereby affecting photosynthesis, and it severely impacts the equilibrium of aquatic ecosystems as a result of their carcinogenic, genotoxic, and mutagenic nature (Vandevivere et al. 1998; Suteu et al. 2009; Hassan and El Nemr 2017). For a long time, scientists have made rigorous attempts to devise efficient, cost-effective techniques to remediate dye-containing wastewater. An array of treatment techniques has been developed, which include physicochemical, biological, and combined treatment processes. Few treatment methods currently being used for dye removal includes flocculation, sedimentation, dissolved air floatation, clarification, neutralization, precipitation, activated sludge process, biological filters, anaerobic treatment systems, granular media filtration, membrane filtration, reverse osmosis systems, ultrafiltration, nanofiltration, ion exchange, activated carbon, and ultraviolet disinfection (Robinson et al. 2001; Wang et al. 2011). These methods can be used as preliminary, primary, secondary, tertiary, or advanced treatments. However, these processes suffer significant drawbacks in terms of their high energy requirement, impractical reaction conditions, high sludge formation, secondary pollution problem, complex procedures, high cost, and low efficiency (Shaw et al. 2002; Zhang et al. 2004; Aravindhan et al. 2007; Sarioglu et al. 2007; Wang et al. 2009). Hence, there remains an urgency for a highly efficient and economical treatment method for dye removal. Environmental engineers put considerable effort into developing new methods or improving the existing technologies (dos Santos et al. 2007; Kabra et al. 2013; Sathishkumar et al. 2014). However, newly developed technologies are not employed at a large scale because they are expensive, labor-intensive, require

extreme operational conditions, secondary pollution, and have a low spectrum of activity for decolorizing dyes with different chemical structures (Fongsatitkul et al. 2004; Fersi and Dhahbi 2008; Hayat et al. 2015).

Although bioremediation is a relatively modern tool, the concept has existed for quite a while. This strategy initially received a lot of enthusiasm but suffered through failures. The accompanying failures in turn helped develop a more practical approach to bioremediation. Continuing research has highlighted some of the limitations of bioremediation, and there remains room for improvement. Biological removal of dyestuff follows three underlying mechanisms: (1) biosorption on biomass, (2) biodegradation by cells, and (3) biodegradation by enzymes (Khan et al. 2013). The large-scale application of bioremediation techniques by industries is hindered by two major drawbacks: stability for long-term operation, and the issue of recovery and reuse. Immobilization of the bioremediation agent improves its survival, stability, and retention. Immobilization is slowly gaining importance in wastewater treatment as it delivers better results when compared to the activity of free cells or enzymes (Bilal and Asgher 2015a; Talha et al. 2017; Bayramoglu and Yilmaz 2018). The efficient use of bioremediation requires patience and, when used appropriately, might deliver substantial long-term benefits.

A considerable amount of research data is available on immobilization and its potential application in the field of environmental remediation. This article intends to provide researchers with a comprehensive review of all aspects of immobilization. This article discusses the types of immobilization and carrier selections for immobilization. Further, the application of this technique for immobilizing cells and enzymes for dye removal will be elaborated. . In contrast to review papers that focus on dye removal of either immobilized enzymes or cells, this article will provide important insights into the potential of immobilization for dye removal by both enzymes and cells. It also attempts to pinpoint the loopholes of this technique and provide a direction for future development.

1. Immobilization

Immobilization refers to the technique of entrapping or anchoring biocatalysts in or on an inert carrier or support, rendering them more stable and functionally reusable. The carrier involved in immobilization allows for the interchange of media, which contain the substrate or effector or inhibitor molecules (Zhu 2007). There is a broad spectrum of immobilization and it has been used with various biocatalysts including enzymes, cellular organelles, microbial, animal, and plant cells. Immobilization is a natural phenomenon, as pointed out by Radwan et al. (2002). In their study, samples of microalgae along the Arabian Gulf coast were examined and

covered by a biofilm of oil-degrading bacteria found in seawater and that are capable of hydrocarbon degradation. In 1969, Japan applied enzyme immobilization for the first time to continuously produce L-amino acids from acyl DL-amino acids. Immobilization methods have been used extensively ever since, in many laboratories (Chibata 1996). Immobilization provides several advantages when applied in the arena of environmental biotechnology including: 1) improvement of biodegradation efficiency, 2) cost reduction as multiple usages of remediation agents is possible, 3) elimination of filtration of the remediation agents, 4) reduction in the risk of undergoing genetic mutations, 5) a stable microenvironment for the remediation agents, 6) resistance to shear forces when operated in a bioreactor, 7) improvement of resistance of the agents towards harsh environmental conditions, 8) extension of storage period for the biocatalysts, and 9) enhancement of the tolerance of remediation agents towards high pollutant concentrations (Kourkoutas et al. 2004; Wojcieszyńska et al. 2012; Rivelli et al. 2013; Bayat et al. 2015).

a. Immobilization Techniques

There are five main immobilization techniques currently in practice: (a) adsorption, (b) binding on a surface (electrostatic or covalent), (c) copolymerization or crosslinking, (d) entrapment, and (e) encapsulation (Kourkoutas et al. 2004; Sun et al. 2011; Bayat et al. 2015) (**Fig.** 1). The following subsections are a brief account of each of these techniques. Table 1. illustrates a comparative analysis of the advantages and disadvantages of the five types of immobilization techniques (Dubey 2010; Elnashar 2011; Bayat et al. 2015).

Adsorption

Adsorption immobilization is the binding of biocatalysts to the outer or inner surface of a carrier by forming low energy bonds such as ionic bonding, hydrogen bonding, and van der Waals forces (Cristóvão et al. 2011; Hou et al. 2014; Jesionowski et al. 2014). This process is reversible. The main reason for successful adsorption is the presence of an enzyme/ cell- carrier affinity. Affinity is the consequence of certain functional groups present on the carrier. However, in the absence of such groups, interactions can be generated by the addition of an intermediate agent (carrier modifiers). There are four main techniques to achieve immobilization by adsorption: the static process, the dynamic batch process, the reactor loading process, and the electrodeposition process (Bilal et al. 2017b). Enzymes of the class oxidoreductase such as tyrosinase, laccase, peroxidase established as potential green catalysts have been successfully immobilized onto carriers via adsorption for dye removal (Valli et al. 1990; Ihekata and Nicell, 2000; Qiu et al. 2009; Faccio et al. 2012; Šekuljica et al. 2016a; Senthivelan et

al. 2016; Tonin et al. 2016; Shaheen et al. 2017; Yehia and Rodriguez-Couto 2017). However, most research has been conducted with laccases (Cristóvão et al. 2011; Bayramoglu et al. 2012b; Zhang et al. 2014). Peralta-Zamora et al. (2003) investigated the dye degradation potential of immobilized fungal laccases by adsorption on different supports. Silica modified with imidazole groups supported an enzyme activity of 12 U g^{-1}. In a continuous study, immobilized laccase was able to decolorize 90% of Reactive Orange 122 in the presence of mediator 1-hydroxy benzotriazole in the eighth cycle. In a recent study, horseradish peroxidase (HRP) was immobilized through adsorption and covalent bonding methods on a novel carrier Purolite® A109 (Šekuljica et al. 2020). Adsorption immobilization yielded comparatively higher enzyme activity (156.21 ± 1.41 U g^{-1}) but lower operational stability (~60% activity after 10 cycles) and lower shelf life (~60% activity after 4weeks of storage). Adsorption immobilized HRP (IM-HRP) showed a slight increase in dye removal (~75%) of Acid Violet 109 compared to covalent IM-HRP (~70%) within 30 min. Relatively few research articles are available on cell immobilization via adsorption. Fungi such as *Phanerochaete chrysosporium*, *Trametes hirsute* have been most commonly examined for their capability to degrade dye (Guimarães et al. 2002; Couto et al. 2004; Pazarlioglu et al. 2005). Lade et al. (2015) immobilized a microbial consortium on polyurethane foam by adsorption. The immobilized consortium was capable of completely removing 100 mg L^{-1} of Congo Red within 12 h, and 92% of real textile effluent from the American Dye Manufacturing Institute within 20 h. In another study, *Brevibacillus parabrevis* was immobilized by adsorption on coconut shell biochar (Abu Talha et al. 2018). Immobilized bacterium operated efficiently in a continuous bioreactor and was capable of 88.92% of dye removal at a concentration of up to 500 ppm.

