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BIOTECHNOLOGY PROGRESS

REVIEW

Formulation and Engineering of Biomaterials

Immobilized enzymes as potent antibiofilm agent

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Abstract

Biofilm has been a point of concern in hospitals and various industries. They not only cause various chronic infections but are also responsible for the degradation of various medical appliances. Since the last decade, various alternate strategies are being adopted to combat the biofilm formed on various biotic and abiotic surfaces. The use of enzymes as a potent anti-fouling agent is proved to be of utmost importance as the enzymes can inhibit biofilm formation in an eco-friendly and cost-effective way. The physical and chemical immobilization of the enzyme not only leads to the improvement of thermostability and reusability of the enzyme, but also gains better efficiency of biofilm removal. Immobilization of amylase, cellobiohydrolase, pectinase, subtilisin A and β-N-acetyl-glucosaminidase (DspB) are proved to be most effective in inhibition of biofilm formation and removal of matured biofilm than their free forms. Hence, these immobilized enzymes provide greater eradication of biofilm formed on various surfaces and are coming up to be the potent antibiofilm agent.

KEYWORDS
antibiofilm, covalent crosslinking, enzyme, gel entrapment, immobilization

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1 | INTRODUCTION

Bacterial biofilms have a manifold harmful effect on human society.[1] Being the foremost cause of biofouling in most industrial systems, and various life-threatening issues in the health care sectors, biofilm is responsible for the loss of more than billions of dollars and serious health crises throughout the world. A biofilm is an agglomeration of bacteria on a surface, where the sessile microcolonies dwelling remain encompassed by self-secreted extracellular polymeric substances (EPS) comprising of carbohydrate, proteins, lipids, nucleic acid, and other minerals that not only provide nourishment to the in-dwelling cells but also protects the cells from environmental stresses.[2] The bacterial species possess the property of adhering to the surface in the form of biofilm and forms an important survival strategy within nature.[3]

It can bring about the development of antimicrobial resistance, damage to the equipment, failure in transplant surgery, energy loss, contamination of products, and the onset of various types of chronic infections.[4] Medical prosthetics like implantable medical devices have become an important part of the modern health care sector providing an enhanced quality of life to millions of people.[5] But, unfortunately, the success of such implantation is severely hindered due to the formation of biofilm on the abiotic surface of the implant[6] and such biofilms are difficult to get eradicated.

Moreover, increased amount of morbidity, mortality, enhanced costs in the healthcare sectors, and prolonged period of hospitalization are mostly associated with the medical device associated biofilm infections.[6]

Hence, the dispersal of multispecies biofilms become the need of the hour. The conventional antimicrobial methods have been shown to eradicate planktonic microbes easily but have proved to be ineffective in the removal of sessile microcolonies.[7] Thus, alternate novel strategies are considered to be essential in fulfilling the requirement of removing the biofilm.

Although the concept of using enzymes to inhibit the formation of unwanted biofilms is not new, the scientific literature still lacks important information about the effects of immobilized biocatalysts and their impacts on biofilm formation as the scientific community underestimated or neglected the impacts of immobilized enzymes on biofilm structure and resistance to traditional antimicrobial agents.[8]

The present review focuses on the elucidation of antibiofilm efficacies of various enzymes, both free and immobilized, their degradation architecture, advantages and instances of immobilized enzyme as antibiofilm agent, use of nanocomposite of immobilized enzymes for treatment of biofilms, mechanism of quorum-quenching.

1.1 | Enzymes as antibiofilm and anti-biofouling agents

Enzymes possess the potential to control the process of biofouling.[9] by removing various types of biomolecular films and proteins from various biotic and abiotic surfaces.[10] The enzymes are considered to be an attractive anti-biofouling agent as they are natural molecules and eco-friendly in nature due to their easy bio-degradability. Due to their nontoxic nature and affordable prices, some of these enzymes are effectively used as antifouling paints in the marine environment as a substitute for biocides.[11]

High substrate affinity of the enzymes along with the economic and environmental friendliness resulted in the use of enzymes within detergent formulations for the purpose of the removal of biofilm.[12] Enzymes are also found to have therapeutic functions in the removal of pathogenic biofilms.[13] In recent times, a variety of enzymes enriched products have been commercialized that include tablets, rinsing solutions, chewing gums for dental treatment, and denitrifies containing enzymes like lysins, dextranase, mutanase, and so forth that can serve to play an effective role in the disintegration of the biofilm matrix[13] formed on different parts of the body.

