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Strategies, challenges and opportunities of enzyme immobilization on porous silicon for biosensing applications

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ABSTRACT

The use of porous silicon (PS) for biosensing applications is now emerging as it offers desired properties such as better surface-volume ratio, tunable pore size, ease of fabrication, and compatibility with the current silicon-based technology. These superior properties make PS as a promising candidate for the immobilization of biomolecules with higher retention, stability, storage, and operational ease. High specificity and catalytic activity of the enzyme for the substrate are advantageous for the detection of a particular analyte with higher accuracy in less time. Immobilization of enzyme on the PS surface alters the surface electrical and optical properties, and can conveniently be used for the sample analysis. In the present review, the strategies of enzyme immobilization on PS and various influencing factors are discussed in detail. The scope of PS for enzyme immobilization and prerequisite surface modifications has been assessed in brief. The review discusses the various methods of enzyme immobilization on PS with their benefits and bottlenecks with special emphasis on the approaches to overcome the difficulties during the immobilization process. Further, various properties of PS influencing the process of immobilization have been outlined in brief. An overall analysis of enzyme immobilization on PS surface has been given for the successful development of PS based enzymatic biosensors.

1. Introduction

Enzymes are a group of proteins that act as a catalyst for the transformation reactions of the specific substrate by lowering a reaction's activation energy barrier, thereby increasing the rate of the reaction. Enzymes increase the reaction rates without changing the chemical equilibrium between reactants and products [1]. The specific structural configuration of amino acids in the active site of enzyme governs enzyme activity. For the maximum activity of the enzyme, optimum reaction conditions, especially pH and temperature are necessary [2,3]. Enzymes are known for efficient catalytic activity and their high specificity for the substrate, which is based on the substrate's chemical and structural configuration in a particular environmental condition without producing any unwanted by-product [4]. Unlike traditional organic catalytic processes, enzymes need no activation or deactivation procedure for functional groups. Different industries (chemical, pharmaceutical, cosmetic, paper, and food) [5,6], biological waste treatment plants, enzymatic bio-analysis [7], and clinical field [8] use enzymes for efficient process and production. Enzyme inhibition based biosensors are becoming popular recently for the detection of the heavy metals concentrations based on the variation in the activity due to the presence of heavy metals [9,10].

Enzymes are used in free-form for most of the processes. Free-form enzymes are more susceptible to the presence of salts, surfactants, alkalis [11], trace levels of substances, can act as inhibitors which add to production costs [12]. Variation in reaction conditions due to the reaction progress, free-form enzymes may lose and result in subsequently reduced enzyme recovery from the reaction mixture. Variation in enzyme activity affects the quality and quantity of products. Since the enzyme production process is relatively expensive, the recovery of enzymes on post-reaction may reduce operating costs. However, the recovery and reuse of enzymes from the reaction solution is challenging and requires expensive purification processes [13].

Immobilization of enzymes is a proven alternative way to overcome ill effects of the use of free enzymes, making the production process more efficient and economical. Aminoacylase was the first enzyme to be immobilized on the support material polymer DEAE-Sephadex (Diethylaminoethyl) for the industrial production of L-methionine in

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1969 [14]. Immobilization of enzymes may define as the attachment of enzymes to support material to restrict the mobility of molecules to reduce loss and natural recovery without/minimal loss of enzyme activity [15]. Immobilization provides enzyme stability against structural deformation and various denaturing organic solvents [16] by engaging the amino acids with activated carrier functional groups [17]. The presence and density of functionalized groups on the enzyme surface determine the efficiency of the immobilization process and stability of immobilized enzymes during storage and operations.

Lyophilized enzymes used in most of the industrial processes tend to denature the native enzymatic structure. Immobilization may offer conformational stability to enzyme molecules by restricting the configurational variations through multipoint covalent binding of enzymes to surface [18]. Immobilization provides stability during storage and operational conditions by preventing the denaturation due to temperature variation or by some organic solvents as well as by autolysis. The use of multiple enzymes via immobilization is possible for simultaneous or sequential production processes to reduce the overall production time [19].

Immobilized enzymes offer great potential for the development of sensors to detect and determine their respective target molecules. In the enzymatic biosensors, the substrate/analyte molecules from the medium are expected to travel towards immobilized enzymes. The catalytic activity of the enzyme converts the substrate into a product with a simultaneous release of the electron. The released electron is transferred to the transducer via an electrode, which can be detected [20]. Improved stability and retained activity extend the use of immobilized enzymes in varying environmental conditions. Passage of the substrate molecule towards the immobilized enzyme can be monitored by a selective membrane to reduce the alteration in the enzymatic process.

Apart from the biosensors, immobilization of enzymes has been used in various other applications such as bioreactors [21], industrial biocatalysts [22], biofuel cells [23]. In the field of medicines, the delivery of therapeutic enzymes is usually carried out in the immobilized form in order to treat deficiency or diseases [24,25]. Also, immobilized enzymes are used in the bioremediation and food industries [26].

With many advantages, there are certain drawbacks of the immobilization process. During the immobilization process, functional groups present on the surface of the enzyme may get inactivated due to bonding with the support material. Enzyme inactivation may lead to unfavorable configurational changes resulting in kinetic variations [27]. Mass transfer limitation is the primary factor affecting the activity of the enzyme, predominantly enzymes with lower specific activity, by limiting the availability of substrate, and hence reduces the yield of the immobilization process. Sometimes, leaching out of enzyme molecules from the surface may occur, adding additional procedures for recovery of leached out enzymes and contamination removal [28]. Table 1 summarizes the overall benefits and bottlenecks of enzyme immobilization.

Table 1
Benefits and bottlenecks of enzyme immobilization.

Benefits	Bottlenecks
• The decrease in enzyme quantity requirement • Usable in the extreme reactor conditions • Broad range pH, and temperature stability • Increased stability by multipoint binding • Applicable to a different mode of production • Multiple enzymes for cascade reactions • High recyclability • Improved activity over the free-form enzyme • Provides better control over the reaction • Reduction of interference by various factors	• Functional group inactivation • Unfavorable changes in kinetics • Contamination due to support • Immobilization cost • Cost of support and linkers • Mass transfer limitations • Occupies space within the reactor • Fouling of supports

2. Why porous silicon (PS) for immobilization of enzymes?

The interaction between the support and the enzymes determines the extent of the effects of specific biochemical, mechanical, and chemical properties [29]. The selection of suitable support for a particular enzyme enhances its binding during immobilization that directly influences the effectiveness of immobilization significantly. The ideal support should have hydrophobicity, biocompatibility, microbial attack resistance, compression resistance, and accessibility at an economical price [30]. The choice of support must be with specific considerations of high affinity for the enzyme, the scope of surface modifications for functional groups, rigid mechanical properties, biodegradability, and nontoxicity [31,32]. Different support offering diverse properties makes the selection of support material more complicated due to the resulting modifications in characteristics of the immobilization system [33]. The advancement of support material properties over the period has enhanced biocompatibility and efficiency of the immobilization process. Fig. 1a illustrates the advancement of the use of immobilization support materials for biomolecules over various generations.

Support materials are of two classes namely organic and inorganic based. Commonly used support materials are organic that includes carboxymethyl-cellulose, collagen, starch, modified sepharose, and agarose [34]. Inorganic materials like diatomaceous earth, hydroxyapatite, titanium, ceramic [35], silica, and treated porous glass surfaces [36] are in use for immobilization of enzyme and various molecules. Fig. 1b represents the detailed classification of support materials based on organic and inorganic nature.

The characteristics of the biosensors are governed by the support material and enzyme selection. Enzymatic biosensors typically require the transfer of the electrons generated by electrochemical reactions for the generation of signals. Support materials with good conductivity enhance the transfer of electrons from the reaction centre to the electrode surface and thus offers better sensitivity [37]. Materials like metal nanoparticles have excellent conductive properties and offer a greater surface area for enzyme immobilization. Therefore, nanomaterials are preferred for the development of sensitive biosensors [38]. Carbon nanotubes and graphene materials also have exhibited extensive mechanical and electrical properties for immobilization of enzymes and rapid electron transfer rates [39,40]. Therefore, these materials are often used for the oxidoreductase based biosensors for the detection of phenols [39].

Porous silicon (PS) constitutes silicon nanocrystals with a high surface area network [41]. Crystalline Si may transform into PS by different established methods: electrochemical etching, strain etching, and plasma dry etching. Electrochemical etching, of a single-crystal silicon wafer in a hydrofluoric acid-containing the electrolyte, is a simple and established a way of fabricating PS [42]. PS offers numerous advantages of the enhanced surface to volume ratio, improved surface reactivity, and luminescence.

Additionally, variation in current density, electrolyte ratio, and silicon wafer type offers control over the pore size, porosity, and thickness of the porous layer [43]. A large surface area achieved by a porous layer can accommodate a large number of biomolecules and results in a change in the refractive index. The effect of the variation on the shift of the reflectance spectrum is applied for the detection and analysis of biomolecules [44]. PS exhibits bright luminescence at room temperature, not observed in the case of crystalline silicon [45]. Some specific semiconductor properties of Si allow the development of a broad range of micropatterning processes for achieving functional features for future

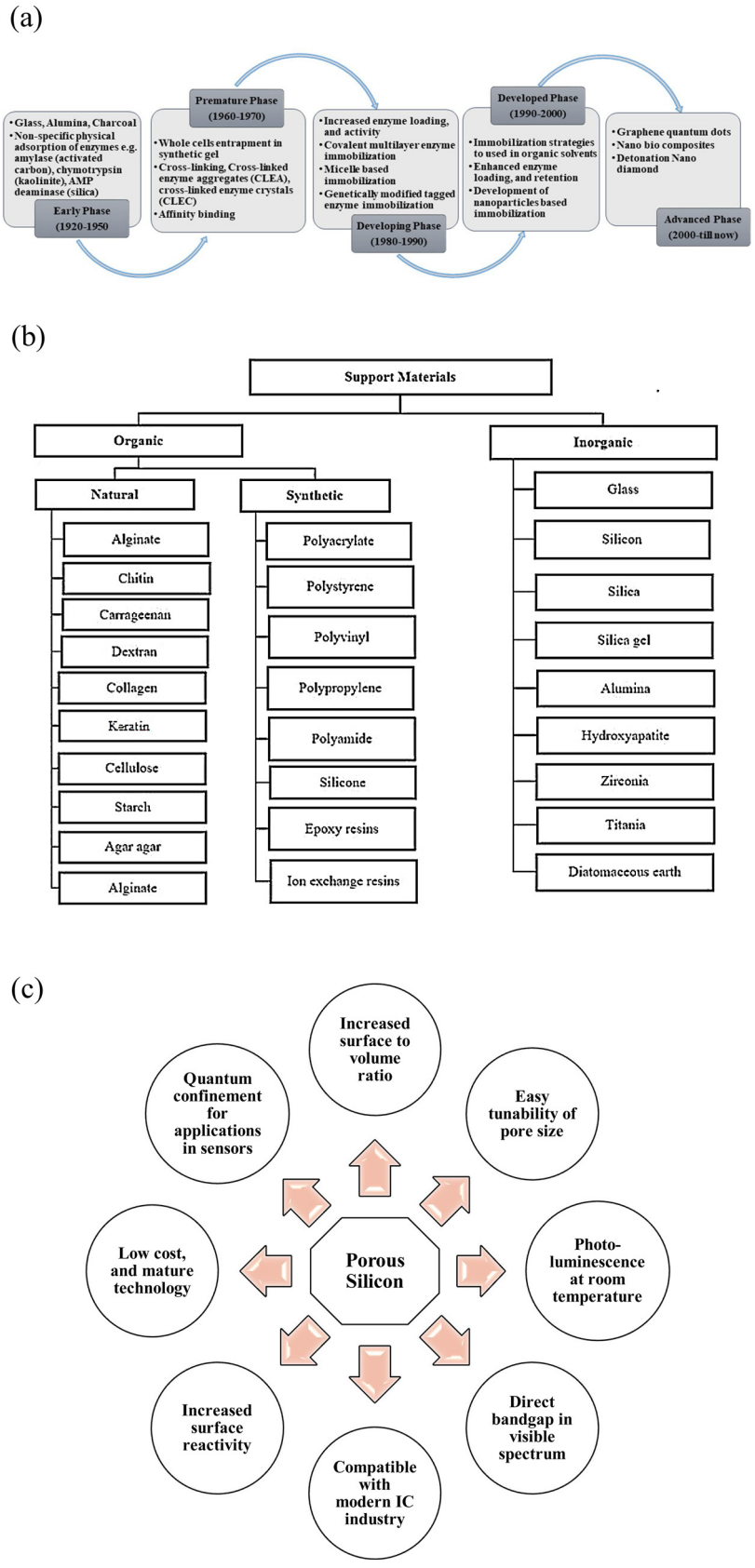


Fig. 1. a. Advances in the support materials for the immobilization of biomolecules over various generations. b. Classification of support materials used for the immobilization of biomolecules. c. Advantages of PS over bulk silicon for enzyme immobilization.

Table 2
Various enzymes immobilized on the porous silicon for different applications.

Enzyme	Materials used	Purpose	References
Physical adsorption			
Urease	–	Enzyme immobilization study	[31]
Lipase	–	Triglycerides detection	[50]
Acetylcholinesterase	–	Study of hydrolytic response for acetylthiocholine iodide	[51]
Penicillinase	–	Capacitive field-effect microsensor	[52]
Glucose oxidase	–	Glucose and heavy metals detection	[53]
Urease	–	Urea and heavy metals detection	[53]
Tyrosinase	–	Catechol detection	[54]
Penicillinase	–	Biosensing applications	[48]
Burkholderia cepacia lipase	–	Improvement of catalytic performance and recyclability potential	[55]
B-Glucosidase	IL	Study the pore morphology on the catalytic performance	[56]
Laccase	–	2,4-dichlorophenol (2,4-DCP) removal	[57]
Acetylcholinesterase	PDDA	Capillary electrophoresis-immobilized enzyme microreactors	[58]
Glucose 6-phosphate dehydrogenase	APTMS	Enzyme immobilization for biocatalyst applications	[59]
β-Galactosidase	–	Enzyme immobilization study	[60]
L-asparaginase	TA	Enzyme immobilization study	[61]
Alcohol dehydrogenase	–	Enhanced Activity of Alcohol Dehydrogenase in Porous Silica	[62]
Horseradish peroxidase	–	Enzyme immobilization study	[63]
Laccase	–	Catalytic phenol removal using enzyme immobilization	[64]
Crosslinking			
Peroxidase	10-undecenoic acid and EDC	Enhancement of enzyme stability by co-immobilization with protein	[65]
Penicillinase	ANB-NOS	Optimization of sensors	[66]
Laccase	EDC and NHS	Oxygen bio electro-reduction study	[67]
Lactate dehydrogenase	MBA, 4-MPA & Sulfo-LCSP	Material biofunctionalization with different crosslinking agents	[68]
Lipase	APTES, MA &EDA	Integrating hollow magnetic fibrous silica with PAMAM into a single nanocomposite for enzyme immobilization	[69]
Lipase	APTMS & GTA	Enzyme immobilization study	[70]
Laccase	GTA	Catalytic phenol removal using enzyme immobilization	[64]
Covalent binding			
Coprinus cinereus peroxidase	APTES and GTA APTES and EDC EDC	Optimization of enzyme immobilization	[71]
Horseradish peroxidase	APTES and GTA APTES and EDC EDC	Optimization of enzyme immobilization	[71]
Urokinase	APTES and Bismaleimide APTES and DSC	Biofunctionalization of silicon	[72]
Urease	3-MPA	Urea biosensor	[73]
Glutathione-S-transferase	Aminosilane and GTA	–	[74]
Peroxidase	APDES and GTA	Enhancement of enzyme stability	[75]
Acetylcholinesterase	APTES and cyanuric chloride	Inhibition effect studies	[76]
Invertase	APTES and GTA	Hydrolysis of sucrose	[77]
Glucose oxidase	APTES and GTA	Improvement of silicon enzyme microreactors performance	[78]
Trypsin	APTES and GTA	Improvement of silicon enzyme microreactors performance	[78]
Lipase	APTES and GTA	Triglycerides detection	[79]
Glucose oxidase	APTES and GTA	Enhancement of immobilized enzyme activity	[80]
Glucose oxidase	APTES and GTA	–	[81]
Glucose oxidase	APTES and GTA	Interaction between biotin and streptavidin	[82]
Alanine aminotransferase	APTES and GTA	Alanine detection for liver diagnosis	[83]
Aspartate aminotransferase	APTES and GTA	Aspartate detection for liver diagnosis	
Cholesterol oxidase	APTES and GTA	Cholesterol detection for liver diagnosis	
Bilirubin oxidase	APTES and GTA	Bilirubin detection for liver diagnosis	
Glutamate oxidase	APTES and GTA	L-glutamate detection for liver diagnosis	
Horseradish peroxidase	APTES, DIEA and DSC grafting	–	[84]
P450	APTMS and GTA	Arachidonic acid detection	[85]
Trypsin	APTMS and GTA	Biosensor for target molecule screening	[86]
β- Glucuronidase	SPDP functionalization and GMBS linking	p-nitrophenol detection for chemical sensors development	[87]
Horseradish peroxidase	EDC and NHS	Phenol detection	[88]
Cytochrome C	APTES and GTA MPTES	Electrochemical evaluation of enzymatic electrode	[89]
Urease	PEI and GTA	Immobilized enzyme stability	[90]
Endoglucanases	PEI and GTA	Cellodextrins and soluble cellulose derivatives	[91]
Carbonic anhydrase	SA and $CuSO_4$	Investigation of the catalytic performance of the enzyme– silica systems	[92]
Horseradish peroxidase	SA and $CuSO_4$	Investigation of the catalytic performance of the enzyme– silica systems	[92]
Trypsin	APTES	Optimization of enzyme activity via Response Surface Methodology	[93]
Naringinase	GTA	Study of the characteristics and hydrolysis properties of enzyme immobilization	[94]
Lipase	GTA	To study the effect of the individual bisepoxides on the catalytic performance of the immobilized Lipase	[95]
Lipase	GTA	Effect of GTA Concentration on catalytic efficiency	[96]
β-Galactosidase	GPTMS	Enzyme immobilization study	[97]
Bilirubin oxidase	APTES & GTA	Dye degradation using enzyme immobilization	[98]
Gold binding proteins	APTES	Enzyme immobilization for gold recovery	[99]
Laccase	APTES	Bisphenol A degradation study	[100]
Bacterial feruloyl esterase	OCTS & PHTS	Enzyme immobilization study	[101]
Acetylcholinesterase	VTMS, GPTMS, and DPSD	Enzyme immobilization study	[102]
Entrapment			

(*continued on next page*)

Table 2 (*continued*)

Enzyme	Materials used	Purpose	References
Glucose oxidase	Gelatin	Comparison of immobilization methods	[103]
Horseradish peroxidase	PEI	Enzyme immobilization study	[104]
Xylanase	–	Enzyme self-immobilization study	[105]

ANB-NOS: N-5-azido-2-nitrobenzoyloxysuccinimide; **APTES**: (3-Aminopropyl)triethoxysilane; **APTMS**: (3-Aminopropyl)trimethoxysilane; **CES**: Carboxyethylsilanetriol sodium salt; **DIEA**: Diisopropylethylamine; **DPSD**: Diphenylsilanediol; **DSC**: Bis(N- succinimidyl)carbonate; **EDA**: Ethylenediamine; **EDC**: Carbaodiimide; **GMBS**: N-maleimidobutyryl-oxysuccinimide ester; **GPTMS**: 3-glycidyloxypropyltrimethoxysilane; **GTA**: Glutaraldehyde; **IL**: phosphonium-based ionic liquids; **MA**: Methyl acrylate; **MBA**: 4-mercaptobutyric acid; **3-MPA**: 3-mercaptopropionic acid; **4-MPA**: 4-mercaptophenylacetic acid; **MPTES**: Mercaptopropyltriethoxysilane; **MSN**: Mesoporous silica nanoparticles; **NHS**: N-Hydroxysuccinimide; **OCTS**: Octyltriethoxysilane; **PDDA**: Poly(diallyldimethylammonium chloride); **PEI**: Polyethyleneimine; **PHTS**: Phenyltriethoxysilane; **SA**: Sodium ascorbate; **SPDP**: N-Succinimidyl-3-(2-Pyridyldithio)propionate; **TA**: Tannic Acid; **VTMS**: Vinyltrimetoxysilane.

integration in complex systems [46].