Covalent Binding

Immobilization through covalent binding occurs by the formation of a covalent bond between chemical groups on enzymes or cells, and chemical groups on the carrier surface. The immobilization procedure follows the binding of a coupling agent followed by activation, or attachment of a chemical group, and finally binding to the enzyme or cell (Martins et al. 2013). This technique is the most irreversible and stable. The different covalent binding methods are diazotization, peptide bond formation, group activation, and polyfunctional reagents (Bilal et al. 2017b). In comparison to enzymes, covalent binding is not commonly used for cell immobilization. Binding or cross-linking involves cytotoxicity and results in cellular death. It is difficult to prevent cellular damage during this process, and hence it is applied mainly for enzyme immobilization (Bayat et al. 2015). Some of the enzymes covalently immobilized for dye degradation include laccase, azoreductase, and HRP (Šekuljica

et al. 2016b; Qi et al. 2017; Zhu et al. 2020). Arica et al. (2009) utilized a spacer arm (1,6-diaminihexane) attached poly (glycidyl methacrylate/ethylene glycol dimetacrylate) beads support to covalently immobilize laccase and experimented with its potential for the degradation of Reactive Red 120 (RR 120). An amount of 4.6 mg g^{-1} of laccase was immobilized on the carrier which yielded an enzyme activity of 88%. In batch mode, 91% of RR 120 was degraded within 10 h. The enzyme activity was over 50% even after six cycles of repeated dye addition. Another study also covalently immobilized laccase on magnetized (Fe_3O_4 nanoparticles) chitosan beads (Nadaroglu et al. 2019). The relative activity of immobilized laccase was increased 2-folds in contrast to free laccase under optimum conditions. Immobilized laccase was capable of degrading several synthetic dyes as Reactive Black 5 (73%), Direct Blue 15 (95%), Evans Blue (96%), and Acid Red 37 (97%) at a contact time of 60 min. HRP covalently immobilized on amine-functionalized glycidyl methacrylate-g-poly (ethylene terephthalate) fibers, removed 98% of Methyl Orange dye, while free enzymes removed only 79% of dyes within a contact time of 45 min. Immobilized HRP retained 69.9% of its relative activity, even after 10 cycles of repeated usage (Arslan 2011). In a similar report, HRP was immobilized covalently on various polysulfone supports (Hydroxyl-terminated bisphenol A- and bisphenol AF-based polysulfones) (Celebi et al. 2013). Immobilized HRP was capable of decolorizing >85% of Reactive Blue 19 and Acid Black 1 within 1 h. The immobilized enzyme retained 70% of its activity even after three reuses and gradually decreased thereafter.

Cross-Linking or Copolymerisation

Cross-linking is described as covalent bond formation between a variety of molecules of an enzyme or cell with the help of polyfunctional reagents such as glutaraldehyde, diazonium salt, hexamethylene diisocyanate, and N-N′ ethylene bismaleimide. The primary methods of immobilization by crosslinking are insoluble aggregate formation by crosslinking of cells or enzymes with glutaraldehyde, enzyme adsorption followed by cross-linking, and enzyme or cell impregnation of a porous carrier followed by enzyme or cellular cross-linking in pores (Dubey 2010; Bilal et al. 2017b). Cross-linking generates high molecular weight insoluble aggregates, and since covalent bond formation is involved, they lead to a conformational change of the biocatalysts, resulting in loss of activity. Another disadvantage that limits the application of cross-linking, is the gelatinous physical appearance of the biocatalyst aggregates. It is often used in combination with other immobilization techniques to prevent biocatalyst leakage. Several enzymes such as laccase, tyrosinase, and peroxidases have been immobilized via crosslinking and used for dye degradation (Wu et al. 2001; Kumar et al. 2014b; Nguyen et al. 2016; Bilal et al. 2017c; Ma et al. 2018; Veismoradi et al. 2019). In a study performed by Bayramoglu et al.

(2012a), HRP was initially adsorbed on polyaniline grafted polyacrylonitrile film and then cross-linked using glutaraldehyde. Immobilized HRP degraded 79% of Direct Black-38 and 53% of Direct Blue 53, whereas the free enzyme could degrade only 48% Direct Black 38 and 39% Direct Blue 53 within 6 h of interaction period. The immobilized enzyme also showed excellent reusability for up to 30 assays. Another interesting study co-immobilized *Phanerochaete chrysosporium* cells with cross-linked fungal enzyme aggregates (lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase) in calcium-alginate beads (Li et al. 2015). The co-immobilized gel beads worked as an efficient biodegradation system and showed enhanced degradation of dyes Acid Violet 7 (93.4%) and Basic Fuchsin (67.9%). However, the reusability of the beads was reduced to ~45% after 4 cycles due to leakage of the cross-linked enzyme aggregates. MnP was crosslinked and immobilized in chitosan beads for the degradation of textile effluent (Bilal et al. 2016b). The immobilized enzyme decolorized diluted and undiluted textile wastewater in the range of ~97% to ~67%, which was much higher compared to free enzyme. The immobilized enzyme was also capable of retaining 60% of its activity even after 10 batches of effluent decolorization. Similarly, laccase was also cross-linked and immobilized in chitosan microspheres (Asgher et al. 2017). Immobilized laccase showed decolorization of five different textile dyes Sandal-fix Red C_4BL, Sandal-fix violet P4RN, Sandal-fix Golden yellow CRL, Sandal-fix Black BR, and Sandal-fix Turq. Blue GWF in the range of 98.7% - 89.36% after 4 h of interaction period. The immobilized enzyme was also capable of retaining more than 60% of its activity after nine cycles of Sandal-fix Red C_4BL decolorization. Research remains sparse on the applicability of cells immobilized by cross-linking in dye degradation.

Entrapment

Enzymes or cells can be physically entrapped inside synthetic or natural polymeric networks. A permeable membrane allows substrate and product molecules to pass through but preserves the biocatalyst. The methods for entrapment immobilization include gel inclusion, fiber inclusion, and microcapsule inclusion (Bernfeld and Wan 1963). The critical point in the entrapment of microorganisms is the ratio of the pore size of the carrier to that of the cell diameter. If the pore size is bigger than the immobilized cells, then it will cause leakage. It is an irreversible technique (Kourkotas et al. 2004; Bayat et al. 2015). The entrapment of microorganisms has been widely used in the field of bioremediation. Organisms like *Pseudomonas luteola*, *T. hirsute*, microbial consortium (containing organisms as *Aeromonas hydrophila*, *Comamonas testosterone,* and *Acinetobacter baumannii*), *Bacillus firmus*, *Enterobacter agglomerans*, *Bacillus* sp., *Lysinibacillus* sp., *Proteus vulgaris* NCIM-2027 were immobilized by entrapment techniques and were capable of successful dye decolorization

(Chen et al. 2003b; Moutaouakkil et al. 2004; Domínguez et al. 2005; Chen and Lin 2007; Saratale et al. 2011; Ogugbue et al. 2012; Pandey et al. 2019). In a recent study, El-Sheekh (2020) used cell immobilization techniques to remediate various types of industrial wastewaters. A bacterial mixture was immobilized in alginate beads to treat the effluent of a textile and dyeing industry. A decolorization of 98% was obtained followed by significant reductions in phosphate content, chemical oxygen demand, biological oxygen demand, total suspended solids, ammonia, oil and grease content, total nitrogen content, total solids, electrical conductivity, and total dissolved solids. Several peroxidases (HRP, bitter gourd peroxidase, soybean peroxidase) and fungal laccases were also immobilized by entrapment and used for dye removal (Mohan et al. 2005; Satar and Hussain 2011; Kalsoom et al. 2013; Zheng et al. 2016; Teerapatsakul et al. 2017). Asgher et al. (2012) immobilized fungal laccase by entrapment in a sol-gel matrix of trimethoxysilane and propyltetramethoxysilane. Immobilized laccase showed complete decolorization of Drimarine blue K2RL dye within 5 h reaction time. It also showed high decolorization rates (97%-100%) for different textile effluents within the same period. In another study, MnP was entrapped in an agarose gel matrix for effluent decolorization (Bilal et al. 2017a). Immobilized enzyme was applied in a packed bed reactor for the decolorization of five different textile effluents. Maximum decolorization for all the effluents was obtained in the range of 78.9% to 98.4% after six consecutive cycles. Entrapped MnP also maintained more than 50% of its activity even after the fifth reaction cycle.

Encapsulation

Encapsulation is comparable to the entrapment technique. This process is also irreversible. In this case, cells or enzymes are trapped within a semipermeable membrane. The immobilized biocatalyst has mobility inside the core space. It is a physicochemical or mechanical process in which the entrapped encapsulated particles (called the core material) lie within a shell or coating, resembling a capsule with few millimeters in thickness (Krishnamoorthi et al. 2015). Similar to the entrapment technique, the semi-permeability of the membrane in encapsulation allows the free flow of substrate and nutrient molecules while retaining the biocatalyst within the membrane. Encapsulation also relies on the ratio of the pore size of the membrane to that of the core material. Enzymes like HRP and laccases have been extensively studied for their ability to degrade dyes after encapsulation (Dai et al. 2010; Jiang et al. 2014; Koyani and Vazquez-Duhalt 2016). Le et al. (2016) constructed a core-shell system of copper alginate incorporated with Fe_2O_3 for encapsulating laccase. Laccase beads decolorized Remazole Brilliant Blue R in the range of 54.2% to 75.8% after an interaction period of 4 h. Additionally, immobilized laccase showed dye decolorization property (37.6% and 54.8%) even in real

wastewater, whereas free laccase activity was inhibited. Salgaonkar et al. (2019) immobilized peroxidase within a metal-organic framework and applied it for the decolorization of Methylene Blue (85.6%) and Congo Red (89.9%). A reusability assay revealed that the immobilized peroxidase retained 48% residual activity after six cycles. In a similar study, Li et al. (2020) encapsulated HRP macro-micropore zeolitic imidazole framework-8. The encapsulated enzyme was able to decolorize Methyl Orange, Congo Red, Rhodamine B, and Rhodamine 6G. Further immobilized enzymes retained more than 90% of its catalytic activity for Cong Red removal after five reuses and more than 50% activity for Methyl Orange and Rhodamine B removal after 4 reuses. Cell immobilization via encapsulation is less researched than enzymes. However, few recent studies have experimented with the potential of cell encapsulation for dye degradation. Sarioglu et al. (2017) encapsulated bacterial cells of *Pseudomonas aeruginosa* ATCC 47085 within nanofibrous webs of polyvinyl alcohol and polyethylene oxide for Methylene Blue removal. Bacterial polyethylene web showed maximal dye removal of 76% at a concentration of 15 mg L^{-1}. In another study cells of *Lysinibacillus* sp. encapsulated in cyclodextrin fibers were able to decolorize 82±0.8% of Reactive Black 5 at a concentration of 30 mg L^{-1} (Keskin et al. 2018). Maniyam et al. (2018) encapsulated *Rhodococcus* cells in gellan gum beads. Encapsulated cells showed complete removal of Methyl Red at a high concentration of 2 g L^{-1} after 7 h of interaction and were able to retain its decolorization property even at a concentration of 5 g L^{-1} after 10 h of interaction. Further, the immobilized cells showed complete dye removal after nine batch studies, indicating its efficient reusability. In another recent study, cells of *Shewanella xiamenesis* were immobilized inside a reduced graphene oxide network (Shen et al. 2019). Encapsulated bacteria decolorized Congo Red (50-400 mg L^{-1}) in the range of 53.6% to 99.9% and Methylene Blue (10-200 mg L^{-1}) in the range of 75.6% to 99.6%.