1.2 | Lysozymes

Lysozymes are the group of hydrolytic enzymes that have shown efficacy in the hydrolysis of the cell wall and have been used to create a coating in most antibacterial agents. The most widely used lysozyme is isolated from hen egg white.[14] They can cleave the β-glycosidic bond between N-acetylmuramic acid of C1 and N-acetylglucosamine of C4 and destabilize the bacterial cell wall structure. Although Gram-negative bacteria show resistance to lysozymes due to the presence of an outer membrane that hinders the accessibility of the enzyme to the peptidoglycan,[14] a large group of Gram-positive bacterial species is susceptible to lysozyme attack, causing cell lysis. However, the susceptibility of the Gram-negative bacteria can be enhanced by pretreating the cells with detergents, chelating agents like EDTA, or in the presence of high hydrostatic pressure.[14] The non-enzymatic mode of lysozyme is based on the amphibolic and cationic properties of the enzyme[15] results in the perturbation of the plasma membrane and thereby activates the autolytic system within the bacteria.[16]

1.3 | Autolysins

Autolysins are a group of membrane-bound enzymes that bring about degradation of the peptidoglycans of the cell wall, resulting in the death of the cells.[17] Lysostaphin and the catalytic domain of LytM, the pentaglycine endopeptidases are responsible for the cleaving of the crosslinking of the peptidoglycan bridges present in the cell wall of *Staphylococcus* sp.[18] It is widely used to eradicate susceptible *Staphylococcus aureus* and *Staphylococcus epidermidis* biofilms.[19]

1.4 | Amylase

Amylase has been also documented to have a potential antibiofilm property by the mechanism of degrading the polysaccharide associated with the biofilm architecture.[7] The α-amylase produced extracellularly by *Bacillus subtilis* S8–18 from the marine environment showed

its antibiofilm efficacy against methicillin-resistant *S. aureus*.[20] It has also been found that enzymes like cellobiose dehydrogenase and amylase act synergistically and showed higher efficacy as antibiofilm and antibacterial agents. The cellobiose dehydrogenase acts upon various types of oligosaccharides and cellulose with the liberation of hydrogen peroxide. They act effectively in the mechanism of eradicating the biofilm. Pectinase also showed a potent enzyme in the mechanism of degrading the adhesive proteins thereby preventing the development of biofilm by *Enterococcus faecalis*, *S. aureus*, and *Pseudomonas aeruginosa*.[21]

1.5 | Glycoside hydrolase

They are usually produced by a group of opportunistic fungi like Aspergillus fumigatus and Gram-negative bacterial specie like *P. aeruginosa* that can be used for the purpose of degrading the biofilm formed by fungal species and thereby help in reducing virulence.[22]

Often, commercial biofilm exclusion requires the synergistic action of complex enzymes like proteases, lipases, and amylases that enhances the efficacy of removing complex biofilm from abiotic surfaces.[23] Various alternative strategies that help in determining the antifouling potential of enzymes comprise screening of the enzymes that help in the cleaving of the specific substrate, helping in cellular adhesion to the surface.[24] Thus, the process of enzyme screening plays a vital role in the process of targeting single or multiple species of foulers.[25]

The enzymes associated with anti-biofouling may be equipped with the power of degradation of compounds that can counteract adhesives, degradation of the extracellular polymeric substance (EPS), and denaturation of intercellular communication molecules leading to inhibition of quorum sensing (QS).[26]