Among microelectronic materials used in biosensor development, silicon (Si) has the most mature, low-cost technology with readily available and better compatibility to modern IC (integrated circuit) industry. Therefore, a comprehensive approach for Si-compatible technologies offers a unique platform for biosensors. The crystalline Si shows a change in a few properties when reduced to nanoscale size due to a change in quantum confinement [47]. The formation of the porous layer on the Si surface helps in achieving the advantages of quantum confinement. The use of PS immobilized with enzyme for biosensing applications was first reported by Thust et al. [48]. Therefore, the combinations of advanced properties of the PS make it a better candidate for the development of biosensors by coupling with efficient transduction and detection systems with high sensitivity [49]. Fig. 1c depicts the numerous advantages of PS as a support medium for enzyme immobilization rather than the bulk silicon in biosensor development. Table 2 lists out the various enzymes immobilized on the porous silicon as support along with their potential applications. Table 3 outlines the different PS based enzyme biosensors and their performance evaluation.

3. Enzyme immobilization techniques

The selection strategy for the immobilization technique has a crucial role as it determines the characteristics and overall success of the enzyme immobilization process. Enzyme activity, efficient substrate consumption, product formation rate, mass transfer rate, and the cost of immobilization process influence the selection of immobilization techniques. Other selection criteria such as the properties of an enzyme, support surface, and purpose of immobilization must be considered for immobilization.

There are two broad classes of immobilization techniques: physical methods and chemical methods [119]. Physical methods include the interactions by weak bonds: hydrogen bonds, hydrophobic bonds, ionic bonds, affinity bonds, and van der Waals forces acting between the support surface and enzyme molecules. As interactions by weak bonds are reversible, the physical methods collectively known as reversible techniques of immobilization. Chemical methods include the bond formation by covalent linkage (either of amide, ether, thioether, and carbamate bonds) or cross-linking between different polymers. Chemical methods are irreversible as the bonds formed are difficult to break due to the high energy requirement [30]. Fig. 2a outlines the classification of various immobilization methods based on the type of interaction between enzyme and support material.

3.1. Physical adsorption

The physical adsorption method is probably the simplest among all of the immobilization techniques. It is a method for reversible immobilization where the enzymes physically adsorbed or attached to the support material. Adsorption involves weak interactions: van der Waal, electrostatic forces (attraction and repulsion forces), hydrogen bonding, and hydrophobic binding. The immobilized enzymes can be removed from the support by exposing it to mild conditions providing altered pH and temperature, and the support can be reloaded with the fresh enzyme [30]. Other interactions, such as entropy-driven hydrophobicity also used to immobilize enzymes to the surfaces of the support. The mechanism involves one enzyme molecule displaces a large number of water molecules both from the support and its surface during immobilization, resulting in entropy gain to produce the hydrophobic interactions between both entities [120]. The strength of the interactions involved depends primarily on the hydrophobic properties of both the adsorbent, i.e., support and the enzyme, regulated by the size of the hydrophobic ligand molecule and the degree of substitution of the support. By adjusting factors such as pH, temperature, and salt concentration during immobilization, further modulation of the hydrophobic interactions between the enzyme and support can be achieved [121,122]. When massive physical adsorption of enzymes is needed to be done, a long spacer arm is commonly used. Polyethylenimine (PEI) or dextran sulfate-coated supports have been described as optimal anionic supports to firmly, but reversibly, immobilize enzyme of interest. By using this method, more than 90 % of an enzyme can be immobilized at pH 7 [123].

The immobilization procedure follows the pouring of enzyme solution on the PS surface for the specific time and air-drying of the solution. During drying, enzyme molecules attach to the PS by weak interactions, and a surplus amount of enzyme is washed away using a buffer or water [31]. Fig. 2a represents the generalized description of enzyme adsorbed on the porous surface. The scanning electron microscope (SEM) confirms the adsorption of the enzyme on the PS surface. Fig. 2b (left image) shows the pores present on the surface of the crystalline silicon wafer. The dimensions of the pores are expected to range from 40 nm to 60 nm in diameter. Fig. 2b (right image) shows the adsorbed enzyme on the PS surface. The porous structure appears to be covered by the enzyme molecules.

After immobilization of an enzyme, some changes in confirmation have been reported. For instance, in the case of immobilization of β-glucosidase enzyme in porous silicon by adsorption shows a change in conformation of enzyme subunits after immobilization when analyzed in FTIR [56]. In another case of Laccase enzyme immobilization by physical adsorption, the secondary structure of enzyme undergone varying degrees of changes, which were found by circular dichroism (CD) and fluorescence spectroscopy analysis. In that, β -sheet ratio decreased significantly, the alpha helix ratio almost disappeared, and the ratio of β-turn and random coil increased markedly, indicating severe damage in the secondary structure of the enzyme [57].

3.1.1. Ionic bonding

In the method of physical adsorption, the enzyme molecules attach to support materials by ionic binding via electrostatic attraction forces on surfaces. pH plays a vital role in the generation of ionic charges on the surface of the enzyme. Change in pH varies the net charges by the

Table 3
Different Porous silicon-based enzymatic biosensors and their scale of performance.

Enzyme	Analyte	Immobilization method	Sensors type	Measure of sensors	Change in	Detection range	Sensitivity	Response time	Storage	Ref
Alanine aminotransferase	Glutamate	APTES and GTA	Amperometric	–	–	1.3U/l to 250.0U/l ALT	0.13698 μA/(U/l)	–	120 days	[83]
Aspartate aminotransferase				–	–	1.3U/l to 250.0U/l	0.45439 μA/(U/l)	–		
Bilirubin oxidase	Bilirubin			–	–	0.002- 0.02 mM bilirubin	0.15354 mA/mM	–		
Cholesterol oxidase	Cholesterol			–	–	1- 50 mM cholesterol	0.2656 μA/mM	–		
Glucose oxidase	Glucose and heavy metals	Physical Adsorption	–	PL	pH	10 nM to 1 mM heavy metals	–	–	–	[53]
Horseradish peroxidase	Hydrogen peroxide	EDC and NHS	–	I-V shift	–	–	–	–	–	[88]
Lipase	Triglycerides	Physical adsorption	EOS	C-V shift	pH	29.1 mM Tributyrin	30 mV/pH	–	6 months	[50]
		APTES and GTA	EISCAP			5 mM Triglyceride	33 mV/pH	210s	–	[106]
					–	1 − 7 mM Tributyrin	–	–	–	[107]
P450	Arachidonic acid	APTMS and GTA	–	Fluorescence	Refractive index	10- 200μM Arachidonic acid	–	–	–	[85]
Penicillinase	Penicillin	Physical Adsorption	EIS	C-V shift	pH	0.2 mM–10 mM Penicillin	54 mV/pH	–	50 days	[52]
			–				30 mV/pH	–	–	[48]
			EIS			0.01 and 0.5 mM Penicillin G	140 mV/Mm	–	12 months	[108]
		crosslinking	EIS	CONCAP	pH	0.025- 0.25 mM Penicillin	138 ± 10 mV/mM	150s	20 days	[66]
Trypsin		APTMS and GTA	FTRIFS	Fluorescence	Refractive index	10- 200 ng/mL trypsin inhibitor	–	–	–	[86]
Tyrosinase	Catechol	Physical adsorption	–	I-V shift	–	0.05 mM to 0.1 mM Catechol	–	120s	20 days	[54]
	pyrocatechol	Undecylenic acid and EDC	–	–	–	1-100 μM pyrocatechol	–	–	–	[109]
Urease	Urea and heavy metals	Physical adsorption	–	PL shift	pH	10 nM to 1 mM heavy metals	–	–	–	[53]
	Urea	Physical adsorption	–	C-V shift	–	0.1–125 mM urea	–	–	–	[110]
		Covalent binding 3-MPA and EDC	–		–	5 − 80 mM Urea	11.21 μA/mMcm^2	50s	30days	[83]
			–		–	0.3–4.5 mM Urea	2.05 μA/mMcm	–	–	[111]
Sarcosine Oxidase with Creatinase & Creatininase	Sarcosine Creatine Creatinine	Crosslinking by GTA and Silanisation by MPTMS	Amperometric	I-V shift		8 uM – 1700 u M Creatinine				[112]
Urease	Chromium (Cr^{6+} & Cr^{3+}) & Copper (Cu^{2+})	Physical adsorption	Spectrometric		Inhibition of Urease	–	–	–	–	[113]
Glucose oxidase	Glucose	Physical adsorption	PS as photoconductor	Voltage	Reflectance, Absorption	1 − 30 mM Glucose	–	90s	–	[114]
Glucose oxidase	Glucose	Surface Functionalization	–	I-V shift	Conductance	–	< 10 MV/cm	–	–	[115]
Trypsin	Myoglobin	Covalent binding	RIFTS	Refractive index	Reflectivity	–	–	–	–	[116]
Choline oxidase	Choline	Covalent binding by GTA	Amperometric	–	Current	LOD: 9 mM/L choline	–	300s	100 days	[117]
Horseradish Peroxidase Glucose Oxidase	Glucose	Covalent Crosslinking of GPTMS and CS	Amperometric	C-V	Current	1 to 351 μmol/L glucose	0.3 μmol/L.	–	4°C for six weeks	[118]

APTES: (3-Aminopropyl)triethoxysilane; **APTMS**: (3-Aminopropyl)trimethoxysilane; **CONCAP**: Constant Capacitance; **C-V**: Change Current per Voltage ; **EDC**: carbodiimide; **EIS**: Electrolyte Insulator Semiconductor; **EISCAP**: Electrolyte Insulator Semiconductor Capacitors; **EOS**: Electrolyte–oxide–semiconductor; **FTRIFS**: Fourier transformed reflectometric interference spectroscopy; **GTA**: Glutaraldehyde; **I-V**: Change Current per Voltage ; **LOD**: Limit of Detection; **3-MPA**: 3-mercaptopropionic acid; **MPTMS**: (3-mercaptopropyl)trimethoxysilane; **NHS**: N-Hydroxysuccinimide; **PL**: Photoluminescence; **RIFTS**: Reflective interferometric Fourier transform spectroscopy.

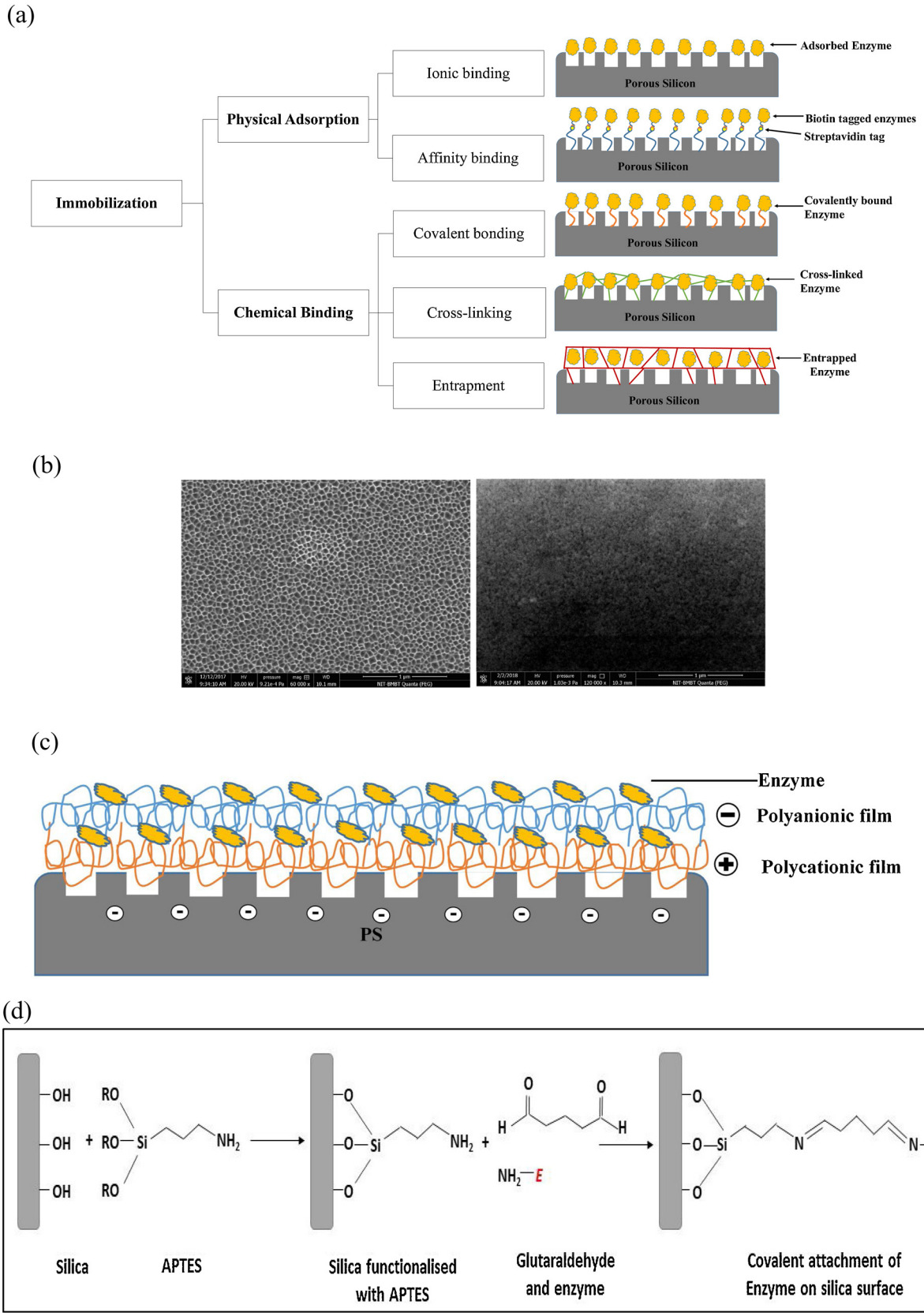


Fig. 2. a. Classification of the immobilization methods based on the nature of the interaction between enzyme and support. b. SEM micrograph of PS (left) and urease physically adsorbed over PS surface (right). c. Immobilization of enzyme on the porous silicon surface by Layer by layer method. d. Porous silicon functionalization using APTES and binding of the enzyme through GTA as linker illustrating the formation of covalent bonds.

Table 4
Various enzymes immobilized on the silicon derived porous materials with their molecular size and isoelectric points.

Enzyme	Size (nm)	pI	Support material	Type of immobilization	Purpose	References
Trypsin	5.48 × 5.87 × 6.76	8–10.5	SBA-15	Covalent binding	Enzyme retention, activity, and reuse study	[142]
Alcohol dehydrogenase	10.3 × 8.04 × 4.76	5.4	Mesoporous cellular siliceous foams	Physical adsorption	enzyme uptakes, stabilities, and leaching behavior study	[16,143,144]
Glucose oxidase	6.0 × 5.2 × 3.7	3.8	Silica sol-gel	Encapsulation	Enzyme immobilization and performance study	[46,145,146]
Horseradish peroxidase	4.0 × 4.4 × 6.8	8.8	SBA-15, MCM-41	Entrapment	To study the retention of the enzyme on the support surface	[147,148]
Horseradish peroxidase	–		Silica porous film	Covalent Binding	To study surface and pore morphology	[149]
Candida antarctica lipase B	6.9 × 5.0 × 8.7	5.1	Fumed silica	Physical adsorption	Optimization of immobilized enzyme retention and activity	[150,151,152]
α-amylase	10.3 × 8.04 × 4.76	4.3	MCM-41, SBA-15	Covalent binding	To study the effect of pore size on enzyme activity	[153,154]
Acetylcholinesterase	4.5 × 6.0 × 6.5	5	MCM-41, FSM-16	Physical adsorption	For better immobilization support study	[155,156]
Superoxide dismutase	–	–	Silica sol-gel	Entrapment	superoxide anion detection	[157]
Glucose oxidase & catalase	–	–	Silica sol-gel	Entrapment	Glucose removal from isomalto-oligosaccharide	[158]
Papain	3.7 × 3.7 × 5.0	8.75	LCS-10 (SBA-1 without –COOH group)	Physical adsorption	To study pH-responsive selective protein immobilization	[159]

isoelectric point (pI) [124]. At the pH range below pI, enzymes molecules acquire net positive charge on the surface and bind to negatively charged silicon surface at pH < 3. However, for attachment of the negatively charged enzyme to the silicon surface, surface functionalizations like a modification of the silica support with amine groups are required [125].

The process of ionic bonding is extended to achieve multiple alternate layers of ionic solution and enzyme called layer-by-layer (LbL) method. The process includes the binding of enzymes to opposite charged polycationic and polyanionic thin films adsorbed on the silicon support [126]. Fig. 2c depicts the schematics for the deposition of different polyionic alternative layers on the PS for enzyme immobilization.

Predominantly used cationic polyelectrolytes are poly(L-lysine), poly(ethyleneimine), poly(allylamine hydrochloride), poly(dimethyldiallylammonium chloride) (PDADMAC), and chitosan derivatives [103]. Whereas, polyanions used are poly(styrenesulfonate) (PSS), poly(acrylic acid), poly(vinyl sulfonate), and poly(methacrylic acid) [37]. LbL method is useful for enzyme immobilization on porous films, nanotubes, and microparticles irrespective of the enzyme size [127]. LbL method offers advantages of controlled thickness, versatility of film assembly [128], less enzyme amount requirement [129], high enzyme loading capacity, and the ionic layers offer protection against enzyme denaturation. Glucose oxidase immobilized on PS with poly(L-lysine) and GTA found ready to use the form and maximum activity obtained during the study due to mild reaction conditions [103]. Enhanced stability and increased lifetime of the enzyme make LbL immobilization a better option for biosensing applications [130]. Ion exchanger can act as a carrier in immobilization by providing ionic and strongly polar interactions. By using the immobilized polymeric-ionic ligands, modulation of protein–support interactions and optimization is made possible. However, in some cases, the highly charged support could present other problems such as kinetics distortion due to partitioning or diffusion phenomena, and subsequently, alter the optimum pH required for the enzyme activity [30]. There are some reports where physical adsorption became very strong due to ionic interactions and a significant rise in enzyme stability. For example, when a multimeric enzyme is immobilized via ion exchange involving all enzyme subunits, and resulting in irreversible dissociation of the immobilized enzyme [131].