b. Carriers for immobilization

The materials used for cell or enzyme immobilization is called a carrier or support matrix. The selection of carriers is the most crucial step in the process of immobilization. Not all carrier materials are suitable for immobilization. The characteristics of the chosen carrier determine the success and result of immobilization. The nature of the application it is being used for is also an important aspect to be considered when choosing a carrier. Carriers chosen for wastewater application should possess characteristics such as: 1) insolubility, 2) absence of toxicity to the immobilized biocatalyst and the environment, 3) environmental safety, 4) non-biodegradability, 5) easy accessibility, 6) ability for easy regeneration, 7) cost-effectiveness, 8) being light-weighted, 9) simplicity of the procedure, 10) high loading capacity of biocatalyst, 11) being capable of an

optimal diffusion rate that would favor the flow of media to the carrier core, 12) ability for a long shelf life, 13) mechanical, biological and chemical stability, and 14) biocompatibility so as not to hamper biocatalyst activity (Leenen et al. 1996; Zacheus et al. 2000; Zhang et al. 2015; Bayat et al. 2015; Weiser et al. 2016). Most of the carriers possess few of these mentioned attributes, and therefore, the pros and cons of a carrier should be considered keeping the particular application in question.

Carriers can be divided into organic, inorganic, and composite carriers based on their chemical constitution. Organic and inorganic carriers can be additionally subdivided into natural or synthetic. Table 2 depicts several examples of carriers used in immobilization for dye decolorization studies.

Inorganic carriers

Inorganic carriers are widely used for both enzyme and cellular immobilization (Verma et al. 2006; Shirisha et al. 2016). They are suitable for immobilization as they: 1) provide extreme stability both mechanically and thermally, 2) have a large surface area, 3) have a porous structure, and 4) have invariant pore diameter to pore volume ratio providing a definitive volume and structure to the support (Thielemann et al. 2011; Carlsson et al. 2014; Bapat et al. 2016). The most significant benefit of using inorganic carriers over others is that they are cost-effective and are naturally abundant. Moreover, biocatalysts are easily attached to inorganic carriers because of the numerous functional groups. The functionalization of the carriers is also possible with surface modifying agents. Some of the inorganic carriers commonly used in the field of bioremediation are activated charcoal, porous glass, silica gel and other silica-based materials, inorganic oxides (titania, zinc oxides, alumina), minerals (bentonite, kaolin, halloysite) (Shirisha et al. 2016; Bouabidi et al. 2019).

Organic carriers

A large number of natural organic polymers are water-insoluble polysaccharides such as chitosan, alginates, cellulose, agarose, k-carrageenan, and gum Arabic (Chang and Juang 2007; Jegannathan et al. 2010; Flores-Maltos et al. 2011; Huang et al. 2011; Shen et al. 2011). Other natural carriers include plant fiber, wood sawdust, sugarcane bagasse, corncob, and tenzotle (Dzionek et al. 2016). The beneficial features of applying natural organic polymers are numerous including they are: 1) hydrophilic, 2) biodegradable, 3) biocompatible, 4) largely derived from food industry wastes, and so are cost-effective, 5) able to stabilize their interaction with the biocatalysts as they have numerous functional groups, 6) able to provide better diffusion rates, and 7) environment friendly. However, their application in bioremediation is limited by their poor mechanical stability,

their predisposition to biodegradation, their sensitivity towards organic solvents, and their restricted function within a narrow pH range (Rivelli et al. 2013; Bayat et al. 2015; Cubitto and Gentili 2015; Paliwal et al. 2015).

Organic synthetic carriers such as polyvinyl alcohol (PVA), polyvinyl chloride (PVC), polyethylene glycol (PEG), polypropylene ammonium, polyurethane, and polyacrylamide (PAM) are often used in bioremediation (Stolarzewicz et al. 2011). The advantages that they offer include: 1) structural modification at the macromolecular level, 2) greater stability than natural organic polymers when applied to wastewater, 3) the presence of a variety of functional moieties which form strong interactions with the biocatalyst, 4) durability, and 5) mechanical stability. However, they have low diffusion rates and are often toxic to the biocatalyst (Zhang et al. 2007; de-Bashan and Bashan 2010).

Composite carriers

Composite carriers are a combination of both inorganic and organic carriers, hence providing an amalgamation of characteristic features from each carrier. Composite carriers are functionally superior when compared to organic and inorganic carriers (Zhang et al. 2016). These types of carriers can be a combination of organic-inorganic, organic-organic, or inorganic-inorganic carriers. They provide advantages including: 1) reusability of the matrix, 2) strong binding interaction with the biocatalyst, 3) high stability, 4) the ability to be tailor-made for a selected biocatalyst, and 5) their consequent catalytic process. Some of the examples of composite carriers include PVA-sodium alginate, PVA-guar gum, chitosan-clay, silica-zinc oxide, silica-magnetite, and chitosan-silica (Ismail and Khudhair 2015; Zdarta et al. 2018).

2. Immobilization of enzymes for dye remediation

For years enzymes have been established as efficient biocatalysts characterized by efficient chemo-, regio-, and stereoselectivity. Enzymes also eliminate the requirement for toxic solvents, lengthy synthesis steps, and activation energy. These attributes make the biocatalytic process inexpensive and environmentally friendly (Cowan and Fernandez-Lafuente 2011). Two enzyme families viz. Azoreductases and Laccases have been proven to be the most efficient for azo dye degradation. Other promising enzymes that have been most frequently applied for dye degradation belong to the class of oxidoreductases including tyrosinase, manganese, lignin, and HRP (Bilal et al. 2017b). Though these biocatalysts are efficient, sustainable, and vigorous, they are often not suitable for industrial exploitation. In this context, enzyme immobilization offers such advantages that

make these biocatalysts industrially desirable. For example, immobilization eliminates instability issues and provides thermostability (Dubey et al. 2010). It allows enzymes to function in the presence of heavy metals, high salt concentration, organic solvents, denaturants, and helps overcome autolysis (Gardossi et al. 2010). It also provides a high enzyme-substrate ratio, reduces contamination risk, allows reusability of enzymes, enhances process control, and reduces reaction time (Dubey et al. 2010; Wang et al. 2012).

Azo reductases are reductive enzymes that reduce azo dyes by cleaving the azo bond, thereby forming colorless amines. This redox reaction requires mediators like NADH or FADH as electron donors. However, specific oxygen insensitive or aerobic azo reductases have also been documented (Solis et al. 2012; Sarkar et al. 2017). Although several researchers have established the potential of azoreductases in dye degradation, its efficiency upon immobilization has been decidedly less researched. Qi et al. (2017) covalently immobilized aerobic oxidoreductase isolated from strain *Rhodococcus opacus* 1CP in meso cellular foams. Enzyme stability increased upon immobilization, and activity was also increased when compared to free enzymes. Lang et al. (2013) immobilized aerobic azoreductase from strain *Brevibacillus laterosporus* TISTR1911 onto a nickel chelating column that was operated in a packed bed reactor. The immobilized enzyme showed efficient degradation of Methyl Orange within 3 h and could be reused over nine cycles. Using a redox mediator resulted in speeding up the catalytic process to 1.5 h and could be reused for over 16 cycles.

Laccases are copper-containing enzymes belonging to the oxidase family and have less substrate specificity. Laccases are widely known for their role in xenobiotic compound degradation (Kalyani et al. 2012). It utilizes a non-specific free radical-mediated process to degrade azo dyes and forms phenolic compounds (Sudha et al. 2014; Singh et al. 2015). The majority of these enzymes have been isolated from fungi, plants, and a few from bacteria (Arregui et al. 2019). Extensive research has been performed to investigate the potential of immobilized laccase to treat dyestuffs. Peralta-Zamora et al. (2003) investigated various carriers for the immobilization of fungal laccase isolated from *T. versicolor*. Silica modified by imidazole SiIm was found to be best suited for immobilization. During the initial stages, dye decolorization was a result of adsorption onto the carrier, but with subsequent dye addition, adsorption decreased, and enzymatic decolorization was observed. A study by Zheng et al. (2016) aimed to enhance the stability and reusability of laccase. Fungal laccase from *T. pubescens* was cross-linked by glutaraldehyde and then entrapped in chitosan beads. The immobilized enzyme showed increased degradation of several synthetic dyes in comparison to free enzymes. Immobilization also considerably enhanced the pH adaptability and thermostability when compared to free enzymes. Even after six cycles of batch decolorization studies, the immobilized enzyme had an activity above 60%. In a similar study by

Sun et al. (2015), laccase from *T. versicolor* was encapsulated within chitosan grafted polyacrylamide hydrogel. The carrier provided efficient diffusion of target substrates due to its uniformly distributed channels, mesopores, and macropores. Immobilized laccase showed increased chemical and thermal stability. In batch decolorization studies, immobilized laccase showed better durability than free laccase even after six cycles. Diverse carriers have been examined by researchers to amplify the efficiency of laccase in dye degradation. Laccases were immobilized onto fibrous polymer-grafted polypropylene chloride film, alginate-chitosan microcapsules, Poly(2-chloroethyl acrylate) grafted zeolite particles, cross-linked magnetic chitosan beads, alumina, silica beads, green coconut fiber, amino-functionalized poly(vinylamine) microbeads, porous glass beads, glutaraldehyde-crosslinked chitosan beads, sol-gel matrix based on methyltrimethoxysilane, and tetramethoxysilane (Zille et al. 2003; Lu et al. 2007; Champagne and Ramsay 2007; Bayramoglu et al. 2010; Champagne and Ramsay 2010; Cristóvão et al. 2011; Karagoz et al. 2011; Lloret et al. 2011; Celikbicak et al. 2014; Nguyen et al. 2016; Arica et al. 2017).