2 | ENZYMES AFFECTING DIFFERENT EVENTS OF BIOFILM FORMATION

2.1 | Cell lysis by enzymes

Various types of lytic enzymes are found to act as antibacterial agents and can release cellular components like proteins and DNA[27] by degradation of the bacterial cell wall with the help of various hydrolytic enzymes. These cell-lysing hydrolytic enzymes can be classified as glycosidases (for cleavage of the polysaccharide chains), endopeptidases (for cleavage of the polypeptide chains), and amidases (for cleavage of peptides and polysaccharides).[28,29] Noteworthy lytic enzymes are: murein hydrolase, a glycosidase enzyme produced by plants and animals part of their defense system, endolysins, produced by bacteriophages and microlysins, produced by most microbes apart from bacteriophages.[28]

2.2 | Degradation of biofilm architecture

Extracellular polymeric substance (EPS) being a complex matrix comprising mainly of carbohydrates, proteins, fats, nucleic acids is the primary target of enzymes[30] and enzymes by degrading the EPS can bring about the dispersion of the biofilm. The enzymes exert their antibiofilm efficacies by degrading the EPS, followed by reducing the mechanical stability of the biofilm.[2] This results in the easy exposure of the sessile communities to antimicrobial agents and antibiotics causing an enhancement in its activity. Enzymes like dispersin B and protease action on the biofilm formed by *S. aureus* result in the elimination of the biofilm. Dispersin B possesses the ability to bring about the degradation of N-acetyl-D-glucosamine residues[31] and degrade the biofilm formed by *S. epidermidis* and *S. aureus*.[32] Cellulase has been found to have efficacy in biofilm removal in the textile, paper, and food industries.[33] Biofilm formed by *P. aeruginosa* is found to be affected in the presence of cellulase. Enzymes have shown their efficacy in removal of the dental plaque infections by bringing about the degradation of the structural components of the plaque.[34] Glycoside hydrolase β-N-acetylhexosaminidase (or dispersin B) is another very useful enzyme that has shown its efficacy in inhibiting the biofilm formed by major group of bacterial cells.[35]

2.3 | Enzymatic degradation of adhesives being produced by the sessile colonies

The adhesion of the bacterial colonies on the biotic and abiotic surfaces is mediated by various types of proteins or glycoproteins and various types of polysaccharides that help in the effective adhesion to the surface.[36] Thus, different enzymes play an effective role in the process of degrading various chemical components, facilitating bacterial adhesions, and thereby preventing the process of biofouling.[37] Various commercially used enzymes like hydrolase, lipases, and proteases prevent the setting of the microbial cells on the surface and degrade the adhesive components. Two important mechanisms that are responsible for the disintegration of the adhesive polymers as well as proteins that help in the surface attachment.[38]

2.4 | Chemical characteristics of enzymes making it a potent antibiofilm/antimicrobial agent

The chemical attributes of the enzymes responsible for antibiofilm activity have a potent site of action like that of the matrix of the biofilm. Enzymes like lysostaphin, beta-*N*-acetylglucosaminidase, DNase I, and dispersin B possess the ability to prevent the adhesion by the sessile microbial communities.[39] Studies have revealed that combinatorial activity of the enzymes dispersin B and DNase I possess the ability to inhibit colonization by *S. aureus*.[40] The enzymes possess the ability to bring about degradation of various types of polysaccharides, eDNA, proteins, and various other types of QS molecules.[41] The various types of enzymes also possess the ability to bring about hydrolysis of various types of autoinducers like acylases, lactonases, and oxidoreductase enzymes.[42] The ability of body to combat against microbial species includes the production of large amounts of superoxides those are associated with the membrane associated NADPH