Some interfacial enzymes like lipase can be selectively immobilized on hydrophobic supports at low ionic strength via their interfacial activation versus the support surface, yielding the stabilized open form of the lipase. Due to the flexibility of the lipase active centre, the enzyme can be easily tuned during immobilization [132]. In a study by Gustafsson et al. [133], the lipase enzyme from *Mucor miehei* was found to be more than four times as active than free form when immobilized at pH 8 in hydrophobic mesoporous silica support.

3.1.2. *Affinity binding*

Affinity binding is a complementary and selective binding of biomolecules in the specific orientation with particular interaction giving minimal conformational changes on the native structure of the enzyme [30]. Affinity binding follows either of two approaches: firstly, incorporation of ligands having an affinity for the enzyme into the support. Alternatively, a linker with functional groups (either of $-NH_3$, COOH, and O−−H) having an affinity for the particular enzyme surface functional groups may adsorb on PS [134]. Secondly, the enzymes are modified to have an affinity for support. Enzymes are attached to the surface via tags having an affinity for the surface. Various affinity tags like histidine, cysteine, mannose-binding protein, and glutathione S-transferase is useful for the affinity-based immobilization [135].

For histidine tag, only six His groups involve in the binding and leave the active sites of enzymes unaffected. All the histidine groups coordinate with a single metal ion, and a secure binding between the tag and enzyme molecules further stabilizes the enzyme molecules. The tagged immobilized enzymes remain at a distance from the support and provide efficient mass transfer for optimal enzyme activity [136,137].

Enzyme affinity for the surface increases structural stability and efficiency with the retention of a higher amount of enzyme on the surface [4,138].

Si-tag, a novel protein discovered by researchers, offers a strong affinity for the silica surface. The Si-tag protein has the potential for the immobilization of the required enzyme on the PS surface [139,140]. Another affinity-based technique is called immobilized-metal affinity chromatography (IMAC), where the enzyme adsorption is based on the coordination between an immobilized metal ion and electron donor groups present in the enzyme surface. Enzyme adsorption to the IMAC support is performed at medium having neutral or slightly basic pH at which imidazole nitrogens in histidyl residues become nonprotonated and subsequently allows efficient adsorption of the enzyme molecule [141].

Table 4 lists the various enzymes immobilized on the different silica-based support materials by the adsorption method.

The physical adsorption method offers advantages: a straightforward approach, protection against aggregation, proteolysis, and almost complete retention of enzyme activity, the favorable orientation of active site during immobilization [16]. However, the weak interacting forces may break as the reaction progresses, resulting in the leaching out of enzymes from the support surface [37]. The significant pore size attributes to the higher activity. However, higher diffusion may result in leaching out of the enzymes form the support [160]. The poor storage stability and non-specific adsorption of proteins and other substances may occur during the process of immobilization [138,161].

3.1.3. Strategies to overcome the disadvantages of physical adsorption

The selection of optimum pH conditions for the immobilization increases the fidelity of the enzyme retention over the silicon surface. The pH offering a higher ionic charge difference between the enzyme and the silicon support causes the strong interaction between the enzyme and support. A study of immobilization of acetylcholinesterase on the mesoporous silicon surface showed higher enzyme retention and improved activity for the pH range of 4–9 [51].

Few surface modifications, including decreasing the pore size after the adsorption, improve the retention of the enzyme on the surface. Pore size over the PS surface may alter by using silanization and provide amine groups attached to the solid surface. Silanes functionalization results in the formation of hydrogen bonds with the strengthening of van der Waals forces [162]. Amine functionalization of surfaces shows two effects: First, protonated amines at lower pH increase the adsorption of hydrophilic enzymes, and second, reduces the pore size preventing the leaching out of the adsorbed enzyme molecules [151,163]. The observations, as mentioned above, were highlighted in the study performed for the immobilization of pepsin in mesoporous SBA-15 (Santa Barbara Amorphous type material) [164]. Modification of enzyme molecules is another perspective for the enhanced binding strength. Glycosylation of enzymes increases the numbers of hydrogen bonds with PS surface and results in more stable enzyme interaction with PS [120].

3.2. Chemical binding

Chemical binding enzyme immobilization is the attachment of the enzyme to the support material via the formation of chemical bonds. The presence of active chemical bonds makes the method more reliable and efficient compared to physical adsorption. The method is further classified as covalent bonding, cross-linking, and entrapment.

3.2.1. Covalent bonding

Covalent bonding involves the formation of covalent bonds between the enzyme functional groups and the PS surface. Covalent attachment can only be recommended if it permits an improvement in the enzyme properties. The covalent bonds retain the enzyme on the support surface and are expected not to interfere with the activity of the enzymes. Peptide-modified surfaces, when used for enzyme linkage with the support, results in higher specific activity and stability with controlled protein orientation [165]. The generalized representation of the enzyme immobilization on the PS by covalent binding is shown in Fig. 2a. Since the enzyme activity directly relies on the structure of active site present within the enzyme, conservation of the enzyme structure is crucial for the optimal activity [166]. The approach of site-specific covalent binding immobilization may avoid the denaturation of the native enzyme structure and active site [18].

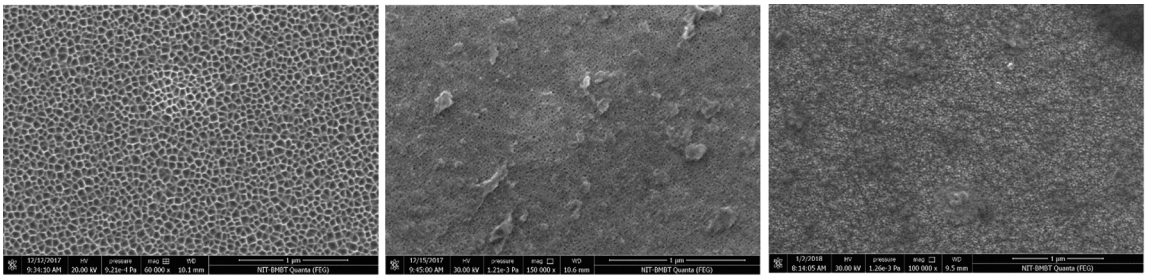
For the formation of covalent bonds, the presence of binding sites on the support material and enzymes is a prerequisite. The bonding sites may already present over the PS surface or need to incorporate into it via specific surface modification techniques without varying its bulk properties [13]. For enzymes, the bonding sites involve the natural functionalizing groups present on the surface.

Immobilization of enzymes by covalent bonding proceeds in two steps: First, PS surface modification by addition of surface functionalization agents either GTA, carbodiimide [167], or organosilanes. Second, the coupling of enzymes to the modified activated surface matrix [37,168]. The PS functionalization agents constitute of different functional groups (amide or epoxy, chloride, alkyl, thiol, cyano/isocyano, vinyl/allyl, organophosphine, phenyl, alkoxy or amino groups [161] and affect the hydrophilic properties of PS membrane surfaces. The addition of functionalizing groups enhances interaction with enzyme functional groups. Activation of surfaces with different reactive compounds results in the formation of the electrophilic groups over the porous surface and can readily react with strong nucleophiles on the enzyme surface [30,168]. The enzymes anchors to the functionalized support surface by forming covalent bonds with residual amino acids like arginine, cysteine, aspartic acid, histidine, and especially with lysine [12].

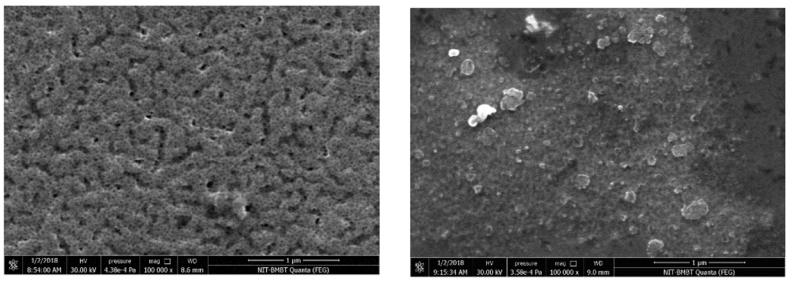
Silanization is a well-known method used for modifying the silicon surface by reacting organosilanes compounds with the silanol groups present on the PS surface [169]. 3-aminopropyl trimethoxysilane (APTMS), 3-aminopropyl triethoxysilane (APTES) [170], 3-mercaptopropyl trimethoxysilane (MPTMS), 3-mercaptopropyl triethoxysilane (MPTES) [171], and different alkoxysilanes or chlorosilanes used in functionalization of the PS surface. Silanes form a uniform monolayer over the surface and offer uniform characteristics to the surface. The uniform surface characteristics allow the even immobilization of the enzyme over the surface and, therefore, suitable for biological and sensing applications [172,173]. The silanization of PS alters the wettability of the surfaces and thus helps to control the orientation of enzymes during immobilization. In an another study by Khaldi et al. [174], acetylcholinesterase was immobilized on PS modified using hydrosilylation and silanization processes. The contact angle revealed the surface that hydrosilyated PS surface achieved hydrophobic characteristics (75°) while the APTES silanized surface showed a comparatively hydrophilic surface (42°). The activity of the immobilized enzyme was observed more for APTES silanized surface owing to the orientation of the enzyme molecules. Acetylcholinesterase has more hydrophobic groups around its active sites, and therefore this site remained facing outwards for the hydrophobic surface groups giving more enzyme activity. Further, different alkoxysilanes offer different surface wettabilities and hydrolytic stability [175]. Thus the selection of silanes for a surface is crucial and depends on the properties of the enzyme and surface reactivity.

The functionalization gives the NH_2 ends on the PS surface. $Si-OH$ and silanol groups (Si-O-Si) groups present on the oxidized PS surface react with the functional groups (epoxy or methoxy) of silanes and undergo hydrolysis of hydroxyl groups leading to the formation of hydrogen bonds [176]. The formation of hydrogen bonding results in the strengthening of van der Waals forces and serves as anchors for covalent bonds formation on the silicon surface [161–163].

Enzyme molecules may directly attach to the functionalized PS surface. However, the direct linking of enzymes to the surface may limit



a) Porous silicon b) Porous silicon oxidized with H_2O_2 c) Porous silicon functionalization with APTES



d) Functionalized Porous silicon treated with Glutaraldehyde e) Porous silicon covalently immobilized with enzyme

Fig. 3. SEM micrograph of urease covalently immobilized over porous silicon.

mass transfer. The introduction of spacer or linker between enzyme molecules and functionalized PS may avoid disadvantages by increasing the distance between two. In covalent immobilization, the spacer arm length plays a crucial role in the enzyme support reaction [123]. The spacer or linker facilitates the enzyme with a high degree of mobility, and enhanced activity compared to direct/without using spacer immobilization [13,103,177]. Polymer linkers, for example, GTA, carbodiimide, polyethyleneimine, Polyethylene glycol (PEG), and spacer of epoxy molecules act as linker molecules for the PS [75,178]. Studies showed more accommodation of enzyme on PS when GTA used as a linker molecule with the PS functionalized with APTES [89].

The PS has the −OH groups on the surface reacts with the silanes and give the amino groups on the surface. The amino groups react with the one end of the GTA, and the other end links with the enzyme functional groups to attach the enzyme covalently to PS. Fig. 2**d** represents the PS surface functionalization using silanes and enzyme attachment via GTA used as a linker [161].

SEM is used to observe the morphological changes occurring on the surface of PS due to chemical modifications. Fig. 3**a** shows the pores on the silicon surface with similar diameters. Fig. 3**b** shows the oxidation of PS surface forms an oxide layer result in a reduction of pore diameter with hydroxyl groups on the surface. Fig. 3**c** shows a layer of functionalizing agent APTES over the oxidized PS. GTA added as a linker molecule changes the surface morphology, as shown in Fig. 3**d**. Enzyme-linked to the functional group of the GTA and attached to the PS surface, as shown in Fig. 3**e**

Attachment of the enzyme to the functionalized PS surface occurs via covalent interaction with the functional groups on the enzyme surface. Fourier Infrared Transform Spectroscopy (FTIR) study gives the confirmations on the bonding between the enzyme and PS surface. Fig. 4 shows the FITR spectrum analysis of the covalently linked urease enzyme to the APTES functionalized PS surface. The peaks at 480 cm^{-1} and 750 cm^{-1} shows the crystalline nature of the PS [179,180]. Slight oxidation before the PS treatment is observable with the stretch of −OH for 3000 − 3600 cm^{-1} [181]. In the next stage of PS-oxidation, the crystalline nature of the PS changes and Si-O-Si peak at 1180 cm^{-1} confirms the formation of the oxide layer [179,180]. During the PS functionalization with APTES, Si-O-Si disappears due to interaction with silanes group from APTES and -NH_2 appears at 1640 cm^{-1} confirming the retention of APTES on PS surface [182]. The appearance of a strong amide bond (C—N) at 2355 cm^{-1} confirms the presence of an enzyme on the surface [181].

Additionally, the use of heterofunctional supports having some groups able to adsorb proteins (via different mechanisms, like an ionic exchange, affinity adsorption), and many groups able to permit enzyme covalent coupling, has opened the opportunity of many different immobilization techniques to have mild selective adsorption of the target enzyme followed by a “strong” covalent coupling, enabling the one-step immobilization and high purification of enzymes at the end of the process. One of the first examples was the use of IMAC-epoxide supports to purify and immobilize the proteins having a poly-His tag [183]. Thus, the use of heterofunctional supports may be an optimal and versatile solution to couple immobilization and purification [184]. The combined technique involving adsorption and covalent binding between the enzyme and support can be used to prevent the enzyme leakage. Use of peptide modified surfaces for the enzyme β-galactosidase immobilization found to be stable and retention of high enzyme activity [165].

Further stabilization of enzyme immobilization can be achieved by multivalent covalent attachment. In this, the relative distances among all enzyme residues involved in immobilization have to remain unaltered during any conformational change induced by any distorting agent. Amino groups are fascinating nucleophiles placed on protein surfaces. The immobilization of enzymes through the region having the highest amount of amino groups (Lys residues) is key behind the successful stabilization. Glyoxyl groups are small aliphatic aldehydes that form very unstable Schiff’s bases with amino groups, and they do not seem to be useful for enzyme immobilization at neutral pH. However, under alkaline conditions, glyoxyl supports can immobilize enzymes via a first multipoint covalent immobilization through the region having the highest amount of Lysine groups [185].

The covalent method offers enhanced enzyme stability by maintaining the structural configuration of the enzyme. The improved structural stability offer resistance to the high-temperature. The higher bond strength ensures the minimal leaching of the enzyme from the surface. Covalently immobilized enzymes offer high storage stability

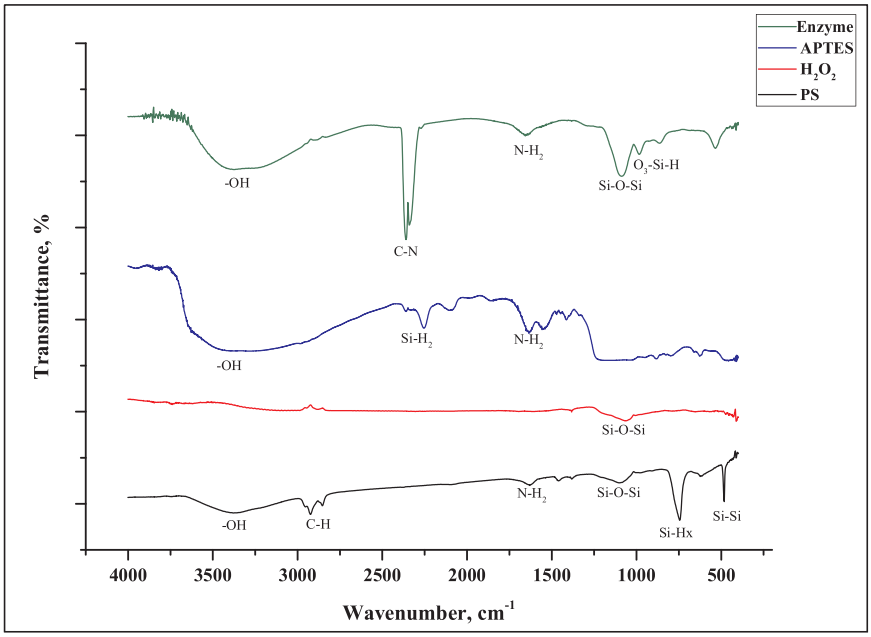


Fig. 4. FTIR spectrum for covalent bonding of urease over porous silicon.

over an extended period. The higher operational stability is useful for scaling up the application of the mentioned method for the industrial processes [186,187].

The complexity of the immobilization process and the cost factor add some disadvantages for the application of covalent bonding immobilization. Some steric conformation changes may occur in the enzyme structure and may affect the activity of the enzyme molecules [18]. As the covalent bonds are irreversible, the reuse of the support is not possible in many of the cases. The requirement of many different chemicals for the immobilization may cause contamination for the enzyme-substrate reaction [188].

3.2.2. Cross-linking

Cross-linking is another method for the immobilization of enzymes where the polymeric chains get linked to each other by covalent bonds. The general principle of cross-linking of the enzyme is to link the enzyme molecules with the help of polymeric crosslinking agents: GTA, bisdiazobenidine [189], and hexamethylene diisocyanate [138]. The confinement of the enzyme within the matrix offers the different and more stable surrounding microenvironment from the bulk solution favoring the optimal activity. Fig. 2a depicts the generalized representation of cross-linked enzyme immobilization on the PS.

Cross-linking ensures retention of catalytic activity and recyclability during the immobilization process as well as reaction with the substrate. The cross-linking agents form covalent bonds by intramolecular and intermolecular aldol condensation of the amino groups of lysine residues of enzyme molecules [190]. When glutaraldehyde used in cross-linking, the reaction involves the formation of Schiff's base formation and Michael-type 1, 4 in addition to α, β-unsaturated aldehyde moieties. The exact mode of crosslinking is dependent on pH [191].

Though the cross-linking method is a carrier-free method, the use of support helps in achieving more stability over the varying conditions of the reactor [33]. The PS support facilitates the deposition of enzyme over its surface and cross-linking between the molecules and surface for prevention from leaching [192]. Experimental studies of carrier-based cross-linked enzymes showed increased enzyme retention and activity with more operational and storage stability for the immobilized enzymes compared to carrier-free cross-linked enzymes [193]. Carrier-based cross-linked enzymes show higher recovery and recyclability. During the immobilization of the enzyme on PS support, the enzyme initially adsorbs on the surface and then crosslinked through a cross-linking reagent. The process ensures the high enzyme loading on the surface.