Tyrosinases are also copper-containing enzymes. They are ubiquitous and can be found in plants, fungi, bacteria, insects, and mammalian tissues. Their catalytic activity requires O_2 and follows a two-step reaction. In the first step, monophenol substrates are hydroxylated to ortho-diphenols. In the next step, ortho-diphenols are oxidized to orthoquinone (Ba and Kumar 2017). The application of immobilized tyrosinases for synthetic dye removal has been decidedly less studied. Veismoradi et al. (2019) analyzed the dye (Acid Blue 113 and Direct Black 22) degradation performance of direct contact membrane reactor (DCMR) and immobilized enzyme membrane reactor (IEMR). Tyrosinase was immobilized onto cross-linked polyacrylonitrile (PAN)/chitosan composite membrane. Initially, both the reactors accounted for ~95% dye removal. However, upon repeated dye degradation, IEMR showed remarkable efficiency. After ten cycles of dye decolorization, IEMR maintained its dye degradation at ~80%, while DCMR catalytic activity dropped to ~60%. Another study conducted by Wu et al (2001) immobilized tyrosinase in swollen chitosan beads. Chitosan beads exhibited a much faster dye adsorption capacity when compared to chitosan flakes. Also, tyrosine immobilization was 14 times greater than chitosan flakes.

Peroxidases are heme-containing enzymes. They are widely found among plants, animals, and microorganisms (Duarte-Vazquez et al. 2003). Secretory plant and fungal peroxidases belonging to the plant peroxidase superfamily have been widely used for the treatment of wastewater containing colored dyestuffs. The common fungal peroxidases immobilized for dye degradation include LiP and MnP. LiP was the first lignin-degrading peroxidase to be discovered. The first step in their catalytic process involves reducing H_2O_2, which

leads to the production of an intermediate compound I and an oxoferryl iron porphyrin radical cation. Ferric (Fe^{3+}) is also oxidized during this step. In the next step, compound I is reduced to compound II and LiP is returned to the ferric oxidation state (Pollegioni et al. 2015). LiP immobilized in chitosan beads showed efficient decolorization (~70- 95%) of six reactive dyes. The immobilized LiP exhibited thermostability, reusability, low K_m, and high V_{max} values (Sofia et al. 2016). Similarly, the immobilization of LiP in Ca-alginate beads enhanced its catalytic activity, thermal stability, and reusability. Further, it efficiently reduced BOD, COD, and TOC levels in wastewater (Shaheen et al. 2017). LiP, when immobilized on carbon nanotubes, exhibited a remarkable increase of 18 to 27- fold specific activity when compared to free enzymes. However, only the immobilized enzyme extracts could be reused; the carbon nanotubes became saturated and had to be discarded over time (Oliviera et al. 2018). The catalytic reaction of MnP shows many similarities to LiP. It involves preliminary oxidation of H_2O_2 to an intermediary compound, which in turn oxidizes Mn^{2+} to Mn^{3+}. Mn^{3+} is stabilized by organic acids and forms an Mn^{3+} organic acid complex that acts as the active oxidant for dyes (Hussain 2010). Several studies have been reported by Bilal et al. (2016a, 2016b, 2016c, 2016d, 2017a) as well as Bilal and Asgher (2015a, 2015b, 2016) on immobilization of MnP. These studies immobilized MnP using different carriers as a sol-gel matrix of tetramethoxysilane and propyltrimethoxysilane, polyvinyl alcohol-alginate beads, Ca-alginate beads, glutaraldehyde activated gelatin matrix, glutaraldehyde activated chitosan beads, and agar-agar. Immobilization using these carriers resulted in enzymes with pH stability, thermal stability, storage stability, and high catalytic activity. Reusability varied among the different carriers; however, chitosan beads exhibited the best result. MnP immobilized in chitosan beads retained more than 60% of its catalytic activity even after ten cycles of batch decolorization studies. Additionally, immobilized MnP reduced BOD, COD, and TOC of the textile wastewaters. Among the plant peroxidases, HRP has been extensively studied for immobilization and its possible application in dye degradation. HRP enzymes obtained from horseradish is a glycoprotein and contains six lysine residues. It is of special interest in biotechnological applications because of its small molecular weight, stability, and cost-effectiveness (Veitch 2004). HRP can cleave azo dyes in the presence of H_2O_2 as well as degrade and precipitate industrial synthetic dyes (Onder et al. 2011; Çelebi et al. 2013). Zamora et al. (1998) immobilized HRP on Amberlite IRA-400 for remediation of effluent from a paper and pulp industry. Immobilized HRP decolorized over 50% of the affluent only within 4 h without significant dye adsorption by the carrier. It was also found that immobilized HRP functioned more efficiently when combined with a pre-treatment of the effluent's photoirradiation. In another study, the HRP enzyme was covalently immobilized onto various carriers as Hydroxyl-terminated bisphenol A- and bisphenol AF-based

polysulfones (Celebi et al. 2013). Immobilized HRP presented good decolorization of dyes Acid Black 1 Reactive Blue 19, thermal and storage- stability was also observed. However, immobilization could not impart the enzyme's pH stability. Kumar et al. (2016) used epoxy functionalized polypropylene (PP) films to immobilize the HRP enzyme for the degradation of Basic Red 29. Immobilized HRP showed increased storage capacity, pH stability, and thermostability. It was capable of ~90% dye degradation and could be reused for five consecutive cycles without any significant loss of activity. Bilal et al. (2018) used a packed bed reactor with immobilized HRP in polyacrylamide gel to study the degradation of Methyl Orange. Immobilized HRP was able to achieve >90% dye degradation. It also reduced the toxicity of the parent dye as established from phytotoxicity assays. Other noteworthy immobilized plant peroxidases applied for dye degradation include bitter gourd peroxidase immobilized onto Con A-Sephadex and concanavalin A layered calcium alginate–starch beads (Akhtar et al. 2005; Matto and Hussain 2009a), soybean peroxidase immobilized on silica monolith (Calza et al. 2016), turnip peroxidase on Concanavalin A-wood shaving, and calcium alginate (Matto and Hussain 2009b; Ahmedi et al. 2015).

3. Immobilization of whole cells for dye remediation

Whole-cell immobilization evolved as an alternative to enzyme immobilization. Cell immobilization can be precisely defined as localization or confinement of viable microbial cells within a particular region to restrict their mobility and carry out catalytic reactions in repeated or continuous mode. The immobilization matrix is characterized by hydrodynamic features differing from that of the surrounding environment (Dervakos and Webb 1991; Amim et al. 2010; Gotovtsev et al. 2015). Cell immobilization drew researchers' attention as an alternative to certain immobilized enzymes since cell immobilization eliminates the laborious and expensive process of enzyme isolation and purification. Initially, immobilized bacterial cells found application in many fields. Eventually, filamentous fungi, algae, and yeasts were also immobilized (Anderson 1975; Šíma et al. 2017; Basak et al. 2019; Abou-El-Souod et al. 2020). Cell immobilization has certain advantages over enzyme immobilization: elimination of the long and expensive procedures of enzyme isolation and purification, elimination of biocatalyst refill since immobilized microbial cells can be operated in the continuous or semi-continuous production process, efficient recovery of the biocatalyst, provides a more extended period of enzyme activity and storage since they remain in their natural cellular environment, and eliminates strain genetic instability problems associated with recombinant strains (Dincbas et al. 1993; Junter and Jouene 2004;

Stolarzewicz et al. 2011; Bouabidi et al. 2019). The numerous advantages of immobilized cell systems have led to their increasing application in wastewater treatment.

Biodegradation of dyes has been studied extensively by scientists over the years. Many microorganisms including bacteria (Anjaneya et al. 2011; Jadhav et al. 2011), fungi (Tapia-Tussell et al. 2011; Cano et al. 2012; Sen et al. 2012), algae (Omar et al. 2008; El-Sheekh et al. 2009), yeasts (Charumathi and Das 2010; Waghmode et al. 2011), actinomycetes (Mane et al., 2008), and microbial consortia (Ndasi et al. 2011; Waghmode et al. 2011) have been applied for this purpose. Although extensive studies on dye biodegradation have been performed with suspended free cells, the scope of immobilized cells in dye degradation has been less explored. The application of immobilized cells for dye biodegradation studies began in the late 1980s with bacteria (Wagner and Hempel 1988). Since then, immobilization studies have been performed using bacteria, fungi, and algae (Das and Adholeya 2015). Comparatively fewer studies are reported on yeast (Mitter and Corso 2013; Dastagir and Padma 2014; Tan et al. 2014a, 2014b).