TABLE 1 Enzymes with anti-QS activities

Name of the enzyme	Source	Function	Reference
AHL Lactonase	Produced from wide varieties of plants, fungi, bacteria and algae	Helps in the breakdown of the HSL ring	73
AHL oxidoreductase	Produced from wide varieties of plants, fungi, bacteria and legumes	It brings about degradation of the acyl chain of HSL by oxidation or reduction	73
AHL-acylase	Produced from wide varieties of plants, fungi, bacteria and legume	It brings about hydrolysis of amide linkage and degradation of HSL	73
2-Alkyl-3-hydroxy4 (1H)-quinolone 2,4-dioxygenase	*Arthrobacter* sp	Inhibits the QS molecules	74
AI-2 kinase	*Escherichia coli*	Brings about degradation of the autoinducers	75
Paraoxonase	Produced from wide varieties of plants, fungi, bacteria and legume	Prevents the development of biofilm by hindering the process of QS	47

oxidase. Xanthine oxidase, cyclooxygenases, and lipoxygenases are responsible for the production of superoxide anion.[43] The superoxides are the group of reactive oxygen species which acts as a source for the production of other ROS.[44] The enzyme superoxide dismutases into hydrogen peroxides that are being used for destroying the invading pathogens. Peroxidases like myeloperoxidases and lactoperoxidases use the hydrogen peroxides for the purpose of oxidizing the halides thereby bringing about reduction in the pathogenic organisms and act as potent antibiofilm and antimicrobial agent.

2.5 | Enzyme-mediated mechanism of quorum-quenching

The sessile microcolonies communicate with each other by a density-dependent cellular communication mechanism known as QS.[45] Various enzymes can obstruct such communication (Table 1) by degrading the QS signal molecules and thereby can prevent the formation of biofilm.[46] Since, Gram-negative bacteria communicate by acylhomoserine lactone (AHL) whereas Gram-positive bacteria by autoinducer peptides,[45] the enzymes required for hindering QS in them are different. AHL acylase breaks the amide bonds present within the acyl chains of the homoserine lactone rings[47] while adenine dinucleotide phosphate oxidase can inactivate the autoinducer peptide signals through oxidation of the C-terminal methionine, associated with the peptides.[48]

2.6 | Immobilization of enzymes

For judicious exploitation, the enzymes may be immobilized, which helps in enhancing the enzyme activity, stability, and selectivity.[49] Immobilized enzymes show their activity over a wider range of various environmental conditions comprising of temperature, pH, and higher stability over a longer period of storage. It also helps in reducing the chances of enzyme inhibition by the reaction product, substrate, and various other components that are present in the environment.[50] Enzyme immobilization supports in localization of enzymes as per the requirement like the fouler-coating interface, which enhances the efficacy of the enzymes.[50]

Although free enzymes are proven to have antibiofilm efficacies, immobilization is found to increase their potential. The augmented storage stability and the reusability of the immobilized catalyst[51] are advantageous for use as an antibiofilm and antifouling agent. Compared to free enzymes in solution, immobilized enzymes are more powerful and more resistant to environmental changes. The covalent immobilization led to the highest amount of enzyme deposited on the surface[52] and thereby maximized the interaction between enzyme molecules and biofilm matrix.

There are various types of immobilization techniques that involve covalent bonding, adsorption, graft-copolymerization, cross-linking, and entrapment.[53] Generally, the mechanism of enzyme immobilization does not provide considerable results and usually trial and error mechanisms help in developing the system of immobilization needed for industrial requirement.[53] The simplest mechanism of enzyme immobilization includes the process of entrapment and physical adsorption that result in the process of enzyme leaching from the immobilized surface, poor performance and low stability.[50] The mechanism of covalent immobilization helps in improving the enzyme stability with minimum loss of enzyme within the aqueous media. The general mechanism of covalent immobilization help in preventing denaturation by the formation of a multiple number of covalent bonds with the enzyme followed by a reduction in the conformational flexibility. The process of thermal vibration prevents the unfolding and denaturation of the enzyme.[53] The chemical modification brought about by covalent binding is one of the drawbacks.[53] The retainment of the stability and activity of the enzyme results in the mechanism of site-directed immobilization scheme by maintaining the accessibility of the active site of the enzyme to the specific substrate. The mechanism of site-directed immobilization is an advantageous technique over the process of random immobilization[53,54] (Figure 1).