There are two ways of immobilization by using the cross-linking method. One of the methods is cross-linking enzyme crystals (CLECs), which includes pure enzyme molecules that are crystallized and held together using crosslinking molecules. The other method is cross-linking enzyme aggregates (CLEAs) which do not require pure crystalline enzymes; instead, they work in an aqueous solution that may or may not need a solid carrier [194]. The enzyme is precipitated by adding salts, organic solvents, or non-ionic polymers: ammonium sulfate, acetone, or polyethylene glycol in mild reaction conditions, generally pH of 7, and temperature range of 35–40 °C [195]. Specific crosslinkers then used for stabilizing the precipitated enzyme aggregates. Linking agents, especially GTA, reacts with the surface amino acids of the enzyme without perturbation of the native structure of the enzyme [12]. Fig. 5 explains the process of the formation of CLEAs using GTA as a cross-linking agent. This method is economical, easy to adopt, and applies to broad-scale applications. The CLEAs formation is a single unit operation that includes purification and immobilization, helping in reducing the time and cost factor. Enzymes present in the crude fermentation broth can directly subject to aggregate formation. The CLEAs may use for the processes with harsh reaction conditions: extreme pH, temperatures, and against organic solvents [163,196]. However, certain disadvantages of the smaller size of aggregates causing severe inconvenience during the purification process, and less stability in stirred reactors. Diffusion limitations for the substrate and products may encounter with the increased size of aggregates [67,197].

The CLEAs formation majorly depends on the pH of the crosslinker solution. Other minor parameters influencing CLEAs formation are temperature, stirring rate, concentration, type of precipitants, cross-linking agents, and additives. The molar ratio of enzyme to crosslinking agent influences the stability, activity as well as aggregates size of CLEAs. The stability of the CLEAs reduces with a reduced concentration of crosslinking agents, while the higher concentrations of the cross-linkers flexibility of CLEAs are reduced. For the reactions where enzyme concentrations are low, fewer lysine residues are available for cross-linking, making the aggregate less stable. The use of additives tackles the insufficiency of binding sites by serving as a source of lysine residues. More stable conformations are achieved by increased cross-

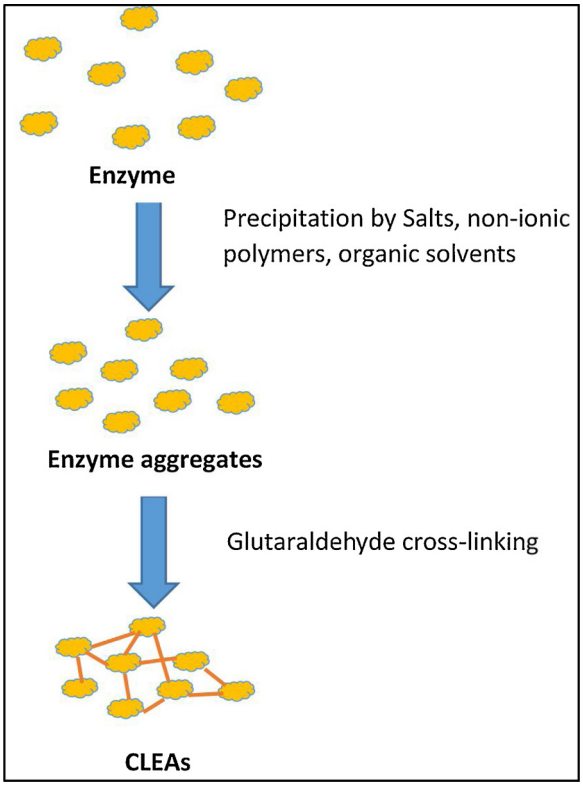


Fig. 5. Formation of crosslinked enzyme aggregates (CLEAs) using glutaraldehyde as a cross-linker.

linking. Since additives do not make covalent binding with the enzymes, they get washed away subsequently with the constant washing.

CLEAs so formed show more activity than free enzymes [198,199]. Some examples of additives are bovine serum albumin (BSA) [200], polyethenimine, and dextran sulfate [28,201]. BSA, a kind of proteic layer, is used popularly for the CLEAs formation, in combination with enzyme followed by GTA cross-linking [202,203]. Different biosensors build upon the cross-linked enzymes by using GTA and BSA are applied for the detection of heavy metals as pollutants [204].

Further, cross-linking agents play a vital role in the formation of CLEAs in a carrier-free as well as carrier-based immobilization [205]. GTA is most commonly used as a bifunctional crosslinker for the immobilization of most of the enzymes [177,191]. Some other agents are *bis*(imidoesters), dextran polysaccharide, bis-isocyanate, bis-diazobenzidine, diazonium salts. Glyoxal and hexamethylenediamine [206] are well-known agents used in biosensors.

With the advancement in technologies for immobilization, binding of multiple enzymes in the aggregates is possible with Combi-CLEAs [194]. Immobilization of multiple enzymes by cross-linking as per the need of reaction may carry out respective transformations by the catalytic activity. Researchers have used Combi-CLEAs for aggregation of pectinase, xylanase, and cellulose. Some study reports the activity of lipase, R-amylase, and phospholipase A2 in Combi-CLEAs found higher compared to free enzymes [207].

3.2.3. Entrapment

Earlier described methods may have direct involvement of enzyme molecules for immobilization and may disturb native configuration of enzyme resulting in loss of catalytic activity of the enzyme. Entrapment of enzyme molecules within a polymeric matrix or sol-gel or fiber mesh is the better means to avoid adverse effects. In some cases, the physical restraints of the polymers are weak to prevent the leakage of the enzyme from the matrix. Additional covalent binding overcomes the weakness of the immobilization of provided to enzymes with the surface. The polymeric networks are usually semipermeable, allows the substrates and products to pass through but restrict the enzyme movements to the inside of the network.

The primary advantages of entrapment are economical, rapid process, and mild process conditions not affecting the catalytic activity of enzymes. Entrapment prevents the direct contact of enzymes with the external environment, thus eliminating the adverse effects of gas bubbles, sheer, and hydrophobic solvents [208]. The entrapment matrix prevents enzymes from other denaturing enzymes such as proteases and may avoid microbial contamination.

The disadvantage of entrapment is mass transfer limitations occurring because of semi-permeability of the membrane due to restricted paths [209], hydrophilic/ hydrophobic nature of support, and substrate solution [144]. Optimal permeability depends on the precise selection and maintenance of membrane pore size. Similarly, sized enzymes and substrate create difficulties in the selection of optimum pore size. Along with this, the vast amount of enzyme loading may cause the deactivation of the enzyme. Besides, the reaction rate of immobilized enzymes is highly dependent on the diffusion rate of substrates and products [197]. Fig. 2a represents the enzyme immobilization by the entrapment matrix incorporated onto the PS surface.

There are two approaches for enzyme entrapment: First, enzymes be incorporated during the membrane manufacturing process. In contrast, in the second approach, the enzyme is made to filter through membrane resulting in entrapment within the network. Some studies showed a reduction in the enzyme activity by the first approach of immobilization compared to the second approach. The process conditions of the synthesis of the membrane in the first approach of entrapment may decrease the enzyme activity. Additives such as GTA, when added to membrane synthesis, helps to retain enzymes within the pores or to interconnect the polymeric fibers of the matrices [13]. Different parameters, for example, as optimization of porosity, particle size, polymeric network, and surface functionalities, may achieve by varying polymerization conditions [208].

As a support material for entrapment of enzymes, PS offers the advantages of tunable pore size and surface properties used for the entrapment. The enzymes can entrap within pores on the surface or polymer matrix over support [125,160]. The commonly used polymer as a matrix is polyacrylamide, alginate, gelatin [210], collagen, silicon rubber, and polyurethane [197]. The polymers attach to the PS surface of the matrix formation in the presence of enzyme molecules under mild conditions. A study of immobilization of glucose oxidase showed the enzyme retention within the gelation matrix formed after dipping the PS in the gelatin for a short time [103].

Silica sol-gels are popularly used for the entrapping enzymes, as it is highly porous, and readily prepared. Chemically sol-gels are inert glass mouldable into various shapes. The porous membrane of sol-gel restricts the pass of the enzyme through it but allows substrates, and products to pass through it. During the synthesis of sol-gel, firstly, hydrolysis of tetraalkoxysilane (tetraethoxysilane, tetramethoxysilane) is carried out, resulting in the formation of (Si − OH) groups. Followed by the condensation, the reaction gives siloxane (Si—O—Si) matrix filled with water or alcohol similar to a gel, which can be used enzymes entrapment [211]. Based on the liquid content of the gel, the gels can be categorized in the aquagel, xerogel, and aerogel. Aquagels are the initial product of the sol-gel reaction with high viscosity and more than 90 % of the liquid content of the total volume. The pore size of the gel is very less and can entrap the enzyme within its matrix. The properties and morphology of the gel are the functions of the liquid fraction of the gel [212]. Upon drying to evaporate the liquid, the size of the aquagel shrinks (< 15 %) to give xerogel with different structures than aquagel. Xerogels show capillary stress by evaporation of water/liquid resulting in shrinkage of the matrix and subsequently pore formations. However, the drying temperature for the synthesis of xerogels should be decided based on the thermal stability of the immobilized enzyme. Again, drying of aquagels under supercritical conditions, xerogels are formed

comprising up to 98 % air of total volume without shrinking of the size of the aquagel [213]. Supercritical drying at high temperatures is not suitable for the enzymes; therefore, liquids like liquid CO_2 with a critical temperature of 31°C can be used to dry the gel [212]. The pore size of the aerogel is comparatively larger than xerogel. However, the mechanical properties of the aerogel are weak, which can be enhanced by introducing some compatible polymers in the reaction mixture [214].

Depending upon the type of enzymes and reaction conditions, sol-gels can be applied for entrapment of enzymes. For the hydrophobic surface of sol-gel, alkyltrialkoxysilanes (for example, methyltrimethoxysilane) added to a synthesis mixture called ambigel showing no contraction of the matrix and pores. Sometimes, additives, for example, polyethylene glycol, polyvinyl alcohol (PVA), or albumin, may be added to stabilize the sol-gel structure. The additives help in reducing the internal stress and shrinkage of the gel matrix. Experimentally, the addition of PVA to the synthesis of sol-gel for immobilization of lipase B influences the surface properties of surface area and hardness [29,120,208].

Summarising all strategies of immobilization, selection of the proper strategy is crucial to maintain the native enzyme structure, and its activity. Fig. 6 shows the generalized process for the enzyme immobilization on the support materials, and the significant parameters that influence the selection of the immobilization method are briefed in Table 5. Table 6 enlists the advantages and disadvantages of the different immobilization methods and emphasized on the strategies to overcome the disadvantages of a particular immobilization method.

Table 5
Conditions affecting the selection of the immobilization method and their respective parameters affecting the immobilization process.

Selection criteria	• Pore size on PS • Enzyme size • Enzyme conformation • Surface charges on enzyme and PS
Conditions process	• pH of the process • The temperature of the process • Stabilizing agents • Solvents for chemicals
Mass-transfer effects	• Viscosity of medium • Hydrophobicity of surface and solvent • The diffusion rate of substrate • Presence of linker/spacer
Efficiency of immobilization	• Enzyme activity retention • Degree of sensitivity • Storage and reaction stability • Enzyme and PS Recyclability • Reproducibility of process

Other techniques, such as electrospinning based immobilization, also been reported. It is an emerging non-invasive technique to fabricate one-dimensional nanofibers, which can be used as a support. Nanofibers produced under this technique have unique properties like long fibre length, uniform diameter, and diversified composition that

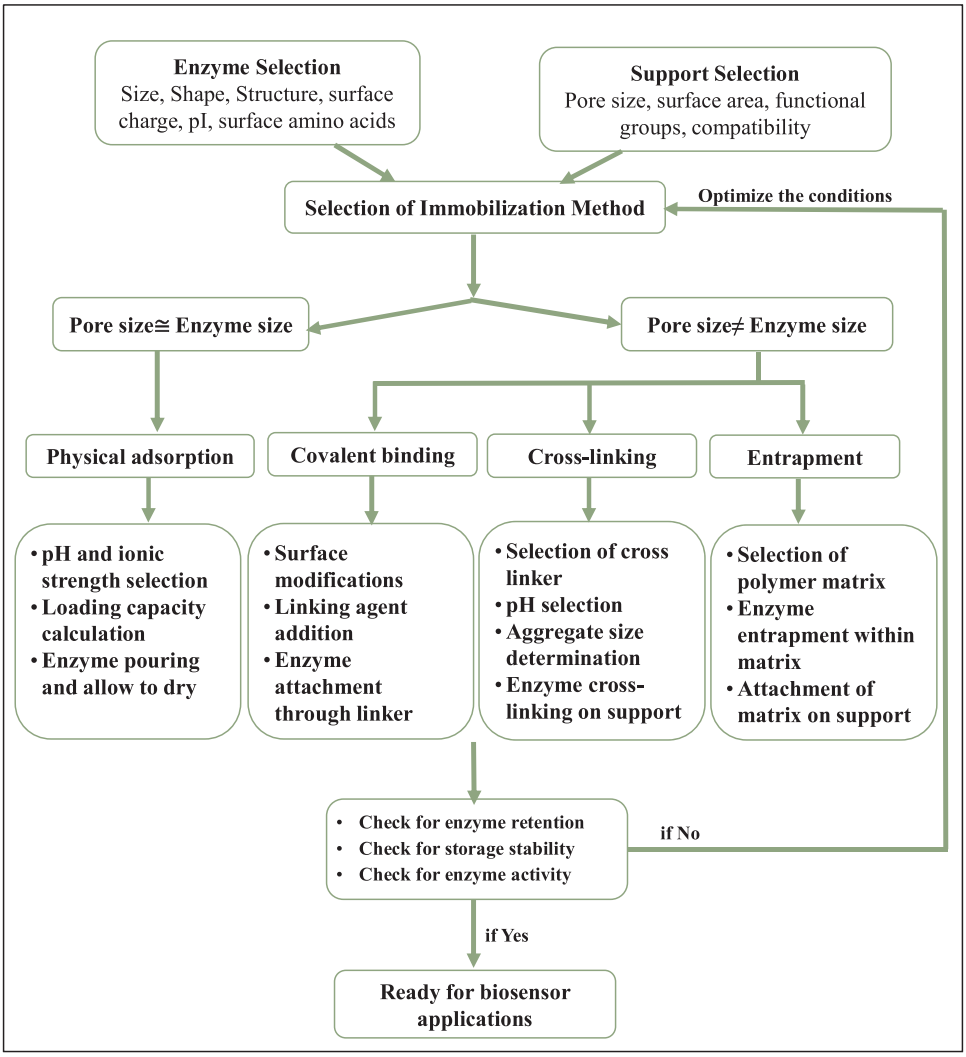


Fig. 6. Generalized process flowchart for enzyme immobilization on porous silicon support for biosensing applications.

Table 6
Benefits and drawbacks of various enzyme immobilization methods and approaches to overcome the drawbacks of the respective method.

Nature of binding	Advantages	Disadvantages	Strategies to overcome disadvantages	References
Physical adsorption				
Electrostatic interactions, van der Waal forces, hydrogen bonding, hydrophobic binding	• Complete retention of activity • Prevention of proteolysis	• Leaching out of enzymes • A small change in reaction conditions affects the activity • Non-specific adsorption • Cost factor for affinity binding	• Pore size reduction after adsorption • Specific pH for charge difference between enzyme and silica support • Use of blocking agent to decrease non-specific interactions	[16,37,138,151,163,215,216]
Covalent binding				
Covalent bonds between functional groups of enzyme and silica support	• Greater bond strength • Reduced mass transfer limitations • Storage and reaction stability	• Active site denaturation • Prerequisite of the binding site • Irreversible binding	• Site-specific binding • Modification of PS and enzyme	[4,77,161,187,217]
Cross-linking				
Binding between enzymes and support with a crosslinker	• Complete activity retention, Higher loading capacity, and recyclability • Higher activity may be achieved for aggregates	• CLECs require pure enzyme • Interference by precipitating agents • Fragile nature of the crosslinking matrix • Diffusion limitations	• Use of CLEAs for partially purified enzymes • Additives to stabilize the structure • Optimum aggregate size by crosslinker to enzyme ratio	• [28,163,197,198,199]
Entrapment				
Enzyme incorporation within support matrices or polymeric matrices	• Eliminates direct contact with the external environment • Mild preparation conditions	• Less physical restrains results in leakage • Mass transfer limitations	• Additional covalent binding • Precise selection of pore size based on enzyme size	[12,120,125,160]

makes them a highly efficient support system for enzyme immobilization [218]. The nanofibers also provide advantages such as high specific surface area and porous structure, along with hydrophilic and biocompatible properties of the immobilization polymer material [219]. In a study by Patel et al. [220], the enzyme horseradish peroxidase was encapsulated into the electrospun mesoporous silica fibres shown a fourfold increase in the enzyme activity (three-fold higher activity than HRP immobilized silica powders) and thermal stability at 60 ℃.

4. PS properties influencing enzyme immobilization

Immobilization of the enzymes offers certain advantages over the use of free form enzymes. Maintaining of enzyme activity during the reaction and the storage is crucial to the success of immobilization. Factors responsible for the retention of enzyme activity and stability include physical and chemical characteristics of enzyme, support surface, and the surroundings. Changes in any of these factors may cause variation in the desired product quality and function. Table 7 gives the critical parameters for the selection of support materials for enzyme immobilization and their effects on the process of immobilization.

Performance parameters of the biosensors are the function crucially dependant on the stable attachment of the enzymes over the support surface. Weak attachments of the enzymes may lead to the leaching out of the molecules during the reaction and storage. Leaching of the enzyme from the surface reduces the overall catalytic rate of sensors and thus reduced the signal generated, sometimes unable to reach the limit of detection of the sensors. Also, with the leaching enzymes, the reproducibility and reusability of the sensors are compromised. Therefore, high affinity or interactions of enzyme and surface is necessary for the successful development of the biosensors.

4.1. Pore size on PS

Retention of enzymes over the support surface in the physical adsorption methods is highly influenced by the pore size of the surface. Molecules of the enzyme get trapped in the surface pores and retained by electrostatic forces. The pore size required for enzyme immobilization depends on the size and concentration of the enzyme [231]. In a study, Takahashi et al. [137] showed limited adsorption of enzymes on the porous surface with the pore diameter less than the molecular size of the enzyme. The small size pores restricted the entry of enzyme in the pore resulting in large unutilized area and weak attachment with the surface. Another study by Hisamatsu et al. [221] on the immobilization of α-amylase over mesoporous silica substrate concluded that pore size should be just matching to the size of the enzyme to accommodate the enzyme within itself and provide adequate support for the attachment. A study by Gimon-Kinsel et al. [232] showed that just matching pore size of the surfaces of all silica MCM-41 (ϕ-3.2 nm), aluminosilicate MCM-41 (ϕ-3.2 nm), all silica MCM-48 (ϕ-3.4 nm) and NbTMS1 (ϕ-3.3 nm) were capable of accommodating a globular enzyme cytochrome C (ϕ-3 nm) without significant leaching at high pH. In another study by Ikemoto et al. [222], the proper pore size reportedly increased the thermal stability of the immobilized on horseradish peroxidase over the SBA-15 surface with a pore diameter of 7.6 nm.

In the PS surfaces prepared by electrochemical etching, the pore size and density is the function of current density and time of current flow. Based on the requirements, PS surface with pore sizes ranging from micropore (< 2 nm), mesopore (2–50 nm) to macropore (> 50 nm) can be prepared [43]. However, macropore range surfaces are avoided as they hold less chance of enzyme retention leading to leaching out of molecules [31].