The application of immobilized bacteria is an essential aspect in the field of azo dye biodegradation. Several reports suggest that immobilized bacteria are more beneficial and efficient compared to free suspended cells. Tuttolomondo et al. (2014) reported that sol-gel immobilized *Pseudomonas* sp. was able to produce seven times more extracellular dye degrading enzymes while protecting bacteria from the aggressive external surroundings, and maintained a high dye decolorization percentage (nearly 80%), even after four cycles of reuse. Immobilized cells of *Enterobacter agglomerans* in a calcium alginate matrix maintained its dye decolorization rate over 95% even after seven cycles of repeated batch decolorization (Moutaouakkil et al. 2004). Chang et al. (2001) examined three different immobilization matrices (calcium alginate, carrageenan, and polyacrylamide) for immobilizing *P. luteola*. When compared to free cells, immobilized *P. luteola* showed stability under various levels of pH, and dissolved oxygen, and maintained its activity after repeated usage for up to four cycles. Free cells showed a higher specific decolorization rate, but immobilization would achieve a higher total decolorization rate since it increased the biomass concentration. It has been reported earlier that bacterial dye degradation is more likely to occur under anaerobic conditions but leads to the production of aromatic amines. Anaerobic degradation is often followed by aerobic degradation to mineralize these amines (Shah et al. 2013). Reports using this strategy in immobilization are scarce. (Kudlich et al. 1996) entrapped *Sphingomonas* sp. BN6 in alginate beads to biodegrade the azo dye Mordant Yellow 3 (MY3). Under aerobic conditions, immobilized cells formed more than equimolar amounts of a 5-aminosalicylate and almost negligible amount of 6-aminonaphthalene-2-sulfonate (6A2NS). Bacterial cells in the anaerobic center of the

alginate beads reduced MY3 to 6A2NS and 5-aminosalicylate. Further, 6A2NS was oxidized to 5-aminosalicylate in the outer aerobic zones of the beads, which were then released in the medium. Also, *Sphingomonas* sp. BN6 was co-immobilized with an aerobic 5-aminosalicylate degrading strain led to the complete mineralization of MY3. Another study was conducted by Hameed and Ismail (2020) for investigating the biodegradation potential of immobilized mixed cells against Reactive Yellow 15 (RY 15) using a sequential anaerobic-aerobic process in bench-scale as well as lab-scale bioreactors. Mixed cells were obtained from activated sludge and were immobilized using three types of carriers (alginate, gelatin, and starch) and cross-linked using polyvinyl alcohol. Immobilized cells showed a better biodegradation potential in both the aerobic and anaerobic phases at increased dye concentrations and showed excellent reusability with the same dye removal potential in the two cycles of reuse.

Fungi are highly effective in breaking down synthetic dyes (Cuoto 2009). They yield an assortment of extracellular proteins, organic acids, and other metabolites, which helps them adapt to adverse environmental conditions (Das and Adholeya 2015). Much consideration has been given to the potential of fungal systems in dye decolorization. Fungi can be used for bioremediation by either utilizing the biomass as a sorbent or by utilizing the biodegradation/ biotransformation enzymes produced by fungi. Biosorption has been mostly practiced with non-ligninolytic fungi. White rot fungi have been extensively studied for dye degradation. Pazarlioglu et al. (2005) immobilized white-rot fungi *P. chrysosporium* on $ZrOCl_2$ activated pumice for direct azo dye degradation. The decolorization ratio for the dyes decreased with the use of immobilized fungi but maintained a high degradation efficiency (100- 95%) even after four cycles of repeated batch decolorization studies. Another white-rot fungus *T. hirsuta* was immobilized on calcium alginate and operated in an airlift bioreactor (Domı´nguez et al. 2005). Dye decolorization was performed in batch mode in the bioreactor and continued for 40 days without any disruption of the bioparticles. High dye decolorization was attained at short duration, indicating the stability of this process. *Dichotomous squalene*, when immobilized on pinewood, resulted in increased laccase production, thereby leading to decolorization of anthraquinone dye Remazol Brilliant Blue R and an azo dye Reactive Orange 16 (Šušla et al. 2007). Other fungi immobilized for dye degradation include *T. pubescens* and *Pleurotus ostreatus* on polyurethane foam (Casieri et al. 2008), *Aspergillus flavus* SA2 on sand particles (Andleeb et al. 2012), *T. versicolor* ATCC 20869 on various natural and synthetic carriers as wheat straw, jute, hemp, maple woodchips, and nylon and polyethylene terephthalate fibers, alginate beads (Shin et al. 2002; Pazarlioglu et al. 2010), *Irpex lacteus* on polyurethane foam (Tavčar et al. 2006), and *Funalia trogii* on calcium alginate (Park et al. 2006).

Algae/ microalgae have been immobilized and used for various biotechnological applications for over 40 years. The extremely high accumulation ability of certain algal species for hazardous pollutants have led to their application in the field of bioremediation. In the majority of cases, algae have benefited from immobilization. Immobilization prevented grazing by aggressive zooplankton; the competition for nutrition with other microbial species is reduced; improved metabolism and function have been observed (de-Bashan et al. 2010). In most studies of algal immobilization for dye degradation, the biomass was entrapped in alginate beads. *Chlorella pyrenoidosa* was immobilized in alginate beads. Immobilization increased algal growth and physiological activity. For example, it was found that there was greater dye decolorization compared to free algae and decolorization was increased further when supplied with bubbling air containing 2% CO_2 (Guolan et al. 2000). Mona et al. (2011) immobilized the spent biomass of *Nostoc linckia* in calcium alginate for the biosorption of Reactive Red 198. Immobilized biomass showed a good absorption of dye concentrations 100-500 mg L^{-1}. Microalgae *Chlamydomonas reinhardtii* immobilized on polysulfone nanofibrous web decolorized Reactive Black 5 and Reactive Blue 221 (Keskin et al. 2015). Immobilized microalgae maintained its decolorization at approximately 51% after the third cycle of batch decolorization. Green algae *Desmodesmus* sp. immobilized in alginate beads showed maximum decolorization of Methylene Blue and Malachite Green compared to free cells (Al-Fawwaz and Abdullah 2016). Few researchers reported comparative studies with different carriers of algal immobilization for dye degradation. For example, Chu et al. (2009) used κ-carrageenan and sodium alginate for immobilization. Alginate immobilized algal cells achieved maximum dye decolorization of textile wastewater. Similar results were obtained by Kassim et al. (2018) where *Chlorella* sp. was immobilized in three different carriers (starch, carboxymethyl cellulose, and alginate). The highest decolorization of effluent was achieved with alginate. Other immobilized algae entrapped in sodium alginate and used for dye degradation include *Scenedesmus quadricauda*, *S. obliquus*, *Chlorella* sp., *C. vulgaris* UMACC 001, *Phormidium* sp. (Ertuğrul et al. 2008; Chia et al. 2014; Kumar et al. 2014a; Revathi et al. 2017; Abou-El-Souod et al. 2020; Wu et al. 2020).

Many researchers have pointed out that the use of pure microbial culture for industrial dye degradation would be ineffective. Pure cultures often degrade dye, which leads to the production of aromatic amines and other toxic products. Also, individual strains are only effective against a particular type of dye. Industrial wastewaters have a complex chemical composition which necessitates the use of several enzymatic reactions (Solís et al. 2012). This led to a culmination of researchers utilizing microbial consortia to search for more practical approaches in dye degradation. Chen et al. (2003a) immobilized a microbial consortium obtained from

the sludge sample of a wastewater treatment plant in PVA gel beads. The immobilized consortium showed efficient decolorization of azo dye Red RBN and maintained decolorization ability even at high dye concentrations. Further, the beads exhibited sufficient stability and were capable of application for six months. The immobilized consortium had a remarkable ability to decolorize various other azo dyes as well as dye mixtures. In another study, a microbial consortium consisting of a fungus and a bacterium was immobilized in PVA for degradation of Direct Fast Scarlet 4BS (Fang et al. 2004). The immobilized consortium showed optimum activity at a broad temperature and pH range and showed efficient decolorization of the dye at a high concentration of 1000 mg L^{-1}. Further, it had an excellent reusability property. The immobilized consortium sustained 30 cycles of batch decolorization without losing its efficiency. Lade et al. (2015) constituted a microbial consortium from the wastewater disposal site of textile effluent and immobilized it in polyurethane foam. The immobilized consortium not only showed complete degradation of Congo Red but also degraded 92% of real textile wastewater. Similarly, Kurade et al. (2019) immobilized a microbial consortium on stainless steel sponge. The immobilized consortium was capable of approximately 90% decolorization of the textile effluent. The decolorization efficiency was maintained above 90% even after three cycles of decolorization. Many recent research strategies have focused on the use of microbial consortium over individual cultures (Patel and Gupte 2015; Yin et al. 2017; Hameed and Ismail 2018; Hameed and Ismail 2020).