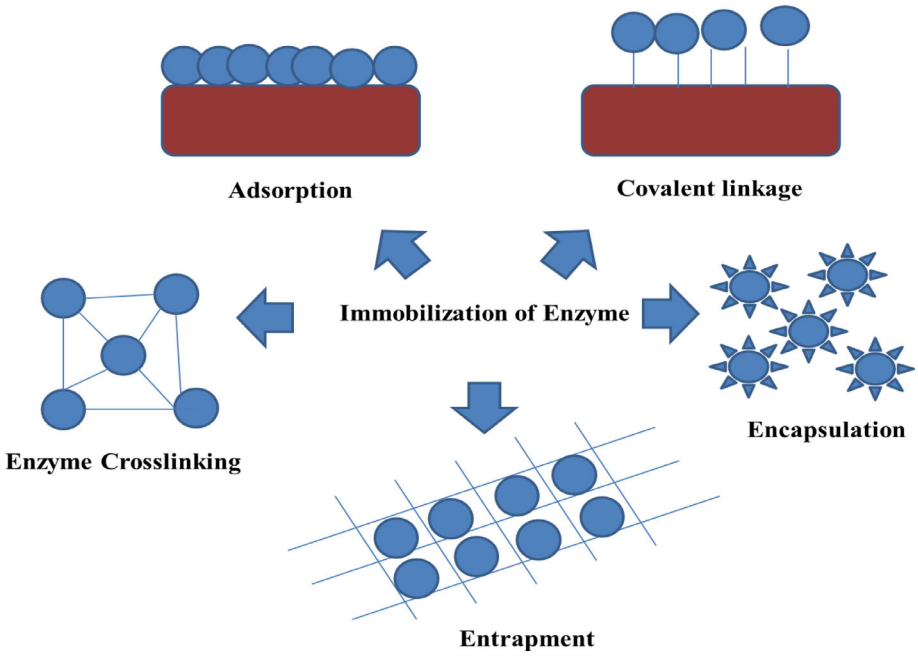

FIGURE 1 Mechanisms of enzyme immobilization

2.7 | Advantages of enzyme immobilization

Enzymes after immobilization, show remarkable chemo as well as thermostability. It will be repeatedly used for a number of subsequent cycles. The recovery of immobilized enzyme, after a reaction becomes much easier with almost no or minimal loss the catalysts. This enhanced the cost-effectiveness of the production process. Use of immobilized enzyme not only curtails the cost of labor, space and more, but also makes the entire handling process more convenient. Immobilization through crosslinking, entrapment, or capsulation can convert the enzyme in a form that can be applied for a variety of purposes. Hence, immobilization allows a consistent supply of products to the market. Other notable benefits of the use of immobilized enzymes in industry include enhanced efficiency of enzyme with minimized reaction time, high enzyme-substrate ratio, and improvement in process control with less labor input. Immobilization can ameliorate the entire system with reduced opportunities for contamination in products formed.

2.8 | Immobilized enzymes as antibiofilm agent

A number of physical adsorption or chemical entrapment matrices are used to immobilize enzymes and are found to gain more antibiofilm efficacy after immobilization (Table 2).

The effects of the glycosidase pectinase and the protease subtilisin A, two commercially available immobilized enzymes were successfully applied as an antibiofilm agent against *Escherichia coli* biofilm. The best antibiofilm performance of solid-supported hydrolases was obtained at the surface concentration of 0.022 and 0.095 U/cm^2 with a reduction of 1.2 and 2.3 log CFU/biofilm for pectinase and subtilisin, respectively.[8]

The papain, an endolytic cysteine protease (EC: 3.4.22.2) isolated from *Carica papaya* latex immobilized on the chitosan matrixes of molecular weight (200 and 350 kDa) showed anti-biofilm activity and increased the antimicrobials efficiency against biofilm-embedded bacterial strains of *S. aureus* and *S. epidermidis*.[55]