Further, pore size also affects the flow of target analyte towards the site where enzymes are immobilized. Target analyte may experience restriction in the mass transfer if the enzyme molecules are retained over the surface due to small pore size. Also, larger pores holding enzyme molecules may accumulate analyte molecules due to the presence

Table 7
Parameters for selection of support material for enzyme immobilization and their effects on the immobilization process.

	Parameter	Effect	References
Physical Parameters	Pore size of PS	• Larger pore size acts as good enzyme carriers but prone to leaching out • Pore size matching with enzyme size is optimum • Optimum size gives stability and prevents leaching	[43,221,222]
	Surface morphology of PS	• Rod-like morphology offers more loading amount with a faster rate of immobilization • Ordered Spherical porous surface morphology offers better retention irregular surfaces	[223,224,225]
	Particle Size	• Reduced particle size offers more enzyme loading due to large pore entrance	[226]
	Mass transfer rate	• Limitations reduce the activity • Dependent on pore size, binding nature, and the hydrophobic or hydrophilic nature of support nature	[144,227]
Chemical parameters	Surface chemistry	• Overall charges provided by functional groups affect the binding • Effects of the loading amount of enzyme • The shape of particles varies with functional groups when added during preparation	[224,228]
	Biocompatibility	• Effect the immobilized enzyme structure and its activity	[229,230]

of excess free space [233]. Therefore, the hindrance in the mass transfer of analyte over the porous surface results in the non-uniform catalytic reactions of enzymes attributing the low sensitivity of the sensors. Similar effects can be observed for the entrapment method of immobilization. The pore size of the matrix also affects the diffusion of target analyte towards the enzyme molecules resulting in the lower signal generation, ultimately low sensitivity of the sensors [234].

4.2. Surface morphology of PS

Morphology of the PS surface significantly influences the enzyme attachment and retention over the support. Different surface morphology offers different loading capacity and the activity [144]. The pores can be cylindrical, funnel-shaped, or square cross-sectioned according to the current density, the electrolyte used, and the type of Si wafer used [235]. In the study by Zhou et al. [225], lipase from *Candida rugosa* was immobilized on the ordered mesoporous silica substrate SBA-15 with rod-shaped and spherical morphology. The immobilization rate was observed higher for rod-shaped morphology compared to spherical morphology. However, the enzyme showed enhanced thermal stability in spherical morphology over the rod-shaped morphology. The unique curvature of the structure and the significant area offers thermal stability. Another study by Manzano et al. [236] showed the effect of surface morphology on the drug (ibuprofen) immobilization and retention on the ordered and irregular mesoporous MCM-1 silica surfaces functionalized with APTES. Higher retention and controlled release were observed on ordered spherical pores compared to the irregular shaped surface.

4.3. Surface chemistry of PS

The surface chemistry of the PS is determined by the chemical composition and surface modifications done over the surface. Functionalization of PS with various silanes gives respective functional groups (amine, carboxylate, alkyl, vinyl, and thiol) that contribute to the net surface charge and reacts with functional groups on the enzyme's surface [237].

Silanes such as APTES and APTMS gave positively charged amine groups over PS surface while MPTES and MPTMS silanes gave negative surface charges via sulfhydryl groups [238,239]. Therefore, better immobilization of enzymes is possible by tuning the net charge of enzymes by altering the pH of the enzyme solution. Considering the pH, two pH values, isoelectric point (*pI*) for enzyme and point of zero charge (*pzc*) for surface, are crucial. pI is the pH of enzyme solution at which net charge of the enzyme is zero while *pzc* is pH value for which support surface exhibits net zero charge in an electrolyte. *pzc* value of the non-functionalized silicon substrate has been reported near to a pH value of 2 [240,241], while *pI* is the exclusive property of the enzyme. Based on the *pzc* and *pI*, immobilization of enzyme is governed by ionic interactions [242]. Similar to pI values, for pH > *pzc* support shows negative charge and for pH < *pzc*, the support surface is positively charged. Takahashi et al. [137] showed the effect of surface charges on the immobilization of HRP on the silica substrates. MCM-41 and FSM-16 surfaces prepared by the cationic template showed more negative charged than non-ionic templated SBA-15. For the MCM-41 with the *pzc* of 3.6, the surface acted as negative for the pH higher than 3.6. Therefore, HRP solution (*pI* = pH 3–9) having positive charges, readily attached to the surface with higher rates [126,137]. In another study, Gómez et al. [243] showed the relation of *pI* and *pzc* on the immobilization of β-glucosidase on SBA-15 surface. The observed *pI* of β-glucosidase was 5, and observed *pzc* of SBA-15 was 2.5-5. The highest immobilization of enzyme was observed at pH 3.5, where the maximum difference of zeta potential was observed between enzyme and SBA-15 surface.

Surface wettability also regulates the attachment of enzymes over the surface. Hydrophobic surfaces are reportedly favorable for adsorption of proteins [244]. In another study by Zou et al. [196] porcine pancreatic lipase was immobilized on non-functionalized and functionalized SBA-15 surfaces, which were modified with n-decyltrimethoxysilane (DE) showed higher hydrophobicity compared to 3-(trimethoxysilyl) propyl methacrylate (MA) modified silica surface. Moreover, the enzyme activity of immobilized lipases was observed, increasing with the increased hydrophobicity of the surface. In another study, Park et al. [245] showed the effect of the hydrophobic silica substrate surface on the immobilization of lysozyme. Periodic mesoporous organosilica surfaces functionalized with either bis [3-(trimethoxysilyl)propyl] amine (BTMS-amine), 1,4-bis (triethoxysilyl) benzene (BTES-benzene), or 4-bis (triethoxysilyl) biphenyl (BTES-biphenyl) were studied in comparison with SBA-15. BTES-biphenyl showed maximum hydrophobicity with the highest immobilization of lysozyme at pH 10.2 (near to pI).

4.4. Structural and optical properties of PS

Enhanced structural and optical properties of PS over planar silicon make the application of PS for the enzyme-based biosensing purposes. PS fabricated from the single crystal silicon wafer is ideal for the application of silicon-based optoelectronic technology. The porous surface of the PS offers a high surface area for enzyme immobilization, attributing to wider applications [246].

Discovery of the visible photoluminescence of PS due to confinement effects during the year 1990 leads to increased demand for PS in the sensing applications based on its optical properties [247]. Surface modifications and enzyme immobilization of PS induce the change in the fringe pattern related to the refractive index of PS in the visible range. The shift change in the Fabry-Perot fringe relates the variation in the surface of PS and can be applied for the biosensing purpose [248,249]. In the study by Benilov et al. [250], the photoluminescence of PS was observed to get affected by the pH of the liquid medium, resulting in the variation of intensity and decay time. Low pH solution

was detected by high photoluminescence intensity with long decay time, while high pH solution was detected by low photoluminescence with short decay time.

Application of PS in sensing is a mature technology, and the structural characteristics of the PS are well known [251]. Surface modification by oxidation and/or functionalization changes the hydrophobic nature of the PS to hydrophilic and improves the enzyme retention as well as enzyme activity [252]. The functionalization causing the change on the PS surface enhances the binding of the molecules with strong interactions. FTIR spectrum and X-ray diffraction data of the pure PS can relate to the changes in the bonding over the PS with the enzyme molecules [253,254].

Along with the properties of PS regulating the immobilization process and retention of the enzyme, properties of the enzymes also influence the success of immobilization and biosensing. A wise selection of enzymes and suitable support for a particular biosensor yields higher immobilization efficiency with maximum catalytic activity required for the estimation of analyte [43]. The retention of the enzyme on the support material relies on the interaction between the molecule and support. Various enzymatic properties need to consider for the modification of surface properties of the support material [136]. Table 8 describes the various influencing enzyme properties and their role in the enzyme immobilization on a support material, especially PS.

The storage stability of enzyme-based biosensors is crucially important for its applications in various fields. Retention of enzyme activity is necessary for the effective performance of biosensors. The change in the pH and drastic change of temperature also reduces the shelf life of the immobilized enzymes. Also, the restriction of the enzyme interaction with other impurities helps to maintain the enzyme activity for a longer duration [263]. Therefore, these biosensors should be stored at 4°C when not in use, and their activity should be analyzed periodically [83]. Further, the stability of the surfaces is also essential in order to maintain the performance of the sensors. PS substrates are mechanically brittle and may break during the handling and storage. Also, oxidation is a major cause of the failure of the PS structure due to inadequate passivation [264]. Such alterations in the properties change the initial surface chemistry and thus interfere with the calibrated performance of the senors. The formation of oxide layers on the PS surface renders its stability. Functionalization of PS with long alkyl chains or formation of Si-C bonds may improve the stability of the PS substrate and thus shelf life with improved chemistry for enzyme immobilization [47]. Therefore, the use of pure grade preparation materials and ambient reaction conditions are needed to maintain during the development of the biosensors [235].

The performance of the enzymatic biosensors relies on efficient immobilization, retention of enzyme activity after immobilization, and transduction of the signals from the redox centre of the enzyme to the electrode. Sensitivity, selectivity, fast response time, low fabrication cost and portability are the main requirements for the development of biosensors [43]. As enzymes are very selective towards their substrate molecules, enzymatic biosensors possess high selectivity for the detection of the analyte. The sensitivity of the biosensors is the function of the amount of enzyme immobilized. PS surface has a higher surface to volume ratio providing for more enzyme immobilization within the pores and thus offers higher sensitivity [53]. The sensitivity of the enzymatic biosensors is dependant on the surface area-volume ratio of the electrode, high enzyme density, high catalytic activity and high mass transfer rate. Porous silicon-based senors have reported higher sensitivity (3.1 folds) than planar silicon electrode [265]. Though tuning of pore size and morphology is easily possible, the optimization of pore size distribution with interface roughness facilitating the efficient transfer of analytes towards the enzyme is still challenging [266]. The limitations in the mass transfer of biomolecules affect the sensitivity of the biosensors and result in delayed response time. Response time can be defined as time taken by the sensors to give a constant value for the minimum amount of analyte. Response time is inversely proportional to the dissociation rate of target analyte binding and dominated by the mass transfer rate [267]. Tuning of pore size and surface chemistry of PS for facilitating accommodating higher enzyme concentration and increased mass transfer should be performed to reduce the response time that achieve greater and reliable performance of the biosensors.

5. Advantages, Challenges, and opportunities of PS-based biosensors

Advantages of the PS based sensors over other materials are as follows: (1) large internal surface of porous silicon as it allows the bonding of active molecules over a large surface in a small amount of volume [268]; (2) PS may be easily prepared either in powder or wafer form, depending on the specific use. Devices integrating PS layers with specific enzymes immobilized or with molecules with specific target allow the realization of label-free biosensors [269]; (3) PS can be fabricated electrochemically easily with simple and inexpensive equipment; and (4) PS is perfectly compatible with conventional microelectronic devices [270]. One of the significant challenges in silicon-based biosensors is the reproducibility of the porous surface. Some chemical and physical methods are in use for the preparation of a porous surface on the silicon wafer. The chemical methods such as electrochemical etching, strain etching, and plasma dry etching are not controllable in the pore formation. The chemical methods use many hazardous chemicals, which creates another pressing issue for the environment and health. However, physical methods preparing PS surfaces such as lithography techniques are under control comparatively, but their applications for the large-scale production of PS surfaces are not economic [271].

The commercialization of the PS based biosensors has to cross the two main obstacles: first, the cost-effective production of PS-based biosensors and second is their ability to respond for the varying degree of analyte concentrations present at the site without influenced by other factors [272]. Compared to other available materials used for the biosensors, PS is relatively expensive. The thin film of PS used for the development of the biosensors reduces the cost but creates a problem

Table 8
Various enzymatic properties and their role in the enzyme immobilization on support materials.

Parameter	Properties of enzyme	Role in enzyme immobilization	References
Physical parameters	Molecular size of the enzyme	Proper entrapment of enzyme within the pore	[43]
	Structure of enzyme	Alternating the structure of the pore	[226]
	Active site orientation	Retention of enzyme activity	[255,256]
Chemical parameters	Surface functional groups	Interaction with the support and functionalized groups	[237]
	pH of the enzyme surface	charge based on pI for ionic interaction optimum enzyme activity	[257]
	The temperature of the process	The efficiency of enzyme action	[258]
Biochemical parameters	Enzyme activity	Carrying out substrate conversion	[259]
	Reaction kinetics	Determination of the rate of the enzyme activity	[260]
	Inhibition kinetics	Determination of contamination limit of the process	[261]
	Saturation kinetics	Determination of the maximum sensitivity limit of enzyme	[262]

during the handling and integration of the brittle PS film in the device.

The preparation of a uniform porous silicon layer by using the fabrication methods is another issue with the use of PS as a support material in the biosensors. Multiple factors affect the PS properties with the slighter change in various parameters. The chemical instability of the PS surface limits its use and applications due to its reactivity with hydrogen give oxide layer altering the characteristics of PS. Oxidation of the surface makes the PS unstable than most of the other support materials used in biosensors. Incorporation of ohmic contacts over the PS surface for the signal transduction and electric studies of the PS biosensors creates different changes in the surface chemistry of the PS. Storage and aging of the PS biosensors create a significant influence on its eligibility as support for biosensors [47,231]. High-temperature application of PS-based biosensors may cause errors due to the oxidation of the surface. Reproducibility and formation of a uniform porous layer on the Si surface are achievable with the well-controlled techniques.

Different studies for the enzyme immobilization on PS showed the improvement in the binding and retention of enzyme molecules with the optimum activity. The orientation of the enzyme on the support is crucial for the enzyme activity. Various studies are undergoing modification of enzymes without altering their native structure and enhancing the complementary for the attachment to the support. Site-directed mutagenesis of enzymes improves their binding properties and lead to the preparation of controlled orientated active sites with the improved resistive enzyme. Surface modification of enzyme increases the immobilization rate includes additions of lysine or histidine residues over the surface, providing sulfide groups and metal chelators [273]. Automated immobilization of the enzyme on the PS with the high reproducible rate will help in reducing the manual errors.

The stability of the immobilized enzymes determines the extent of the success of the process. Maintaining the enzyme activity during the operation, purity, presence of unwanted inhibitors, and the storage affects the performance of the biosensor. The sensitivity of the enzyme for a low concentration of analyte determines the working range of the biosensors. The specific enzyme activity governs the sensitivity towards the substrate and may raise the problem with the enzyme with a less specific activity. The signal detection method from the enzyme reaction plays a critical role in the sensitivity of the biosensors. Signals obtained from the reactions are usually low strength and need amplification for the output. The selection of the proper detection method for the maximum performance of the PS biosensor is crucial [37]. The response time taken by the biosensor to give the output determines the possible applications for various purposes.

The further development of the PS biosensors might give an ability to the detection of multiple analytes from actual samples *in-vivo* and *in-vitro* with high accuracy and real-time. The PS based biosensors will emerge as the promising option over the currently available devices and commercialize for the broad range applications from laboratory analysis, enzyme-substrate reaction studies to the environmental monitoring studies, and diagnostics clinical applications. Fig. 7 outlines the challenges and possible scope for the advancements of enzymatic PS based biosensors.

6. Summary

Since the early times, enzymes are in use for the production of various food, pharmaceuticals, and other products. Development in the enzymology has ensured the fidelity of the effective yield by the use of enzyme-based reactions. Though the enzymes are known for their high catalytic activity, and specificity for the substrate, the conditions required for the enzymes to work optimally are particular and need to be maintained throughout the reaction. Enzymes are very susceptible to various factors: temperature, pH, and the presence of organic solvents. After the completion of the reaction, a considerable amount of the enzymes gets converted into the products. At the end of an enzymatic reaction, some amount of enzyme gets into the residual part of the reaction mixture, which is economically unacceptable. Recovery of the enzymes from the reaction mixture is a bit difficult and expensive process. Thus, the applications of enzymes for production have tackled many problems.

Enzyme immobilization helps in reducing the loss of enzymes caused by washing out during downstream processing of products and restricting the mobility of the enzymes within a specific area with a confined boundary or by binding them to support materials. Studies showed that the stability of the enzymes increases when immobilized. Immobilization offers protection to enzymes against the organic solvents, salts, surfactants, alkalis, and other factors, which may cause the destruction of the bonds in the enzyme structure. Being immobilized, the recovery of the enzymes from the reaction mixture becomes easy, and thus reduces the cost of the overall production, and purification processes.

Immobilization on the solid surfaces is convenient since the handling and recovery are easy compared to other support mechanisms. In the areas of biosensors, PS has very much crucial for the development of enzyme-based biosensors. PS offers the advantages of having an enhanced surface area-volume ratio, effective bandgap for the visible light range, specific semiconducting properties for the broad range of micropatterning for integration in the complex integrated systems, biocompatibility, and inertness towards biological molecules. The PS-based enzymatic biosensors are immensely popular for the detection and analysis of various analytes within the samples.

Based on the type of immobilization, strategies such as physical adsorption, chemical binding, cross-linking, and entrapment of enzymes are used. Physical adsorption works on the simple principles of weak interaction forces: van der Waal forces, ionic interaction, and hydrogen bonds. The weak interacting forces are subjected to alter with the progress of a reaction, which may result in the leaching out of

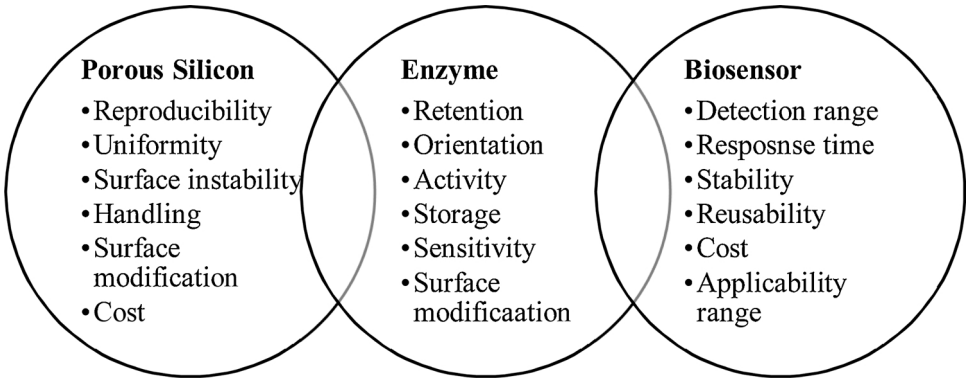


Fig. 7. Challenges to tackle for the development and the possible scope of advancements in the area of PS-based biosensors.

enzymes form the PS to the reaction mixture. Comparatively, chemical binding works based on the binding of enzymes to surface through strong covalent bonds. The chemical binding process needs some surface modifications on the PS surface for providing the functional groups on the surface for interacting with the enzyme molecules. Modifications include the functionalization of PS with the organosilanes, carbodiimide that gives amino and carboxyl groups on the surface, respectively. Cross-linking based immobilization of enzymes needs the binding of enzyme molecules on the surface by formation of cross-linkage with the help of cross-linking agents like GTA, and bisdiazobenidine. The association keeps the enzymes retained to PS and protects the enzyme activity. Entrapment involves the confining of enzyme molecules within the polymeric matrix, which allows the substrate to pass through while restricting the enzyme movement.