Comparative Assessment of Immobilized Biocatalysts for Industrial Applications

Real industrial dye-laden wastewater contains a myriad of chemicals and dye mixtures. They are characterized as being hazardous due to their intense color, high COD contributed by the dyes (20%) and auxiliary dyeing agents (80%), and their complex chemical constitution. Hence it is important to examine the degradation potential of real wastewater to determine the applicability of immobilized systems. Another critical parameter for industrial application is the ability to treat large volumes of wastewater. Table 3 presents a brief overview of the potential industrial applications of different immobilized systems. Studies that tested the potential of biocatalysts for effluent removal or utilized bioreactors for color removal were considered. Only a few studies have tested the potential of immobilized biocatalysts for effluent color removal in bioreactors. Immobilized microbial consortia showed more potential for industrial applications (Lade et al. 2015; Kurade et al. 2019; Hameed and Ismail 2020). The concoction of microorganisms in a consortium probably provides a combination of enzymes that is suitable for treating the dye mixtures in an effluent. However, the presence of different microorganisms makes the process unreliable and difficult to control. Several studies were undertaken on the

development of novel carriers for enzyme immobilization but its potential for industrial application has been less explored. The high specificity of an enzymatic process makes these biocatalysts relatively unlikely for effluent treatment. Studies performed with enzymes like laccase in bioreactors for effluent treatment resulted in lower dye decolorization properties when compared to results using single dyes (Sondhi et al. 2018; Katuri et al. 2009). This hurdle can be overcome by using the co-immobilization of enzymes for effluent treatment. Selecting a reactor for immobilized systems is essential in making effluent treatment cost-effective and efficient. An efficient bioreactor should possess optimum fluid dynamic conditions, effective mass, and heat transfer to and from the bioreactor surface. Other factors such as the intensity of shear stress, flow, and mixing outlines are also crucial, especially for immobilized cells. The majority of the studies performed by researchers have utilized anaerobic/microaerophilic reactors. A few studies utilized aerobic reactors such as rotating biological contractors and airlift reactors. In most cases, aerobic reactors suffer from auto-oxidation and reaction with compounds of sludge matrix which hinders the dye degradation process. Researchers mostly reported the use of packed bed reactors for effluent treatment with immobilized systems (Senan et al. 2003; Manikandan et al. 2009; Lang et al. 2013; Torres-Farradá et al. 2018).

Conclusions

Environmental deterioration poses significant challenges for researchers to find effective treatment technology to combat pollution from various dye utilizing industries. The generation of colored effluents from various industries is a significant contributor to the growing worldwide water crisis. Preliminary studies have already revealed the potential of various bioremediating agents to control dye pollution. Immobilization is an efficient way to enhance the potential of these agents for treating large-scale dyestuff containing wastewater. A crucial step in successful immobilization is the choice of carriers for a particular biocatalyst. The application for wastewater treatment necessitates the development of carriers that have characteristics such as: 1) inexpensiveness, 2) biocompatibility, 3) mechanical and chemical stability, 4) porosity with optimum diffusion rate, and 5) high surface area. Hence, researchers should focus on carrier development extensively. Another difficulty in choosing a carrier is the generation of varied results among researchers using the same carrier. Thus, it is necessary to standardize the use of carriers in terms of their physical and chemical properties, purity, composition, and source reproducibility. Another key point to successful immobilization is the method of immobilization. Current studies are based on developing a particular method of immobilization by screening the biocatalysts; hence, most biocatalysts function below optimum operating conditions. It is necessary to design the

immobilization method with consideration given to the biocatalyst, the carrier, and the application. Immobilization was initially performed with enzymes, and ever since, many studies have been conducted to examine its potential for dye degradation. Cellular immobilization is a relatively new technology and has been less researched in comparison to enzymes. Immobilized enzymes are often preferred over intact cells owing to their high specificity, ease in activity optimization, effortless handling, and ease of storage. The majority of research articles have studied laccase immobilization, followed by fungal and plant peroxidases for dye degradation. The main reason for their widespread application is their low specificity making them adept at degrading a wide range of dyestuffs such as azo, heterocyclic, and polymeric dyes. The significant advantages of applying cellular immobilization over enzymes are the lower bioprocessing cost, The broader spectrum of activity of these multi-enzyme systems, and their comparatively extended duration of function and storage. Lignin degrading white-rot fungi were extensively studied for immobilization and their broad spectrum of activity holds much potential in dye degradation. The vast and interesting research on the immobilization of cells and enzymes has helped establish the efficiency of immobilization systems over free biocatalysts due to increasing their physical, chemical, storage-stability, reusability, and enhanced catalytic activity. However, the application of large-scale wastewater treatment technology is still in its infancy. Only a few research articles have explored the potential of immobilized systems for real industrial effluent. Experimental biotreatment research must focus on the capacity and function of these systems to handle the massive volume of industrial effluents over longer periods so that these methods may be commercially feasible.

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Table 1. Comparative analysis of the different immobilization techniques

Technique	Advantages	Disadvantages
1. Adsorption	• Mild technique • Eliminates the use of chemical additives • Simplicity in operation • Less time consuming • Reversibility which allows for purification of the biocatalyst and reloading of the support • The active site of the enzyme is unaltered • Cost-effective • Eco-friendly	• Heavy leakage of enzyme or cells from the carrier due to weak binding forces • Interactions involved between the biocatalyst and the carrier are unstable • Loading cannot be controlled • Steric hindrance by the carrier • Low reproducibility
2. Covalent Binding	• Improves catalytic activity • Strong bond formation between biocatalyst and the carrier. • Elimination of desorption or leakage problems. • Improved thermal stability • Ease in substrate contact • Comparatively simpler in operation • Cost-effective	• The usage of toxic coupling agents leads to loss of cell viability or enzyme activity. • Biocatalyst loading occurs only in small amounts (0.02 g per g of carrier) • Can be costly as some of the good supports are expensive (eg. Eupergit C and Agaroses)
3. Cross-linking or Copolymerisation	• Carrier free immobilization • Better application when used in conjunction with other methods • Stability increases as enzyme molecules or cells are cross-linked resulting in a rigid structure • Simpler operation	• Usage of polyfunctional agents can denature biocatalyst thereby resulting in loss of enzyme activity.

	• Cost-effective	
4. Entrapment	• Rapid and a versatile method • Protection from external harsh conditions • Enzyme loading can be very high • Mild conditions required during the reaction process • Inexpensive	• Possibility of cellular and enzyme leakage from the matrix • Deactivation during immobilization • Limited substrate accessibility and reduced electron transport • Injury of the carrier during usage
5. Encapsulation	• Protection of biocatalyst from external harsh conditions • No leakage issues • An inexpensive and simple method • Core material does not require any chemical modification hence the activity of biocatalyst is unharmed • Co-immobilization is possible. Biocatalyst can be immobilized in any desired combination	• Damage to the capsule by growing cells • Damage to capsule due to product build-up from enzyme reactions • Hardly used for ex-situ bioremediation

Table 2. Selected examples of some carriers used for dye degradation studies

Carrier	Biocatalyst	Immobilization technique	Features	References
Inorganic Carriers				
Graphene oxide nanosheet	Laccase	Covalent binding, Adsorption	• Large surfaces area • Physical and mechanical stability • Increased stability in a wide pH and temperature range • Increased dye decolorization ability • Decrease in enzyme affinity towards substrate • Comparatively lower reusability potential	Skoronski et al. 2017; Kashefi et al. 2019
Silica gel, Silica beads, epoxy activated silica	*Pseudomonas* sp., laccase	Encapsulation, Covalent binding	• Higher protein production per bacteria • Rapid and high immobilization yield • Moderate pH stability • Good thermal stability • Lower substrate affinity • moderate reusability • Excellent storage stability • High adsorption efficiency • Inexpensive	Champagne and Ramsay 2007; Tuttolomondo et al. 2014; Salami et al. 2018

			• Mechanically stable	
Kissiris	*Phanerochaete chrysosporium*	Adsorption	• A high degree of roughness and porosity provides a large surface area for cell attachment • Mechanical and biological stability • Inert in nature • Inexpensive	Karimi et al. 2006
Nanoporous Zeolite X	Laccase	Adsorption	• Stability over a wide pH range • Thermostable • Good reusability • Non-toxic • Inexpensive • Large surface area • Excellent loading capacity	Zamel et al. 2019
Fly ash	*Pseudomonas* sp.	Adsorption	• Inexpensive • Better dye decolorization • Non-toxic	Roy et al. 2018
γ-aluminum oxide pellets	Fungal laccase	Covalent bonding	• Enhanced decolorization due to unspecific adsorption by carrier • moderate thermostability • low pH stability • Protection from enzyme inhibitors • Increased enzyme tolerance towards halides	Abadulla et al. 2000, Kandelbauer et al. 2004
Celite R-646	Horseradish peroxidase	Covalent attachment	• Microporous structure • Inexpensive • Mechanical stability	Shim et al. 2007

			• Enhanced pH stability • Moderate thermostability • High dye decolorization potential	
Mesocellular foams	Bacterial Azoreductase	Covalent bonding	• Open and rigid structure • large surface area • Uniformly porous • Moderate thermostability • Moderate pH stability • Storage stability • Increased range of substrate reaction	Qi et al. 2017
Phospholipid-templated titania particles	Horseradish peroxide	Encapsulation	• Higher mechanical strength • Stable chemical structure • Good biocompatibility • Enhanced thermal stability • Negligible swelling in organic solvent • Overcomes diffusion limitation • No leaching of adsorbed biomolecules • Can act both as anion and cation exchanger at acidic and alkaline pH • Increased tolerance towards inactivating agents • Highly decreased substrate affinity	Jiang et al. 2014

			• Moderate reusability	
Organic Carriers				
Alginate	Horseradish peroxidase, Lignin peroxidase, Laccase, *Cucurbita pepo* (courgette) peroxidase, White-rot fungi (*Coriolopsis gallica*, *Bjerkandera adusta*, *Trametes versicolor* and *T. trogii*), *Funalia trogii*, *P. aeruginosa*, *Bacillus subtilis*, g-C3N4-P25/photosynthetic bacteria, Consortium (*Geotrichum candidum* and *B. cereus*)	Entrapment	• Low availability of enzyme to dye • Low thermostability • Low pH stability • Long shelf-life • Higher dye decolorization potential than free cells • Good reusability	Mohan et al. 2005; Park et al. 2006; Boucherit et al. 2013; Daâssi et al. 2013; Daâssi et al. 2014; Zhang et al. 2017; Akpor 2018
Chitosan	Laccase, Lignin peroxidase	Entrapment, cross-linking	• Environmentally benign • Efficient biosorption property • Cost-effective • Protects enzyme and enhanced stability • High biocompatibility • Low toxicity • Conformation rigidity • Enhanced shelf life	Sofia et al. 2016; Ma et al. 2018; Nguyen et al. 2016