The detachment of biofilm already formed by *S. epidermidis*, *S. aureus*, and *Aggregatibacter actinomycetemcomitans* was efficiently accomplished by the recombinant enzyme β-N-acetyl-glucosaminidase (DspB) originally cloned from *A. actinomycetemcomitans* CU1000 and immobilized on carboxymethyl chitosan (CMCS) modified by linoleic acid (LA) after sonication.[56]

A remarkable reduction of the protein and carbohydrate content of the biofilm matrix of *S. aureus* and *S. aureus* was brought about by lipase immobilized polycaprolactam (LIP).[57] Similarly when polycaprolactam is used to immobilize the proteolytic enzyme Subtilisin, shows antimicrobial activity against both Gram-positive as well as negative microbes.[58]

Immobilization of subtilisin A is found to play an important role in the process of the biofilm removal.[59] On the other hand, the enzymes, subtilisin A and the glycoside hydrolase cellulose, when immobilized through covalent crosslinking onto poly(ethylene-alt-maleic) anhydride copolymer films, the biofilm attachment was reduced by 44% in *P. aeruginosa*.[60]

Langumir Blodgett (LB) immobilized lipase showed about a 20% increase in antimicrobial and antibiofilm activity in comparison to its free form. Moreover, the immobilized enzyme achieved immense thermostability.[57] A significant reduction in the carbohydrate and protein content of EPS of *S. aureus* and *E. coli* was found after treatment with lipase immobilized on polycaprolactam (LIP), a porous polymer, resembling natural polypeptide.

The immobilization of cellulase within glutaraldehyde has been found to bring about partial removal of the biofilm formed by *P. aeruginosa*.[49]

TABLE 2 The immobilization of enzymes with antibiofilm activities and their mode of action

Name of the Enzyme	Immobilization material	Mode of action as antibiofilm agent	Reference
Lysostaphin	Polydopamine is used for the purpose of immobilization that attaches the enzyme by covalent crosslinking	They inhibit the biofilm by degrading the cell wall of *Staphylococcus aureus*.	76
Ficin	Immobilized on the surface of chitosan	They help in the degradation of biofilm by *S. aureus* and enhance the susceptibility of the microbial cells to the antimicrobial agents.	77
Protease	Immobilized on the surface of chitosan	They act effectively in eradicating the biofilm formed by *Pseudomonas aeruginosa*, *Listeria monocytogenes* and *S. aureus*	78
Papain	Immobilized on the surface of chitosan	They effectively remove the biofilm formed by *S. aureus* and *S. epidermidis*	66
β-N-acetyl-glucosaminidase	Immobilized on the surface of carboxymethyl chitosan	They effectively remove the biofilm formed by *S. aureus* and *S. epidermidis*	56
Lipase	Immobilized in polycaprolactum	Help in reducing the biofilm formed by *Escherichia coli* and *S. aureus* by bring about marked reduction of the architectural component of extracellular polymeric substances	57
Alginate lyase	Immobilized on the surface of chitosan nanoparticles	It helps in the degradation of alginate associated with the biofilm of *P. aeruginosa*.	61
DNase I	Immobilized on the surface of polydimethylsiloxane	Inhibition of the biofilm formed by *P. aeruginosa* and *S. aureus*	79
Alginate lyase	Immobilized on the surface of chitosan nanoparticles of ciprofloxacin	Helps in inhibiting the biofilm formed by *P. aeruginosa* thereby helps in preventing cystic flibrosis.	80
Hydrolase	Immobilized upon solid surface	Helps in inhibiting the biofilm formed by *E. coli*	8
Acylase	Immobilized in polyurethane	It helps in a 60% reduction in the biofilm formed by *P. aeruginosa*.	81
Cellobiose dehydrogenase	Immobilized on plasma-activated urinary polydimethylsiloxane	Helps in the eradication of *S. aureus* biofilm	82
Lysozyme	poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate)	Helps in the eradication of the biofilm *E. coli*	83
Deoxyribonuclease I and cellobiose dehydrogenase	Surface of chitosan nanoparticles	Helps in the eradication of biofilm formed by *S. aureus* and *Candida albicans*	84
α-Chymotryps	Immobilized on the surface of immobilized polyethylene	Helps in the eradication of biofilm formed by *E. coli*	85
Protease	Immobilization on the surface of polypropylene	Helps in the eradication of biofilm formed by *Candida albicans*	86
Glycoside Hydrolase Dispersin B	Immobilized on the surface of magnetic nanoparticles	Helps in the removal of the biofilm formed by *S. aureus*	87
Dextranase	Immobilized on the surface of the alginate	Helps in the mechanism of eradicating the biofilm formed by *Streptococcus mutans*	88
Glycoside hydrolase	Immobilized by the cross-linking of glutaraldehyde and amine functionalization	Inhibits the formation of biofilm by *P. aeruginosa*	89
α-amylase	Immobilized on the surface of silver nanoparticles	Helps in the purpose of eradicating the biofilm formed by multidrug resistant bacteria	90