Immobilization offers numerous advantages over the conventional use of free-form enzymes. The uncontrolled orientation of enzymes during the immobilization remains the major drawback of the process. The involvement of active site in the bonds formed with the support materials may result in the inactivation of the active site, and thus reduce or loss in the overall activity of the enzyme. Similarly, the controlled microenvironment surrounding the molecules profoundly affects the catalytic efficiency of the molecules. Oxidation of the PS surface is one of the major obstacles which limit the applications. The immobilization of enzymes offers efficient retention of the activity with stable storage and operating capabilities. Post-immobilization, the enzyme can be used for the development of PS-based enzymatic biosensors having the potential for the accurate detection of the desired constituent.

Future prospects of PS based biosensor emerges due to the major developments in the areas of immobilization, surface chemistry, and fabrication. Over the past few decades, advances in the use of PS based sensing devices are proving to be an interesting platform for a variety of sensing applications. The advantage of manipulating the pore size, porosity, and surface functionalization of PS with ease allows one to fabricate sensors to detect analyte at very low concentrations. The remarkable growth of interest in PS sensors has started from the huge internal surface area, surface reactivity, and its unique optical and electrical properties. However, some arising challenges still exist, delaying the commercialization. In the future, more number of studies will be focused on Multiplexed detection of different analytes on a microarray of PS for parallel biosensing and PS optical biosensors integrated with microfluidic systems for highly sensitive and rapid detections of analytes. In the end, the PS based Biosensor using immobilization still holds a very promising research area in the sensing fields and open for advancements.

CRediT authorship contribution statement

Rushikesh Fopase: Data curation, Writing - original draft. **Santhosh Paramasivam:** Data curation, Writing - original draft. **Paresh Kale:** Supervision, Funding acquisition, Writing - review & editing. **Balasubramanian Paramasivan:** Conceptualization, Supervision, Funding acquisition, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] G.M. Cooper, The Central Role of Enzymes as Biological Catalysts, Sinauer Associates, 2000.
[2] G.J. Bartlett, C.T. Porter, N. Borkakoti, J.M. Thornton, Analysis of catalytic residues in enzyme active sites, J. Mol. Biol. 324 (1) (2002) 105–121, https://doi.org/10.1016/S0022-2836(02)01036-7.
[3] G.L. Holliday, J.B. Mitchell, J.M. Thornton, Understanding the functional roles of amino acid residues in enzyme catalysis, J. Mol. Biol. 390 (3) (2009) 560–577, https://doi.org/10.1016/j.jmb.2009.05.015.
[4] S. Nisha, S.A. Karthick, N. Gobi, A review on methods, application and properties of immobilized enzyme, Chem. Sci. Rev. Lett. 1 (3) (2012) 148–155.
[5] O. Kirk, T.V. Borchert, C.C. Fuglsang, Industrial enzyme applications, Curr. Opin. Biotechnol. 13 (4) (2002) 345–351, https://doi.org/10.1016/S0958-1669(02)00328-2.
[6] B. Das, P. Balasubramanian, R. Jayabalan, N. Lekshmi, S. Thomas, Strategies behind biosensors for food and waterborne pathogens, Quorum Sensing and Its Biotechnological Applications, Springer, 2018, pp. 107–141, https://doi.org/10.1007/978-981-13-0848-2_8.
[7] C.R. Ispas, G. Crivat, S. Andreescu, Recent developments in enzyme-based biosensors for biomedical analysis, Anal. Lett. 45 (2–3) (2012) 168–186, https://doi.org/10.1080/00032719.2011.633188.
[8] S. Li, X. Yang, S. Yang, M. Zhu, X. Wang, "Technology prospecting on enzymes: application, marketing and engineering, Comput. Struct. Biotechnol. J. 2 (3) (2012) e201209017, https://doi.org/10.5936/csbj.201209017.
[9] R. Fopase, S. Nayak, M. Mohanta, P. Kale, P. Balasubramanian, Inhibition assays of urease for detecting trivalent chromium in drinking water, Green Buildings and Sustainable Engineering, Springer, 2019, pp. 313–323, https://doi.org/10.1007/978-981-13-1202-1_27.
[10] S. Nayak, P. Kale, Inhibition assays of horseradish peroxidase by hexavalent chromium and other heavy metals, Int. J. Environ. Anal. Chem. (2020) 1–13, https://doi.org/10.1080/03067319.2020.1776864.
[11] V.V. Mozhaev, et al., Catalytic activity and denaturation of enzymes in water/organic cosolvent mixtures: α-Chymotrypsin and laccase in mixed water/alcohol, water/glycol and water/formamide solvents, Eur. J. Biochem. 184 (3) (1989) 597–602, https://doi.org/10.1111/j.1432-1033.1989.tb15055.x.
[12] A.A. Homaei, R. Sariri, F. Vianello, R. Stevanato, Enzyme immobilization: an update, J. Chem. Biol. 6 (4) (2013) 185–205, https://doi.org/10.1007/s12154-013-0102-9.
[13] P. Jochems, Y. Satyawali, L. Diels, W. Dejonghe, Enzyme immobilization on/in polymeric membranes: status, challenges and perspectives in biocatalytic membrane reactors (BMRs), Green Chem. 13 (7) (2011) 1609–1623, https://doi.org/10.1039/c1gc15178a.
[14] I. Chibata, T. Tosa, T. Sato, Biocatalysis: immobilized cells and enzymes, J. Mol. Catal. 37 (1) (1986) 1–24, https://doi.org/10.1016/0304-5102(86)85134-3.
[15] A.A. Khan, M.A. Alzohairy, Recent advances and applications of immobilized enzyme technologies: a review, Res J Biol Sci 5 (8) (2010) 565–575, https://doi.org/10.3923/rjbsci.2010.565.575.
[16] M. Hartmann, D. Jung, Biocatalysis with enzymes immobilized on mesoporous hosts: the status quo and future trends, J. Mater. Chem. 20 (5) (2010) 844–857, https://doi.org/10.1039/B907869J.
[17] W. Tischer, V. Kasche, Immobilized enzymes: crystals or carriers? Trends Biotechnol. 17 (8) (1999) 326–335, https://doi.org/10.1016/S0167-7799(99)01322-0.
[18] F. Secundo, Conformational changes of enzymes upon immobilisation, Chem. Soc. Rev. 42 (15) (2013) 6250–6261, https://doi.org/10.1039/c3cs35495d.
[19] R. DiCosimo, J. McAuliffe, A.J. Poulose, G. Bohlmann, Industrial use of immobilized enzymes, Chem. Soc. Rev. 42 (15) (2013) 6437–6474, https://doi.org/10.1039/c3cs35506c.
[20] H.H. Nguyen, S.H. Lee, U.J. Lee, C.D. Fermin, M. Kim, Immobilized enzymes in biosensor applications, Materials 12 (1) (2019) 121, https://doi.org/10.3390/ma12010121.
[21] Z. Al-Qodah, M. Al-Shannag, M. Al-Busoul, I. Penchev, W. Orfali, Immobilized enzymes bioreactors utilizing a magnetic field: a review, Biochem. Eng. J. 121 (2017) 94–106, https://doi.org/10.1016/j.bej.2017.02.003.
[22] C. Bernal, K. Rodriguez, R. Martinez, Integrating enzyme immobilization and protein engineering: an alternative path for the development of novel and improved industrial biocatalysts, Biotechnol. Adv. 36 (5) (2018) 1470–1480, https://doi.org/10.1016/j.biotechadv.2018.06.002.
[23] X.-Y. Yang, G. Tian, N. Jiang, B.-L. Su, Immobilization technology: a sustainable solution for biofuel cell design, Energy Environ. Sci. 5 (2) (2012) 5540–5563, https://doi.org/10.1039/C1EE02391H.
[24] M. Sharifi, et al., Strategies of enzyme immobilization on nanomatrix supports and their intracellular delivery, J. Biomol. Struct. Dyn. 38 (9) (2020) 2746–2762, https://doi.org/10.1080/07391102.2019.1643787.
[25] N. Mokhtari, M. Vaidyanathan, S. Esener, Y.-S. Yeh, Nanoparticle-mediated enzyme delivery for application in cancer therapy, Drug Target Rev. 4 (1) (2017) 38–41.
[26] V. Sirisha, A. Jain, A. Jain, Enzyme immobilization: an overview on methods, support material, and applications of immobilized enzymes, Advances in Food and

Nutrition Research vol. 79, Elsevier, 2016, pp. 179–211, https://doi.org/10.1016/bs.afnr.2016.07.004.
[27] I. Eş, J.D.G. Vieira, A.C. Amaral, Principles, techniques, and applications of biocatalyst immobilization for industrial application, Appl. Microbiol. Biotechnol. 99 (5) (2015) 2065–2082, https://doi.org/10.1007/s00253-015-6390-y.
[28] U. Roessl, J. Nahálka, B. Nidetzky, Carrier-free immobilized enzymes for biocatalysis, Biotechnol. Lett. 32 (3) (2010) 341–350, https://doi.org/10.1007/s10529-009-0173-4.
[29] R.A. Sheldon, Enzyme immobilization: the quest for optimum performance,", Adv. Synth. Catal. 349 (8–9) (2007) 1289–1307, https://doi.org/10.1002/adsc.200700082.
[30] N.R. Mohamad, N.H.C. Marzuki, N.A. Buang, F. Huyop, R.A. Wahab, An overview of technologies for immobilization of enzymes and surface analysis techniques for immobilized enzymes, Biotechnol. Biotechnol. Equip. 29 (2) (2015) 205–220, https://doi.org/10.1080/13102818.2015.1008192.
[31] P.S. Chaudhari, A. Gokarna, M. Kulkarni, M. Karve, S. Bhoraskar, Porous silicon as an entrapping matrix for the immobilization of urease, Sens. Actuators B Chem. 107 (1) (2005) 258–263, https://doi.org/10.1016/j.snb.2004.10.009.
[32] Q. Shabir, Biodegradability of porous silicon, Handbook of Porous Silicon (2017) 1–8, https://doi.org/10.1007/978-3-319-05744-6_39.
[33] L. Cao, L. van Langen, R.A. Sheldon, Immobilised enzymes: carrier-bound or carrier-free? Curr. Opin. Biotechnol. 14 (4) (2003) 387–394, https://doi.org/10.1016/S0958-1669(03)00096-X.
[34] J. Kim, J.W. Grate, P. Wang, Nanostructures for enzyme stabilization, Chem. Eng. Sci. 61 (3) (2006) 1017–1026, https://doi.org/10.1016/j.ces.2005.05.067.
[35] A.A. Khan, S. Akhtar, Q. Husain, Direct immobilization of polyphenol oxidases on Celite 545 from ammonium sulphate fractionated proteins of potato (Solanum tuberosum,)," Journal of Molecular Catalysis B: Enzymatic 40 (1-2) (2006) 58–63, https://doi.org/10.1016/j.molcatb.2006.03.001.
[36] C. Wu, G. Zhou, X. Jiang, J. Ma, H. Zhang, H. Song, Active biocatalysts based on Candida rugosa lipase immobilized in vesicular silica, Process. Biochem. 47 (6) (2012) 953–959, https://doi.org/10.1016/j.procbio.2012.03.004.
[37] A. Sassolas, L.J. Blum, B.D. Leca-Bouvier, Immobilization strategies to develop enzymatic biosensors, Biotechnol. Adv. 30 (3) (2012) 489–511, https://doi.org/10.1016/j.biotechadv.2011.09.003.
[38] W. Putzbach, N.J. Ronkainen, Immobilization techniques in the fabrication of nanomaterial-based electrochemical biosensors: a review, Sensors 13 (4) (2013) 4811–4840, https://doi.org/10.3390/s130404811.
[39] W. Feng, P. Ji, Enzymes immobilized on carbon nanotubes, Biotechnol. Adv. 29 (6) (2011) 889–895, https://doi.org/10.1016/j.biotechadv.2011.07.007.
[40] A.T. Lawal, Progress in utilisation of graphene for electrochemical biosensors, Biosens. Bioelectron. 106 (2018) 149–178, https://doi.org/10.1016/j.bios.2018.01.030.
[41] J.H. Song, M.J. Sailor, Chemical modification of crystalline porous silicon surfaces, Comments Inorg. Chem. 21 (1–3) (1999) 69–84, https://doi.org/10.1080/02603599908020416.
[42] P.G. Kale, S. Pratibha, C.S. Solanki, Synthesis and characterization of Si nanoparticles obtained on sonication of porous silicon multilayer films, J. Nano Res. 17 (2012) 13–25, https://doi.org/10.4028/www.scientific.net/jnanor.17.13 Trans Tech Publ.
[43] P. Biswas, A.K. Karn, P. Balasubramanian, P.G. Kale, Biosensor for detection of dissolved chromium in potable water: a review, Biosens. Bioelectron. 94 (2017) 589–604, https://doi.org/10.1016/j.bios.2017.03.043.
[44] C. Lv, Z. Jia, J. Lv, H. Zhang, Y. Li, High sensitivity detection of CdSe/ZnS quantum dot-labeled DNA based on N-type porous silicon microcavities, Sensors 17 (1) (2017) 80, https://doi.org/10.3390/s17010080.
[45] R. Boukherroub, J. Wojtyk, D.D. Wayner, D.J. Lockwood, Thermal hydrosilylation of undecylenic acid with porous silicon, J. Electrochem. Soc. 149 (2) (2002) H59–H63, https://doi.org/10.1149/1.1432679.
[46] J. Drott, K. Lindström, L. Rosengren, T. Laurell, Porous silicon as the carrier matrix in microstructured enzyme reactors yielding high enzyme activities, J. Micromechanics Microengineering 7 (1) (1997) 14, https://doi.org/10.1088/0960-1317/7/1/004.
[47] S. Dhanekar, S. Jain, Porous silicon biosensor: current status, Biosens. Bioelectron. 41 (2013) 54–64, https://doi.org/10.1016/j.bios.2012.09.045.
[48] M. Thust, M.J. Schöning, S. Frohnhoff, R. Arens-Fischer, P. Kordos, H. Lüth, Porous silicon as a substrate material for potentiometric biosensors, Meas. Sci. Technol. 7 (1) (1996) 26, https://doi.org/10.1088/0957-0233/7/1/003.
[49] R. Chhasatia, et al., Non-invasive, in vitro analysis of islet insulin production enabled by an optical porous silicon biosensor, Biosens. Bioelectron. 91 (2017) 515–522, https://doi.org/10.1016/j.bios.2017.01.004.
[50] R.R.K. Reddy, A. Chadha, E. Bhattacharya, Porous silicon based potentiometric triglyceride biosensor, Biosens. Bioelectron. 16 (4–5) (2001) 313–317, https://doi.org/10.1016/S0956-5663(01)00129-4.
[51] M. Saleem, M. Rafiq, S.-Y. Seo, K.H. Lee, Acetylcholinesterase immobilization, characterization and comparison of porous silicon immobilized enzyme activity with the free counter part, Biosci. Rep. (2016), https://doi.org/10.1042/BSR20150154 p. BSR20150154.
[52] M.J. Schöning, et al., Miniaturization of potentiometric sensors using porous silicon microtechnology, Electrochim. Acta 42 (20–22) (1997) 3185–3193, https://doi.org/10.1016/S0013-4686(97)00172-2.
[53] O. Syshchyk, V.A. Skryshevsky, O.O. Soldatkin, A.P. Soldatkin, Enzyme biosensor systems based on porous silicon photoluminescence for detection of glucose, urea and heavy metals, Biosens. Bioelectron. 66 (2015) 89–94, https://doi.org/10.1016/j.bios.2014.10.075.
[54] S. Tembe, P.S. Chaudhari, S. Bhoraskar, S. D'Souza, M.S. Karve, Conductivity-based catechol sensor using tyrosinase immobilized in porous silicon, IEEE Sens. J. 8 (9) (2008) 1593–1597, https://doi.org/10.1109/JSEN.2008.928925.
[55] M.S. Barbosa, et al., Enhanced activity of immobilized lipase by phosphonium-based ionic liquids used in the support preparation and immobilization process, ACS Sustain. Chem. Eng. 7 (18) (2019) 15648–15659, https://doi.org/10.1021/acssuschemeng.9b03741.
[56] V. Califano, A. Costantini, B. Silvestri, V. Venezia, S. Cimino, F. Sannino, The effect of pore morphology on the catalytic performance of β-glucosidase immobilized into mesoporous silica, Pure Appl. Chem. 91 (10) (2019) 1583–1592, https://doi.org/10.1515/pac-2018-1202.
[57] X. Qiu, J. Qin, M. Xu, L. Kang, Y. Hu, Organic-inorganic nanocomposites fabricated via functional ionic liquid as the bridging agent for Laccase immobilization and its application in 2, 4-dichlorophenol removal, Colloids Surf. B Biointerfaces 179 (2019) 260–269, https://doi.org/10.1016/j.colsurfb.2019.04.002.
[58] X. Liu, I. Azhar, H. Khan, Q. Qu, M. Tian, L. Yang, Capillary electrophoresis-immobilized enzyme microreactors for acetylcholinesterase assay with surface modification by highly-homogeneous microporous layer, J. Chromatogr. A 1609 (2020) 460454, https://doi.org/10.1016/j.chroma.2019.460454.
[59] X. Briones, et al., Surfaces based on amino acid functionalized polyelectrolyte films towards active surfaces for enzyme immobilization, Mater. Sci. Eng. C 104 (2019) 109938, https://doi.org/10.1016/j.msec.2019.109938.
[60] S.F. Prazeres, F. Zapata, N. Canilho, A. Pasc, C. García-Ruiz, G. Montalvo, Probing the confinement of β-galactosidase into meso-macro porous silica by Raman spectroscopy, Microporous Mesoporous Mater. 278 (2019) 149–155, https://doi.org/10.1016/j.micromeso.2018.11.032.
[61] S.A.A. Noma, et al., Comparative study of ASNase immobilization on tannic acid-modified magnetic Fe 3 O 4/SBA-15 nanoparticles to enhance stability and reusability, New J. Chem. 44 (11) (2020) 4440–4451, https://doi.org/10.1039/D0NJ00127A.
[62] J. Sun, D. Zhang, W. Zhao, Q. Ji, K. Ariga, Enhanced activity of alcohol dehydrogenase in porous silica nanosheets with wide size distributed mesopores, Bull. Chem. Soc. Jpn. 92 (2) (2019) 275–282, https://doi.org/10.1246/bcsj.20180201.
[63] M.N. El-Nahass, M.M. El-keiy, E.M. Ali, Immobilization of horseradish peroxidase into cubic mesoporous silicate, SBA-16 with high activity and enhanced stability, Int. J. Biol. Macromol. 116 (2018) 1304–1309, https://doi.org/10.1016/j.ijbiomac.2018.05.025.
[64] Z. Fathali, S. Rezaei, M.A. Faramarzi, M. Habibi-Rezaei, Catalytic phenol removal using entrapped cross-linked laccase aggregates, Int. J. Biol. Macromol. 122 (2019) 359–366, https://doi.org/10.1016/j.ijbiomac.2018.10.147.
[65] P. Sahare, et al., Enhancement of peroxidase stability against oxidative self-inactivation by co-immobilization with a redox-active protein in mesoporous silicon and silica microparticles, Nanoscale Res. Lett. 11 (1) (2016) 417, https://doi.org/10.1186/s11671-016-1605-4.
[66] M. Thust, et al., Enzyme immobilisation on planar and porous silicon substrates for biosensor applications, J. Mol. Catal., B Enzym. 7 (1–4) (1999) 77–83, https://doi.org/10.1016/S1381-1177(99)00023-5.
[67] A. Ressine, et al., Bioelectrochemical studies of azurin and laccase confined in three-dimensional chips based on gold-modified nano-/microstructured silicon, Biosens. Bioelectron. 25 (5) (2010) 1001–1007, https://doi.org/10.1016/J.BIOS.2009.09.014.
[68] M. González Álvarez, Chemical Surface Modification of Bio-functionalized Thin Films Using Cross-linking Agents, (2019) Bachelor thesis.
[69] D. Wan, C. Yan, Y. Liu, K. Zhu, Q. Zhang, A novel mesoporous nanocarrier: Integrating hollow magnetic fibrous silica with PAMAM into a single nanocomposite for enzyme immobilization, Microporous Mesoporous Mater. 280 (2019) 46–56, https://doi.org/10.1016/j.micromeso.2019.01.025.
[70] J. Li, L. Xu, Z.-g. Shi, Waxberry-like hierarchically porous ethyl-bridged hybrid silica microsphere: a substrate for enzyme catalysis and high-performance liquid chromatography, J. Chromatogr. A 1587 (2019) 79–87, https://doi.org/10.1016/j.chroma.2018.11.073.
[71] A. Sola-Rabada, et al., Biogenic porous silica and silicon sourced from Mexican Giant Horsetail (Equisetum myriochaetum) and their application as supports for enzyme immobilization, Colloids Surf. B Biointerfaces 166 (2018) 195–202, https://doi.org/10.1016/j.colsurfb.2018.02.047.
[72] B. Xia, et al., Biofunctionalisation of porous silicon (PS) surfaces by using homobifunctional cross-linkers, J. Mater. Chem. 16 (6) (2006) 570–578, https://doi.org/10.1039/B511175G.
[73] D. Yun, M. Song, S. Hong, M. Kang, N. Min, Highly sensitive and renewable amperometric urea sensor based on self-assembled monolayer using porous silicon substrate, J. Korean Phys. Soc. 47 (2005) S445.
[74] L.A. DeLouise, B.L. Miller, Enzyme immobilization in porous silicon: quantitative analysis of the kinetic parameters for glutathione-S-transferases, Anal. Chem. 77 (7) (2005) 1950–1956, https://doi.org/10.1021/ac0486185.
[75] P. Sahare, M. Ayala, R. Vazquez-Duhalt, V. Agrawal, Immobilization of peroxidase enzyme onto the porous silicon structure for enhancing its activity and stability, Nanoscale Res. Lett. 9 (1) (2014) 409, https://doi.org/10.1186/1556-276X-9-409.
[76] M. Saleem, M. Rafiq, S.-Y. Seo, K.H. Lee, Influence of acetylcholinesterase immobilization on the photoluminescence properties of mesoporous silicon surface, Appl. Surf. Sci. 308 (2014) 148–154, https://doi.org/10.1016/j.apsusc.2014.04.122.
[77] M. Azodi, C. Falamaki, A. Mohsenifar, Sucrose hydrolysis by invertase immobilized on functionalized porous silicon, J. Mol. Catal., B Enzym. 69 (3–4) (2011) 154–160, https://doi.org/10.1016/j.molcatb.2011.01.011.
[78] M. Bengtsson, S. Ekström, G. Marko-Varga, T. Laurell, Improved performance in silicon enzyme microreactors obtained by homogeneous porous silicon carrier matrix, Talanta 56 (2) (2002) 341–353, https://doi.org/10.1016/S0039-9140(01)