Calcium pectate	*Ziziphus mauritiana* peroxidase	Co-immobilization (Adsorption and crosslinking)	• Shift in optimum pH of enzyme activity • Good thermostability • Good storage stability • Excellent reusability • Decreased substrate affinity	Khan and Husain 2019
Acrylamide	Horseradish peroxidase	Entrapment	• Non-ionic nature of beads resulted in minimum enzyme modification • Activity at a narrow pH range	Mohan et al. 2005
Sugarcane bagasse	*Saccharomyces cerevisiae*	Adsorption	• Mechanically stable • Biocompatible • Inexpensive	Mitter and Corso 2013
Coconut shell Biochar	*Brevibacillus parabrevis*	Adsorption	• Better dye degradation potential than free cells at a higher dye concentration • Non-toxic to microorganisms • Efficient at continuous dye removal • inexpensive	Abu Talha et al. 2018
Green coconut fiber	Laccase	Covalent bonding	• Inexpensive • Moderate thermal stability • Moderate reusability and storage stability • Low substrate affinity	Cristo´va˜o et al. 2012
Terminalia arjuna seeds biochar	*Providencia stuartii*	Adsorption	• Inexpensive • Environment friendly • Large surface area • Enhanced dye decolorization	Goswami et al. 2020

Loofa (Dried fruits of *Luffa cylindrica*)	*Lysinibacillus sp*. RGS, Fungal laccase	Entrapment	• Specific pore size • Mechanically stable • Constant material characteristics • Non-toxic • Inexpensive • Eco-friendly	Bedkar et al. 2014; Mohammed et al. 2018
Agar-Agar	Bacterial consortium (*Enterobacter dissolvens* and *P. aeruginosa*)	Entrapment	• Excellent reusability • Enhanced shelf life • Activity at a wide pH range • Thermostable	Patel and Gupte 2015
Oxidized bacterial cellulose membrane	Laccase co-immobilized with TiO_2 nanoparticles	Covalent bonding	• High surface area-volume ratio • High operational stability • Higher pH stability • Relatively more stable at temperature changes • Poor thermal stability • Low affinity towards substrate	Li et al. 2017
PVA	Laccase, Microbial consortium (*Aeromonas hydrophila, Comamonas testosteroni*, and *Acinetobacter baumannii*), *A. jandaei* strain SCS5 coimmibolized with	Entrapment	• High decolorization activity • Excellent reusability • Inexpensive • Biocompatible • Good storage stability • Good Reusability	Chen et al. 2003b; Chhabra et al. 2015; Sharma et al. 2016

	anthraquinone-2,6-disulphonate and Fe_3O_4			
Con A sephadex	*Momordica charantia* peroxidase	Adsroption	• High Decolorization activity of dye mixtures • Good reusability • Enhanced storage stability • No thermal stability • Active at a narrow pH range	Akhtar et al. 2005
Polyurethane foam	Horseradish peroxidase, *Paenibacillus alvei*, *B. subtilis*, *B. cohnii*, Bacterial consortium (*Actinomycetes* sp., *P. aeruginosa*, *Stenotrophomonas rhizophila* and *Staphylococcus pasteuri*)	Adsorption	• High dye decolorization • Stability across a wide pH range • Cost-effective • Compatible for microbial growth • Renewable • Good storage stability	Malani et al. 2013; Pokharia and Ahluwalia 2016, Padmanaban et al. 2016; Setty 2019, Rajendran et al. 2015
Composite Carriers				
Alginate-chitosan	Laccase	Entrapment	• High thermostability • Poor pH stability • Lower affinity for substrate • Reduced dye decolorization • Lower resuability	Lu et al. 2007
Agarose-Chitosan	Horseradish peroxidase	Entrapment	• Biocompatible • Renewable • Biodegradable	Wang et al. 2020

			• Cross-linked three dimensional network structure • Moderate pH stability and thermostability • Good Reusability • Good storage stability	
Alginate-silicate beads	*P. luteola*	Entrapment	• High biomass loading • Good substrate diffusion • High mechanical stability • Poor thermal stability • Less sensitive to pH change • Non-swelling • Enhanced efficiency at higher dye concentrations • Lower substrate affinity • Good reusability	Chen and Lin 2007
Calcium alginate-pectin	Bitter gourd peroxidase	Entrapment	• Enhanced dye decolorization • Good thermostability • Higher stability to pH variation • Good reusability and storage stability	Satar and Hussain 2011
Calcium alginate-starch	Bitter gourd peroxidase	Entrapment	• pH stable • Thermostable • Good reusability • Good storage property • Enhanced dye decolorization property	Matto et al. 2009

PVA–sodium alginate–kaolin	*Burkholderia vietnamiensis*	Entrapment	• Physiologically and mechanically stable	Cheng et al. 2012
Chitosan membrane grafted with itaconic acid polymer and chelated with Cu(II) ion	Laccase	Adsorption	• Moderate pH stability • Very less thermostability • Good storage stability • Good reusability • Moderate decolorization property	Bayramoglu et al. 2012b
Thiolated chitosan Fe_3O_4	Laccase	Adsorption	• Large surface area • Biocompatible • Enhanced dye decolorization • High thermostability • Increased substrate affinity • Excellent reusability • Good storage property	Ulu et al. 2020
Polyvinyl alcohol/polyethylene oxide hydrogels	*Lentinus concinnus*	Adsorption	• Inexpensive • Thermostable • Reusability lead to a decrease in activity similar to that of the free cells • Increase in ionic strength decreased dye adsorption • Activity in a narrow pH range	Baryamoglu and Yilmaz 2018

Alginate coated with polyacrylamide	Microbial consortium	Entrapment	• Non-toxic to microorganisms • Mechanically stable • Low biomass leakage	Steffan et al. 2005
Polyvinyl alcohol-Alginate	Manganese peroxidase	Entrapment	• Good enzyme activity in a broad pH range • Thermostable • Efficient dye decolorization potential when compared to free enzyme • Good reusability property	Bilal and Asgher 2015a
Polyvinyl alcohol–calcium alginate–activated carbon beads	*Bacillus* sp.	Entrapment	• Efficient adsorption property • Mechanically stable • Biocompatible • Enhanced dye decolorization property • Good reusability	Cai et al. 2020
Cellulose acetate - poly(ethylene oxide) nanofibrous membrane	*B. paramycoides*	Encapsulation	• A large number of cells adhered to the membrane • Cells uniformly dispersed throughout the membrane • Good dispersion • Easy handling • Moderate reusability	Zamel et al. 2019
Magnetic zeolitic imidazolate	Laccase	Adsorption	• Large surface area • Highly porous • Structurally flexible	Wang et al. 2019

framework-8 nanoparticles			• Numerous functional sites • High thermo-stablilty • Moderate storage stability • High substrate affinity • Good reusability	
ZnO nanowires/macroporous SiO_2	Horseradish peroxidase, Fungal laccase, Co-immobilization of chloroperoxidase and horseradish peroxidase	Covalent bonding, Adsorption, in-situ cross linking	• Macroporous structure • High surface area • High adsorption potential • High dye decolorization potential • Good pH stability • High thermostability • Enhanced enzyme affinity towards substrate • Long-term storage stability • Excellent reusability	Li et al. 2015; Jin et al. 2017; Sun et al. 2017
2D Copper oxide nanosheet-polymethyl mathacrylate	Horseradish peroxidase	Co-immobilization (Adsorption and Encapsulation)	• Good reusability • High loading capacity • Increased dye decolorization property • Excellent thermostability • moderate pH stability • Highly resistant to metal ions and organic compounds • Reduced substrate affinity	Aldhahri et al. 2020

Table 3: Comparative Analysis of the potential of Different Immobilized Biocatalysts for Industrial scale implementation

Biocatalyst	Immobilization Technique	Bioreactor	Dye Removal efficiency	References
Microbial consortium (*Brevibacillus laterosporus* and *Galactomyces geotrichum*)	Adsorption on Stainless Steel Sponge	Upflow Fixed Bed Reactor	>90% of textile effluent	Kurade et al. 2019
Microbial consortium	Adsorption on Polyurethane foam	Upflow Column Bioreactor	92% of real textile effluent	Lade et al. 2015
Bacterial strains of *Aeromonas*, *Alkaligenes*, *Pseudomonas*	Adsorbed and entrapped in highly perforated brick fragments	Fixed Bed Bioreactor	>95% of dye factory wastewater	Jian et al. 1994
Bacterial consortium	Entrapped in polyvinyl alcohol-sodium alginate	Sequential anaerobic/aerobic bioreactor	>95% of real textile wastewater	Hameed and Ismail 2020
Bacterial consortium (*Enterobacter dissolvens* and	Entrapped in agar agar	Packed bed reactor	89-91% of synthetic dye wastewater	Patel and Gupte 2015