The antibiofilm efficacy of alginate lyase, Aly08, cloned from the marine bacterium *Vibrio* sp. SY01, was found to be enhanced after immobilization on low molecule weight (LMW) CS-NPs, as the immobilized enzyme achieved more inhibitory potential for biofilm formation and eradication of mature biofilm of *P. aeruginosa*, making the bacteria more sensitive to antibiotics.[61–63]

Amylase and Cellobiose dehydrogenase were immobilized on the surface of urinary catheters using different techniques including ultrasound, layer by layer and covalent binding, of which the maximum enzyme deposition on the surface could be achieved by covalent binding but the highest antibiofilm activities were shown when enzymes were immobilized using polyelectrolyte layer by layer technique. In

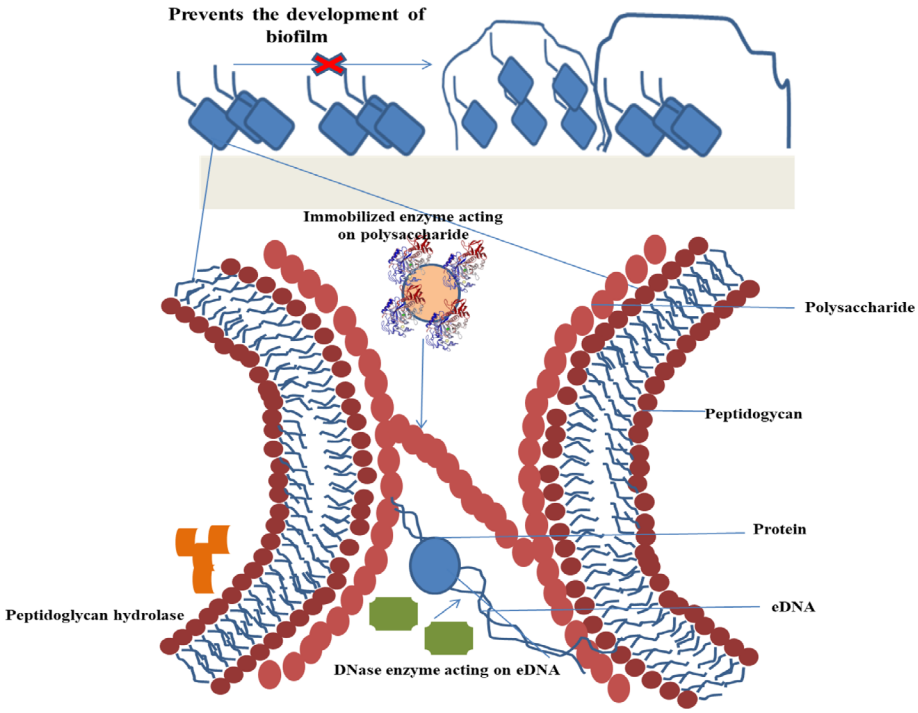

FIGURE 2 Mechanism of inhibition of biofilm by the immobilized enzyme

this technique, enzymes are co-assembled with polyelectrolytes and complementary functionalization with tailored protective inert polymers comprising cationic anchor groups and zwitterionic functional groups, and a coating method has been established and also successfully used for enzyme immobilization (Figure 2). Another strategy is the combination of the nonchemical modification with immobilization of CDH and/or PIPs to make an anti-biofilm coating on the catheter.[64]