00600-2.
[79] R.E. Fernandez, S. Stolyarova, A. Chadha, E. Bhattacharya, Y. Nemirovsky, MEMS composite porous silicon/polysilicon cantilever sensor for enhanced triglycerides biosensing, IEEE Sens. J. 9 (12) (2009) 1660–1666, https://doi.org/10.1109/JSEN.2009.2030643.
[80] T. Laurell, J. Drott, L. Rosengren, Silicon wafer integrated enzyme reactors, Biosens. Bioelectron. 10 (3–4) (1995) 289–299, https://doi.org/10.1016/0956-5663(95)96848-S.
[81] S. Libertino, V. Aiello, A. Scandurra, M. Renis, F. Sinatra, Immobilization of the enzyme glucose oxidase on both bulk and porous SiO2 surfaces, Sensors 8 (9) (2008) 5637–5648, https://doi.org/10.3390/s8095637.
[82] G. Palestino, V. Agarwal, R. Aulombard, E. Perez, C. Gergely, Biosensing and protein fluorescence enhancement by functionalized porous silicon devices, Langmuir 24 (23) (2008) 13765–13771, https://doi.org/10.1021/la8015707.
[83] M.-J. Song, D.-H. Yun, N.-K. Min, S.-I. Hong, Electrochemical biosensor array for liver diagnosis using silanization technique on nanoporous silicon electrode, J. Biosci. Bioeng. 103 (1) (2007) 32–37, https://doi.org/10.1109/NMDC.2006.4388768.
[84] G. Shtenberg, N. Massad-Ivanir, L. Fruk, E. Segal, Nanostructured porous Si optical biosensors: effect of thermal oxidation on their performance and properties, ACS Appl. Mater. Interfaces 6 (18) (2014) 16049–16055, https://doi.org/10.1021/am503987j.
[85] A. Giovannozzi, V. Ferrero, F. Pennecchi, S. Sadeghi, G. Gilardi, A. Rossi, P450-based porous silicon biosensor for arachidonic acid detection, Biosens. Bioelectron. 28 (1) (2011) 320–325, https://doi.org/10.1016/j.bios.2011.07.046.
[86] Y. Shang, W. Zhao, E. Xu, C. Tong, J. Wu, FTRIFS biosensor based on double layer porous silicon as a LC detector for target molecule screening from complex samples, Biosens. Bioelectron. 25 (5) (2010) 1056–1063, https://doi.org/10.1016/j.bios.2009.09.029.
[87] S.E. Letant, B.R. Hart, S.R. Kane, M.Z. Hadi, S.J. Shields, J.G. Reynolds, Enzyme immobilization on porous silicon surfaces, Adv. Mater. 16 (8) (2004) 689–693, https://doi.org/10.1002/adma.200306173.
[88] A. Kermad, S. Sam, N. Ghellai, K. Khaldi, N. Gabouze, Horseradish peroxidase-modified porous silicon for phenol monitoring, Mater. Sci. Eng. B 178 (18) (2013) 1159–1164, https://doi.org/10.1016/j.mseb.2013.07.010.
[89] J. Márquez, L.F. Cházaro-Ruiz, L. Zimányi, G. Palestino, Immobilization strategies and electrochemical evaluation of porous silicon based cytochrome c electrode, Electrochim. Acta 140 (2014) 550–556, https://doi.org/10.1016/j.electacta.2014.05.065.
[90] Md.M.D. Maia, et al., Immobilization of urease on vapour phase stain etched porous silicon, Process. Biochem. 42 (3) (2007) 429–433, https://doi.org/10.1016/j.procbio.2006.08.008.
[91] C. Melander, M. Bengtsson, H. Schagerlöf, F. Tjerneld, T. Laurell, L. Gorton, Investigation of micro-immobilised enzyme reactors containing endoglucanases for efficient hydrolysis of cellodextrins and cellulose derivatives, Analytica Chimica Acta 550 (1–2) (2005) 182–190, https://doi.org/10.1016/j.aca.2005.06.070.
[92] D. Gößl, et al., Highly active enzymes immobilized in large pore colloidal mesoporous silica nanoparticles, New J. Chem. 43 (4) (2019) 1671–1680, https://doi.org/10.1039/C8NJ04585B.
[93] J. Liu, et al., Immobilization of trypsin onto large-pore mesoporous silica and optimization enzyme activity via response surface methodology, Solid State Sci. 89 (2019) 15–24, https://doi.org/10.1016/j.solidstatesciences.2018.12.014.
[94] J. Luo, et al., The study of the characteristics and hydrolysis properties of naringinase immobilized by porous silica material, RSC Adv. 9 (8) (2019) 4514–4520, https://doi.org/10.1039/c9ra00075e.
[95] F. Nagy, K. Szabó, P. Bugovics, G. Hornyánszky, Bisepoxide-activated hollow silica microspheres for covalent immobilization of lipase from Burkholderia cepacia, Period. Polytech. Chem. Eng. 63 (3) (2019) 414–424, https://doi.org/10.3311/PPch.12665.
[96] E. Onoja, R.A. Wahab, Effect of glutaraldehyde concentration on catalytic efficacy of Candida rugosa lipase immobilized onto silica from oil palm leaves, Indones. J. Chem. 19 (4) (2019) 1043–1054, https://doi.org/10.22146/ijc.42177.
[97] P.C. Tiosso, A.Hd.S. Venzel, F.F. de Moraes, P.W. Tardioli, G.M. Zanin, Immobilization of β-galactosidase on Porous Silica Activated with Glyoxyl Groups, (2017).
[98] A. Roucher, et al., Bilirubin oxidase-based silica macrocellular robust catalyst for on line dyes degradation, Enzyme Microb. Technol. 120 (2019) 77–83, https://doi.org/10.1016/j.enzmictec.2018.10.004.
[99] Y.-L. Han, P.-J. Gao, C.-L. Cheng, P.-Y. Wu, J.-S. Chang, Recovery of gold from industrial wastewater by immobilized gold-binding proteins on porous silica carriers grafted with amino group, Biochem. Eng. J. 152 (2019) 107388, https://doi.org/10.1016/j.bej.2019.107388.
[100] K.-L. Chang, T.-C. Teng, C.-K. Fu, C.-H. Liu, Improving biodegradation of Bisphenol A by immobilization and inducer, Process. Saf. Environ. Prot. 128 (2019) 128–134, https://doi.org/10.1016/j.psep.2019.05.038.
[101] S.L. Chong, V. Cardoso, J.L. Brás, M.Z. do Valle Gomes, C.M. Fontes, L. Olsson, Immobilization of bacterial feruloyl esterase on mesoporous silica particles and enhancement of synthetic activity by hydrophobic-modified surface, Bioresour. Technol. 293 (2019) 122009, https://doi.org/10.1016/j.biortech.2019.122009.
[102] E. Çakmakçi, S. Demir, Nonhydrolytic sol–gel synthesized oligosiloxane resin reinforced thiol-ene photocured coatings for the immobilization of acetylcholinesterase, J. Solgel Sci. Technol. 91 (1) (2019) 72–81, https://doi.org/10.1007/s10971-019-05006-2.
[103] A. Subramanian, S.J. Kennel, P.I. Oden, K.B. Jacobson, J. Woodward, M.J. Doktycz, Comparison of techniques for enzyme immobilization on silicon supports, Enzyme Microb. Technol. 24 (1–2) (1999) 26–34, https://doi.org/10.1016/s0141-0229(98)00084-2.
[104] S. Correa, et al., Design of stable magnetic hybrid nanoparticles of Si-entrapped HRP, PLoS One 14 (4) (2019), https://doi.org/10.1371/journal.pone.0214004.
[105] Y. Lin, W. Jin, Y. Qiu, G. Zhang, Programmable stimuli-responsive polypeptides for biomimetic synthesis of silica nanocomposites and enzyme self-immobilization, Int. J. Biol. Macromol. 134 (2019) 1156–1169, https://doi.org/10.1016/j.ijbiomac.2019.05.159.
[106] H. Vemulachedu, R.E. Fernandez, E. Bhattacharya, A. Chadha, Miniaturization of EISCAP sensor for triglyceride detection, J. Mater. Sci. Mater. Med. 20 (1) (2009) 229, https://doi.org/10.1007/s10856-008-3534-y.
[107] R.E. Fernandez, V. Hareesh, E. Bhattacharya, A. Chadha, Comparison of a potentiometric and a micromechanical triglyceride biosensor, Biosens. Bioelectron. 24 (5) (2009) 1276–1280, https://doi.org/10.1016/j.bios.2008.07.054.
[108] A. Simonis, C. Ruge, M. Müller-Veggian, H. Lüth, M.J. Schöning, A long-term stable macroporous-type EIS structure for electrochemical sensor applications, Sens. Actuators B Chem. 91 (1–3) (2003) 21–25, https://doi.org/10.1016/S0925-4005(03)00061-3.
[109] K. Lasmi, et al., Tyrosinase immobilization on functionalized porous silicon surface for optical monitoring of pyrocatechol, Appl. Surf. Sci. 446 (2018) 3–9, https://doi.org/10.1016/j.apsusc.2018.03.037.
[110] J.-H. Jin, S.-I. Hong, N.K. Min, Integrated urea sensor module based on poly (3-methylthiophene)-modified p-type porous silicon substrate, J. Porous Mater. 16 (4) (2009) 379–386, https://doi.org/10.1007/s10934-008-9210-2.
[111] D. Yun, M.-J. Song, S. Hwang, S.-I. Hong, Fabrication and electrochemical characterization of nanoporous silicon electrode for amperometric urea biosensor,", J. Appl. Phys. 51 (6S) (2012) 06FG02, https://doi.org/10.1143/JJAP.51.06FG02.
[112] S. Chen, E.A. Hall, A biosilification fusion protein for a 'self-immobilising'sarcosine oxidase amperometric enzyme biosensor, Electroanalysis 32 (4) (2020) 874–884, https://doi.org/10.1002/elan.202000032.
[113] R. Fopase, S. Nayak, M. Mohanta, P. Kale, B. Paramasivan, Inhibition assays of free and immobilized urease for detecting hexavalent chromium in water samples, 3 Biotech 9 (4) (2019) 124, https://doi.org/10.1007/s13205-019-1661-4.
[114] D. Basu, T. Sarkar, K. Sen, S.M. Hossain, J. Das, Multi-parametric optical glucose sensor based on surface functionalized nano-porous silicon, IEEE Sens. J. 18 (24) (2018) 9940–9947, https://doi.org/10.1109/JSEN.2018.2872846.
[115] T. Sarkar, D. Basu, N. Mukherjee, J. Das, Comparison of glucose sensitivity of nano and macro porous silicon, Mater. Today Proc. 5 (3) (2018) 9798–9803, https://doi.org/10.1016/j.matpr.2017.10.169.
[116] G. Shtenberg, N. Massad-Ivanir, A. Khabibullin, I. Zharov, E. Segal, Label-free optical monitoring of proteolytic reaction products using nanoporous silica colloidal assembly, Sens. Actuators B Chem. 262 (2018) 796–800, https://doi.org/10.1016/j.snb.2018.02.057.
[117] S. Tvorynska, J. Barek, B. Josypčuk, Amperometric biosensor based on enzymatic reactor for choline determination in flow systems, Electroanalysis 31 (10) (2019) 1901–1912, https://doi.org/10.1002/elan.201900237.
[118] F. Li, Z. Wang, Y. Feng, Construction of bienzyme biosensors based on combination of the one-step electrodeposition and covalent-coupled sol-gel process, Sci. China Ser. B Chem. 52 (12) (2009) 2269, https://doi.org/10.1007/s11426-009-0158-0.
[119] S.-H. Chiou, W.-T. Wu, Immobilization of Candida rugosa lipase on chitosan with activation of the hydroxyl groups, Biomaterials 25 (2) (2004) 197–204, https://doi.org/10.1016/S0142-9612(03)00482-4.
[120] U. Hanefeld, L. Gardossi, E. Magner, Understanding enzyme immobilisation, Chem. Soc. Rev. 38 (2) (2009) 453–468, https://doi.org/10.1039/B711564B.
[121] J.M. Guisan, Immobilization of Enzymes and Cells, Springer, 2006, https://doi.org/10.1007/978-1-0716-0215-7.
[122] J. Porath, Salting-out adsorption techniques for protein purification, Biopolymers: Original Research on Biomolecules 26 (S0) (1987) S193–S204, https://doi.org/10.1002/bip.360260017.
[123] J.C.Sd. Santos, O. Barbosa, C. Ortiz, A. Berenguer-Murcia, R.C. Rodrigues, R. Fernandez-Lafuente, Importance of the support properties for immobilization or purification of enzymes, ChemCatChem 7 (16) (2015) 2413–2432, https://doi.org/10.1002/cctc.201500310.
[124] J. Lee, et al., Simple synthesis of hierarchically ordered mesocellular mesoporous silica materials hosting crosslinked enzyme aggregates, Small 1 (7) (2005) 744–753, https://doi.org/10.1002/smll.200500035.
[125] C.-H. Lee, T.-S. Lin, C.-Y. Mou, Mesoporous materials for encapsulating enzymes, Nano Today 4 (2) (2009) 165–179, https://doi.org/10.1016/j.nantod.2009.02.001.
[126] Z. Zhou, M. Hartmann, Progress in enzyme immobilization in ordered mesoporous materials and related applications, Chem. Soc. Rev. 42 (9) (2013) 3894–3912, https://doi.org/10.1039/c3cs60059a.
[127] T. Komatsu, Protein-based nanotubes for biomedical applications, Nanoscale 4 (6) (2012) 1910–1918, https://doi.org/10.1039/C1NR11224D.
[128] H. Yao, Q. Gan, J. Peng, S. Huang, M. Zhu, K. Shi, A stimuli-responsive biosensor of glucose on layer-by-layer films assembled through specific lectin-glycoenzyme recognition, Sensors 16 (4) (2016) 563, https://doi.org/10.3390/s16040563.
[129] M. Ferreira, P.A. Fiorito, O.N. Oliveira Jr., S.I.C. de Torresi, Enzyme-mediated amperometric biosensors prepared with the Layer-by-Layer (LbL) adsorption technique, Biosens. Bioelectron. 19 (12) (2004) 1611–1615, https://doi.org/10.1016/j.bios.2003.12.025.
[130] O.S. Sakr, G. Borchard, Encapsulation of enzymes in Layer-by-Layer (LbL) structures: latest advances and applications, Biomacromolecules 14 (7) (2013) 2117–2135, https://doi.org/10.1021/bm400198p.
[131] J.J. Virgen-Ortíz, et al., Desorption of lipases immobilized on octyl-agarose beads