Pseudomonas aeruginosa)				
Microbial consortium	Biomass attachment on Laterite stones	Packed Bed Reactor	61.7% of simulated textile effluent	Senan et al. 2003
Bacterial consortium	Adsorbed on polyurethane foam	Packed Bed Bioreactor	80-90% of textile dye effluent	Manikandan et al. 2009
Anaerobic bacteria	Entrapped in Reticulated sintered glass	Fixed Bed Reactor	Complete decolorization of textile effluent	Georgiou and Aivasidis 2005
Plant-Bacterium (*B. pumilus*) consortium	Bacterium entrapped in sodium alginate	Phytoreactor	93% of textile effluent	Watharkar et al. 2015
Lysinibacillus sp.	Entrapped in Loofa	Upflow column bioreactor	69% real textile effluent	Bedekar et al. 2014
Bacillus cohnii	Adsorbed on Polyurethane foam	Packed bed reactor	Complete decolorization of Reactive Red 120	Padmanaban et al. 2016
Pseudomonas sp.	Fly ash	Fluidized bed Bioreactor	98.2% congo red	Roy et al. 2019
Stentrophomonas maltophilia	Entrapped in alginate gel	Down flow column reactor	82% of textile effluent	Galai et al. 2010

Aspergillus flavus	Adsorbed on sand particles	Fluidized Bed Bioreactor	71.3% of Drimarene blue K2RL in simulated textile effluent	Andleeb et al. 2011
Pleurotus flabellatus	Luffa sponge	Continuous packed bed reactor	60-70% real textile wastewater	Nilsson et al. 2006
Ganoderma weberianum	Adsorbed on Sugarcane baggase	Packed Bed Bioreactor	74-80% textile effluent	Torres-Farradá et al. 2018
Geotrichum sp.	Entrapped in calcium alginate	Fluidized Bed Bioreactor	>95% of azo dyes orange G, trypan blue, azorubine, methyl red	Zeroual et al. 2007
Stropharia sp.	*Luffa cylindrica*	Column Bioreactor	>65% of anthraquinone violet R (AQVR)	Agarwal and Verma 2019
Penicllium glabrum	Macroporous polymeric sponge	Upflow Packed Bed Reactor	78.8% of textile wastewater	Arikan et al. 2019
Aspergillus terreus	*L. cylindrica*	Stirred tank bioreactor	92% congo red	Laraib et al. 2020

Lentinus arcularius	Entrapped in calcium alginate beads	Immersion Bioreactor	~90% of artificial textile effluent	Bayburt et al. 2020
Trametes versicolor	Entrapped in Calcium alginate	Fluidized Bed Continuous Bioreactor	61%- 72% of kraft bleach plant effluent	Pallerla and Chambers 1996; Lemus-Gómez et al. 2018
	Polyurethane foam	Sequencing Batch Reactor	>95% of vinyl sulfone azo dye RB5	
Phanerochaete chrysosporium	Adsorbed on Kissiris	Packed Bed Bioreactor	87% of Astrazon Red FBL in textile effluent	Cammarota and Sant'Anna Jr 1992; Guimarães et al. 2005; Sedighi et al. 2009; Pakshirajan and Kheria 2012; Pakshirajan et al. 2011
	Adsorbed on polyurethane foam	Rotating biological contactor (RBBC) reactor	55% of sugar refinery wastewater	
	Polyurethane foam and polystyrene mesh as biosupport medium	Rotating biological contactor (RBBC) reactor	Complete decolorization of Direct Red-80 and Mordant Blue-9 in synthetic wastewater,	

			>64% of diluted raw wastewater	
	Adsorbed on polyurethane foam	Continuous Packed Bed Reactor	70% of kraft pulp bleach plant effluent	
Coriolus versicolor	Adsorbed in Polyurethane foam	Air Bubble Bioreactor	80% textile wastewater	Kapdan et al. 2000; Srikanlayanukul et al. 2006
	Entrapped on metal mesh particles	Packed Column Reactor	99% of textile dyestuff Everzol Turquoise Blue G	
Laccase	Entrapped in Copper alginate	Airlift Bioreactor	Complete decolorization of indigo carmine dye	Katuri et al. 2009; Osma et al. 2010; Teerapatsakul et al. 2017; Sondhi et al. 2018; Yuan et al. 2020
	Bacterial nanocellulose	Horizontal Rotating Reactor	~88% of Reactive Blue 19	
	Encapsulated in chitosan membrane	Membrane Reactor	>95% decolorization of acid black 10 BX	
	Covalently bonded alumina pellets	Fluidized bed bioreactor	Complete decolorization of Reactive Black 5	

			in simulated textile effluent	
	Entrapped in Cu-alginate beads	Continuous packed bed reactor	66% of textile effluent	
Manganese peroxidase	Entrapped in agarose beads	Packed Bed Reactor	~79%- ~95% of different textile effluent	Asgher et al. 2013; Bilal et al. 2017a
	Sol-gel entrapment using tetramethoxysilane and propyltrimethoxysilane	-	~98%- 100% decolorization of textile industrial effluent	
Lignin and manganese peroxidase from *P. chrysosporium*	Amberlite IRA-400	-	50% Kraft E1 effluent decolorization	Peralta-Zamora et al. 1998
Crude enzyme extract from *Trametes versicolor* and *Pestalotiopsis* sp.	Entrapped in double layer alginate beads	Vertical Bioreactor system MasterFlex®L/S® (Cole-Parmer Instrument Company)	45-84% of textile dyes Lefavix Blue 16, Reactive Remazol Violet 9, and Reactive Remazol Navy 4	Yanto et al. 2014
Horseradish	Entrapped in chitosan beads	Packed Bed Reactor	~82% to ~97% of Remazol	Bilal et al. 2016e; Bilal et al. 2017d

peroxidase			Brilliant Blue R, Reactive Black 5, Congo Red, and Crystal Violet	
	Entrapped in calcium alginate	Packed Bed Reactor	~72%- ~87% of reactive dyes (Reactive Red 120, Reactive Blue 4, Reactive Orange 16)	
Bitter gourd (*Momordica charantia*) peroxidase	Entrapment in calcium alginate-starch	Two Reactor System	>80% textile effluent decolorization	Matto et al. 2009
Turnip peroxidase	Adsorption to Concavalin-A-wood cheving	Two-reactor system	64% of Direct Red 23 and 50% of direct dye mixture	Matto and Husain 2009b
Ginger peroxidase	Entrapped into two hydrogels of guar gum-alginate/agarose	Continuous Packed Bed Reactor	69% and 80% textile effluent	Ali and Husain 2018
Peroxidase	Porous celite	Membraneless electrochemical reactor	92% simulated textile wastewater	Cho et al. 2009; Darwesh et al. 2019

	Magnetic nanoparticles	Prototype Sequential Bioreactor	Complete decolorization of Direct Green dye, and Reactive Red dye	
Recombinant oxygen insensitive azoreductase	Nickel chelating column	Packed Bed Reactor	Complete decolorization of methyl orange	Lang et al. 2013

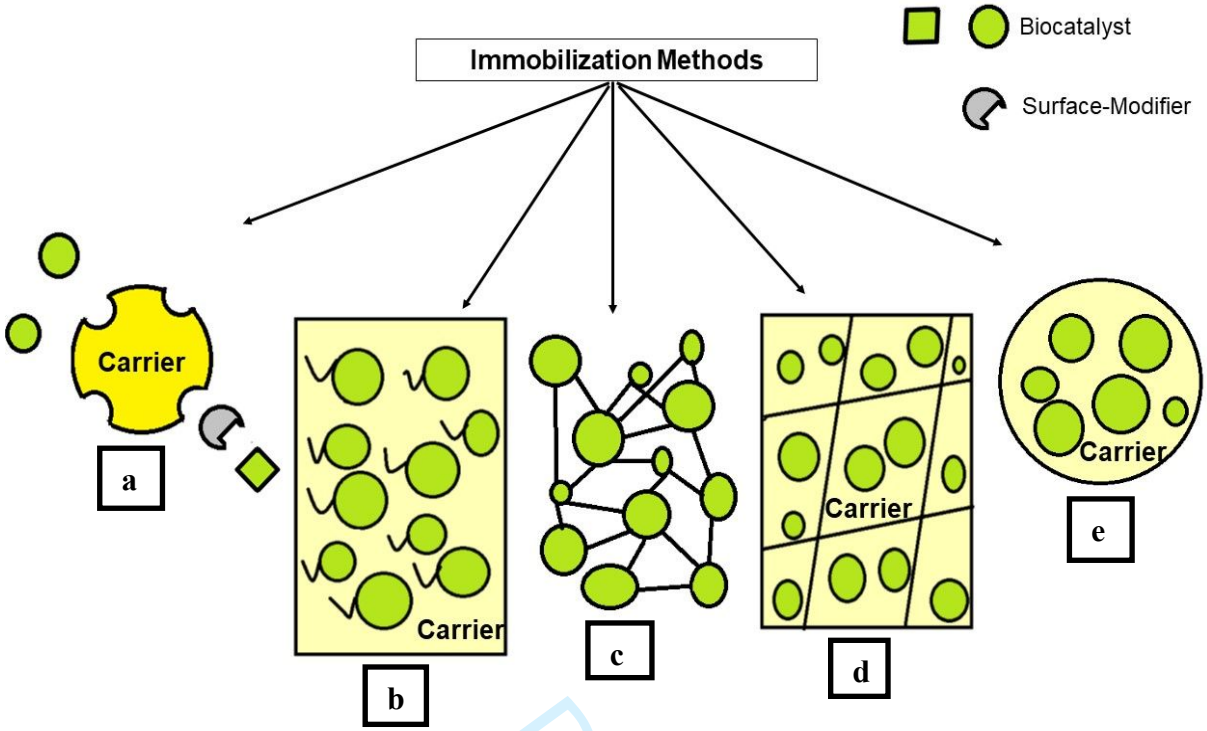

Fig. 1 Different types of Immobilization a. Adsorption; b. Covalent Binding; c. Cross-linking; d. Entrapment; e. Encapsulation