The mechanism of immobilization of lysostaphin (Lst) on the surface of polystyrene and fluorinated ethylene propylene catheters inhibits the adherence of *S. aureus* thereby preventing the formation of biofilm.[65] It has been found that Lst-coated equipment surfaces may be used to kill nosocomial strains of *S. aureus* in less than 15 min and prevent biofilm formation.[55,66]

2.9 | Use of nanocomposite of immobilized enzymes for treatment of biofilms

The use of nanotechnology makes the use of nanoparticles as the carriers of biocatalysts that enhances the catalytic effect due to enhanced volume to surface ratio. These nanomaterials possess the ability to immobilize various types of enzymes including lipases and cellulases that provide an innovative catalytic property.[67,68]

The nanoparticles possess very high efficiency to support various types of immobilized enzymes due to its ideal characteristics for bringing about balance in the determining factors including surface area, effective enzyme loading, and mass transfer resistance. Gold nanoparticle (GNP) were developed functionalized with enzyme proteinase K (denoted as GNP + PK) and were found effective against the biofilm formed by Pseudomonas fluorescens for 72 h.[69]

The antibiofilm activity of chitosan NP are remarkably increased when linoleic acid-modified chitosan NPs were used to immobilize the enzyme β-N-acetyl-glucosaminidase (DspB) as immobilized enzyme remained active for a long time.[56]

The biofilm formed by *S. aureus* on titanium could be removed by the combination of self-immobilization chemistry of dopamine with a biofilm-lysing enzyme, α-amylase, and an antimicrobial agent, silver nitrate.[70]

Nanozymes being the type of nanomaterials that possess various types of enzyme-like properties and also exhibit various types of physicochemical properties pertaining to nanomaterials.[71]

Studies have shown protein/inorganic hybrid nanozymes by being oriented in the form of immobilized structure on the surface of inorganic grapheme nanoparticles.[72]

3 | CONCLUSION

The recalcitrance of biofilm-associated bacteria makes them almost impossible to tackle and the surface-attached colonies pose a potent threat to health sectors and industries. Since almost all attempts to eradicate biofilm with conventional antibiotics are found to be ineffective, researchers are trying to explore new approaches to remove them. Among the aqueous-soluble macromolecules, enzymes are proved to be with significant antibiofilm efficacy. In order to increase the activity, shelf life, thermostability, and reusability, the researchers prefer to immobilize the enzymes through numerous processes like adsorption, gel entrapment, covalent crosslinking, and ionotropic gelation and use them for complete eradication of biofilm. The immobilized enzymes not only inhibit biofilm formation but also can remove

the already formed mature biofilm. Immobilization of enzymes amylase, cellobiohydrolase, pectinase, subtilisin A and β-N-acetyl-glucosaminidase (DspB) have proved to have maximum efficiency in the eradication of the biofilm. Hence one or multiple enzymes co-immobilized on a gel matrix may be successfully used to remove biofilm from various biotic and abiotic surfaces in a nontoxic and cost-effective way.

AUTHOR CONTRIBUTIONS

Dibyajit Lahiri: Investigation (equal). **Moupriya Nag:** Investigation (equal). **Ankita Dey:** Investigation (equal). **Tanmay Sarkar:** Investigation (equal). **Rina Rani Ray:** Investigation (equal). **Maksim Rebezov:** Investigation (equal). **Mohammad Ali Shariati:** Investigation (equal). **Muthu Thiruvengadam:** Investigation (equal). **Jesus Simal-Gándara:** Investigation (equal).

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

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