and coated with ionic polymers after thermal inactivation. Stronger adsorption of polymers/unfolded protein composites, Molecules 22 (1) (2017) 91 https://dx.doi.org/10.3390%2Fmolecules22010091.
[132] R.C. Rodrigues, et al., Immobilization of lipases on hydrophobic supports: immobilization mechanism, advantages, problems, and solutions, Biotechnol. Adv. (2019), https://doi.org/10.1016/j.biotechadv.2019.04.003.
[133] H. Gustafsson, E.M. Johansson, A. Barrabino, M. Odén, K. Holmberg, Immobilization of lipase from Mucor miehei and Rhizopus oryzae into mesoporous silica—the effect of varied particle size and morphology, Colloids Surf. B Biointerfaces 100 (2012) 22–30, https://doi.org/10.1016/j.colsurfb.2012.04.042.
[134] B. Brena, P. González-Pombo, F. Batista-Viera, Immobilization of enzymes: a literature survey, Immobilization of Enzymes and Cells, Springer, 2013, pp. 15–31, https://doi.org/10.1007/978-1-62703-550-7_2.
[135] T. Ikeda, K.-i. Ninomiya, R. Hirota, A. Kuroda, Single-step affinity purification of recombinant proteins using the silica-binding Si-tag as a fusion partner, Protein Expr. Purif. 71 (1) (2010) 91–95, https://doi.org/10.1016/j.pep.2009.12.009.
[136] D. Wasserberg, et al., Controlling protein surface orientation by strategic placement of oligo-histidine tags, ACS Nano 11 (9) (2017) 9068–9083, https://doi.org/10.1021/acsna7b03717.
[137] H. Takahashi, B. Li, T. Sasaki, C. Miyazaki, T. Kajino, S. Inagaki, Catalytic activity in organic solvents and stability of immobilized enzymes depend on the pore size and surface characteristics of mesoporous silica, Chem. Mater. 12 (11) (2000) 3301–3305, https://doi.org/10.1021/cm000487a.
[138] S. Datta, L.R. Christena, Y.R.S. Rajaram, Enzyme immobilization: an overview on techniques and support materials, 3 Biotech 3 (1) (2013) 1–9, https://doi.org/10.1007/s13205-012-0071-7.
[139] T. Ikeda, et al., Oriented immobilization of antibodies on a silicon wafer using Si-tagged protein A, Anal. Biochem. 385 (1) (2009) 132–137, https://doi.org/10.1016/j.ab.2008.11.001.
[140] T. Ikeda, A. Kuroda, Why does the silica-binding protein “Si-tag” bind strongly to silica surfaces? Implications of conformational adaptation of the intrinsically disordered polypeptide to solid surfaces, Colloids Surf. B Biointerfaces 86 (2) (2011) 359–363, https://doi.org/10.1016/j.colsurfb.2011.04.020.
[141] V. Gaberc-Porekar, V. Menart, Perspectives of immobilized-metal affinity chromatography, J. Biochem. Biophys. Methods 49 (1–3) (2001) 335–360, https://doi.org/10.1016/S0165-022X(01)00207-X.
[142] H.H. Yiu, P.A. Wright, N.P. Botting, Enzyme immobilisation using SBA-15 mesoporous molecular sieves with functionalised surfaces, J. Mol. Catal., B Enzym. 15 (1–3) (2001) 81–92, https://doi.org/10.1016/S1381-1177(01)00011-X.
[143] M. Dreifke, F.J. Brieler, M. Fröba, Immobilization of alcohol dehydrogenase from E. Coli onto mesoporous silica for application as a cofactor recycling system, ChemCatChem 9 (7) (2017) 1197–1210, https://doi.org/10.1002/cctc.201700468.
[144] S. Hudson, J. Cooney, E. Magner, Proteins in mesoporous silicates, Angew. Chemie Int. Ed. 47 (45) (2008) 8582–8594, https://doi.org/10.1002/anie.200705238.
[145] Q. Chen, G.L. Kenausis, A. Heller, Stability of oxidases immobilized in silica gels, J. Am. Chem. Soc. 120 (19) (1998) 4582–4585, https://doi.org/10.1021/ja971750k.
[146] X. Zhang, R.-F. Guan, D.-Q. Wu, K.-Y. Chan, Enzyme immobilization on amino-functionalized mesostructured cellular foam surfaces, characterization and catalytic properties, J. Mol. Catal., B Enzym. 33 (1–2) (2005) 43–50, https://doi.org/10.1016/j.molcatb.2005.02.001.
[147] J.F. Diaz, K.J. Balkus Jr., Enzyme immobilization in MCM-41 molecular sieve, J. Mol. Catal., B Enzym. 2 (2–3) (1996) 115–126, https://doi.org/10.1016/S1381-1177(96)00017-3.
[148] F. Pitzalis, M. Monduzzi, A. Salis, A bienzymatic biocatalyst constituted by glucose oxidase and Horseradish peroxidase immobilized on ordered mesoporous silica, Microporous Mesoporous Mater. 241 (2017) 145–154, https://doi.org/10.1016/j.micromeso.2016.12.023.
[149] O. Pérez-Anguiano, B. Wenger, Rl. Pugin, H. Hofmann, E. Scolan, Controlling mesopore size and processability of transparent enzyme-loaded silica films for biosensing applications, ACS Appl. Mater. Interfaces 7 (4) (2015) 2960–2971, https://doi.org/10.1021/am508630c.
[150] R.M. Blanco, P. Terreros, M. Fernández-Pérez, C. Otero, G. Dıaz-González, Functionalization of mesoporous silica for lipase immobilization: characterization of the support and the catalysts, J. Mol. Catal., B Enzym. 30 (2) (2004) 83–93, https://doi.org/10.1016/j.molcatb.2004.03.012.
[151] T. Jesionowski, J. Zdarta, B. Krajewska, Enzyme immobilization by adsorption: a review, Adsorption 20 (5-6) (2014) 801–821, https://doi.org/10.1007/s10450-014-9623-y.
[152] A. Sugihara, T. Tani, Y. Tominaga, Purification and characterization of a novel thermostable lipase from Bacillus sp, J. Biochem. 109 (2) (1991) 211–216, https://doi.org/10.1093/oxfordjournals.jbchem.a123363.
[153] K.A. Laderman, et al., The purification and characterization of an extremely thermostable alpha-amylase from the hyperthermophilic archaebacterium Pyrococcus furiosus, J. Biol. Chem. 268 (32) (1993) 24394–24401.
[154] P.H. Pandya, R.V. Jasra, B.L. Newalkar, P.N. Bhatt, Studies on the activity and stability of immobilized α-amylase in ordered mesoporous silicas, Microporous Mesoporous Mater. 77 (1) (2005) 67–77, https://doi.org/10.1016/j.micromeso.2004.08.018.
[155] F.B. Hasan, J. Elkind, S. Cohen, J. Cohen, Cationic and uncharged substrates and reversible inhibitors in hydrolysis by acetylcholinesterase (EC 3.1. 1.7). The trimethyl subsite, J. Biol. Chem. 256 (15) (1981) 7781–7785.
[156] M.C. Rosales-Hernandez, J.E. Mendieta-Wejebe, J. Correa-Basurto, J. Vazquez-Alcantara, E. Terres-Rojas, J. Trujillo-Ferrara, Catalytic activity of acetylcholinesterase immobilized on mesoporous molecular sieves, Int. J. Biol. Macromol. 40 (5) (2007) 444–448, https://doi.org/10.1016/j.ijbiomac.2006.11.004.
[157] J. Di, S. Bi, M. Zhang, Third-generation superoxide anion sensor based on superoxide dismutase directly immobilized by sol–gel thin film on gold electrode, Biosens. Bioelectron. 19 (11) (2004) 1479–1486, https://doi.org/10.1016/j.bios.2003.12.006.
[158] B. Zhao, et al., Co-immobilization of glucose oxidase and catalase in silica inverse opals for glucose removal from commercial isomaltooligosaccharide, Int. J. Biol. Macromol. 107 (2018) 2034–2043, https://doi.org/10.1016/j.ijbiomac.2017.10.074.
[159] D. Saikia, J.R. Deka, C.-E. Wu, Y.-C. Yang, H.-M. Kao, pH responsive selective protein adsorption by carboxylic acid functionalized large pore mesoporous silica nanoparticles SBA-1, Mater. Sci. Eng. C 94 (2019) 344–356, https://doi.org/10.1016/j.msec.2018.09.043.
[160] L. Betancor, H.R. Luckarift, Bioinspired enzyme encapsulation for biocatalysis, Trends Biotechnol. 26 (10) (2008) 566–572, https://doi.org/10.1016/j.tibtech.2008.06.009.
[161] M. Hartmann, X. Kostrov, Immobilization of enzymes on porous silicas–benefits and challenges, Chem. Soc. Rev. 42 (15) (2013) 6277–6289, https://doi.org/10.1039/c3cs60021a.
[162] N. Majoul, S. Aouida, B. Bessaïs, Progress of porous silicon APTES-functionalization by FTIR investigations, Appl. Surf. Sci. 331 (2015) 388–391, https://doi.org/10.1016/j.apsusc.2015.01.107.
[163] D.N. Tran, K.J. Balkus Jr., Perspective of recent progress in immobilization of enzymes, ACS Catal. 1 (8) (2011) 956–968, https://doi.org/10.1021/cs200124a.
[164] H.G. Manyar, E. Gianotti, Y. Sakamoto, O. Terasaki, S. Coluccia, S. Tumbiolo, Active biocatalysts based on pepsin immobilized in mesoporous SBA-15, J. Phys. Chem. C 112 (46) (2008) 18110–18116, https://doi.org/10.1021/jp802420t.
[165] J. Fu, J. Reinhold, N.W. Woodbury, Peptide-modified surfaces for enzyme immobilization, PLoS One 6 (4) (2011), https://doi.org/10.1371/journal.pone.0018692.
[166] I. Schechter, Reprint of “on the size of the active site in proteases. I. Papain”, Biochem. Biophys. Res. Commun. 425 (3) (2012) 497–502, https://doi.org/10.1016/j.bbrc.2012.08.015.
[167] A.M. Rossi, L. Wang, V. Reipa, T.E. Murphy, Porous silicon biosensor for detection of viruses, Biosens. Bioelectron. 23 (5) (2007) 741–745, https://doi.org/10.1016/j.bios.2007.06.004.
[168] V. Singh, P.C. Mondal, A.K. Singh, M. Zharnikov, Molecular sensors confined on SiOx substrates, Coord. Chem. Rev. 330 (2017) 144–163, https://doi.org/10.1016/j.ccr.2016.09.015.
[169] G. Priano, L.N. Acquaroli, L.C. Lasave, F. Battaglini, R.D. Arce, R.R. Koropecki, Rationally designed porous silicon as platform for optical biosensors, Thin Solid Films 520 (20) (2012) 6434–6439, https://doi.org/10.1016/J.TSF.2012.06.055.
[170] Q. Weiping, et al., Controlled site-directed assembly of antibodies by their oligosaccharide moieties onto APTES derivatized surfaces, J. Colloid Interface Sci. 214 (1) (1999) 16–19, https://doi.org/10.1006/JCIS.1999.6151.
[171] E. Finocchio, E. Macis, R. Raiteri, G. Busca, Adsorption of trimethoxysilane and of 3-mercaptopropyltrimethoxysilane on silica and on silicon wafers from vapor phase: an IR study, Langmuir 23 (5) (2007) 2505–2509, https://doi.org/10.1021/la062972b.
[172] P. Yang, X. Li, R. Zhang, N. Liu, Y. Zhang, Nanocrystals encapsulated in SiO2 particles: silanization and homogenous coating for bio applications, J. Nanosci. Nanotechnol. 13 (3) (2013) 1784–1788, https://doi.org/10.1166/jnn.2013.7121.
[173] Y. Han, D. Mayer, A. Offenhäusser, S. Ingebrandt, Surface activation of thin silicon oxides by wet cleaning and silanization, Thin Solid Films 510 (1-2) (2006) 175–180, https://doi.org/10.1016/j.tsf.2005.11.048.
[174] K. Khaldi, S. Sam, A. Lounas, C. Yaddaden, N.-E. Gabouze, Comparative investigation of two methods for acetylcholinesterase enzyme immobilization on modified porous silicon, Appl. Surf. Sci. 421 (2017) 148–154, https://doi.org/10.1016/j.apsusc.2016.12.169.
[175] M. Zhu, M.Z. Lerum, W. Chen, How to prepare reproducible, homogeneous, and hydrolytically stable aminosilane-derived layers on silica, Langmuir 28 (1) (2012) 416–423, https://doi.org/10.1021/la203638g.
[176] J. Kim, P. Seidler, L.S. Wan, C. Fill, Formation, structure, and reactivity of amino-terminated organic films on silicon substrates, J. Colloid Interface Sci. 329 (1) (2009) 114–119, https://doi.org/10.1016/j.jcis.2008.09.031.
[177] I. Migneault, C. Dartiguenave, M.J. Bertrand, K.C. Waldron, Glutaraldehyde: behavior in aqueous solution, reaction with proteins, and application to enzyme crosslinking, Biotechniques 37 (5) (2004) 790–802, https://doi.org/10.2144/3705A0790.
[178] C. Manta, et al., Polyethylene glycol as a spacer for solid-phase enzyme immobilization, Enzyme Microb. Technol. 33 (7) (2003) 890–898, https://doi.org/10.1016/S0141-0229(03)00221-7.
[179] D.B. Mawhinney, J.A. Glass, J.T. Yates, FTIR study of the oxidation of porous silicon, J. Phys. Chem. B 101 (7) (1997) 1202–1206, https://doi.org/10.1021/jp963322r.
[180] Y. Seo, et al., Photoluminescence, Raman scattering, and infrared absorption studies of porous silicon, Appl. Phys. Lett. 62 (15) (1993) 1812–1814, https://doi.org/10.1063/1.109557.
[181] J. Salonen, V.-P. Lehto, E. Laine, The room temperature oxidation of porous silicon, Appl. Surf. Sci. 120 (3–4) (1997) 191–198, https://doi.org/10.1016/S0169-4332(97)00385-1.
[182] R. Ramesh, P. Puhazhendi, J. Kumar, M.K. Gowthaman, S.F. D’Souza, N.R. Kamini, Potentiometric biosensor for determination of urea in milk using immobilized Arthrobacter creatinolyticus urease, Mater. Sci. Eng. C 49 (2015) 786–792, https://doi.org/10.1016/j.msec.2015.01.048.
[183] B.C. Pessela, et al., One-step purification, covalent immobilization, and additional

stabilization of a thermophilic poly-His-tagged β-galactosidase from Thermus sp. Strain T2 by using novel heterofunctional chelate− epoxy Sepabeads, Biomacromolecules 4 (1) (2003) 107–113, https://doi.org/10.1021/bm020086j.
[184] S. Mohapatra, D. Pal, S.K. Ghosh, P. Pramanik, Design of Superparamagnetic Iron Oxide Nanoparticle for Purification of Histidine-Tagged Recombinant Proteins, (2007), https://doi.org/10.1166/jnn.2007.869.
[185] F. López-Gallego, G. Fernandez-Lorente, J. Rocha-Martin, J.M. Bolivar, C. Mateo, J.M. Guisan, Stabilization of enzymes by multipoint covalent immobilization on supports activated with glyoxyl groups, Immobilization of Enzymes and Cells, Springer, 2013, pp. 59–71, https://doi.org/10.1007/978-1-62703-550-7_5.
[186] L.D. Unsworth, J. van der Oost, S. Koutsopoulos, Hyperthermophilic enzymes−stability, activity and implementation strategies for high temperature applications, FEBS J. 274 (16) (2007) 4044–4056, https://doi.org/10.1111/j.1742-4658.2007.05954.x.
[187] P. Zucca, E. Sanjust, Inorganic materials as supports for covalent enzyme immobilization: methods and mechanisms, Molecules 19 (9) (2014) 14139–14194, https://doi.org/10.3390/molecules190914139.
[188] L.S. Wong, F. Khan, J. Micklefield, Selective covalent protein immobilization: strategies and applications, Chem. Rev. 109 (9) (2009) 4025–4053, https://doi.org/10.1021/cr8004668.
[189] B. Yu and F. Moussy, "Epoxy enhanced polymer membrane to increase durability of biosensors," ed: Google Patents, 2009.
[190] Chemical cross-linking by glutaraldehyde between amino groups: its mechanism and effects, Polymer Modification, Springer, 1997, pp. 119–131, https://doi.org/10.1007/978-1-4899-1477-4_11.
[191] O. Barbosa, C. Ortiz, Á. Berenguer-Murcia, R. Torres, R.C. Rodrigues, R. Fernandez-Lafuente, Glutaraldehyde in bio-catalysts design: a useful crosslinker and a versatile tool in enzyme immobilization, RSC Adv. 4 (4) (2014) 1583–1600, https://doi.org/10.1039/C3RA45991H.
[192] C.-H. Lee, J. Lang, C.-W. Yen, P.-C. Shih, T.-S. Lin, C.-Y. Mou, Enhancing stability and oxidation activity of cytochrome c by immobilization in the nanochannels of mesoporous aluminosilicates, J. Phys. Chem. B 109 (25) (2005) 12277–12286, https://doi.org/10.1021/jp050535k.
[193] M.I. Kim, et al., Crosslinked enzyme aggregates in hierarchically-ordered mesoporous silica: a simple and effective method for enzyme stabilization, Biotechnol. Bioeng. 96 (2) (2007) 210–218, https://doi.org/10.1002/bit.21107.
[194] Z. Chen, Y. Wang, W. Liu, J. Wang, H. Chen, A novel cross-linked enzyme aggregates (CLEAs) of papain and neutrase-production, partial characterization and application, Int. J. Biol. Macromol. 95 (2017) 650–657, https://doi.org/10.1016/j.ijbiomac.2016.11.083.
[195] R. Sheldon, Cross-linked Enzyme Aggregates (CLEA® S): Stable and Recyclable Biocatalysts, ed: Portland Press Limited, 2007, https://doi.org/10.1042/BST0351583.
[196] B. Zou, et al., Immobilization of porcine pancreatic lipase onto ionic liquid modified mesoporous silica SBA-15, Biochem. Eng. J. 53 (1) (2010) 150–153, https://doi.org/10.1016/j.bej.2010.09.005.
[197] S. Krishnamoorthi, A. Banerjee, A. Roychoudhury, Immobilized enzyme technology: potentiality and prospects, J. Enzymol. Metabol. 1 (1) (2015) 010–104.
[198] B.S. Aytar, U. Bakir, Preparation of cross-linked tyrosinase aggregates, Process. Biochem. 43 (2) (2008) 125–131, https://doi.org/10.1016/j.procbio.2007.11.001.
[199] P. Lopez-Serrano, L. Cao, F. Van Rantwijk, R. Sheldon, Cross-linked enzyme aggregates with enhanced activity: application to lipases, Biotechnol. Lett. 24 (16) (2002) 1379–1383, https://doi.org/10.1023/A:1019863314646.
[200] C.S. Karamitros, N.E. Labrou, Preserving enzymatic activity and enhancing biochemical stability of glutathione transferase by soluble additives under free and tethered conditions, Biotechnol. Appl. Biochem. 64 (5) (2017) 754–764, https://doi.org/10.1002/bab.1535.
[201] S. Shah, A. Sharma, M.N. Gupta, Preparation of cross-linked enzyme aggregates by using bovine serum albumin as a proteic feeder, Anal. Biochem. 351 (2) (2006) 207–213, https://doi.org/10.1016/j.ab.2006.01.028.
[202] R.A. Sheldon, Characteristic features and biotechnological applications of cross-linked enzyme aggregates (CLEAs), Appl. Microbiol. Biotechnol. 92 (3) (2011) 467–477, https://doi.org/10.1007/s00253-011-3554-2.
[203] R.A. Sheldon, S. van Pelt, Enzyme immobilisation in biocatalysis: why, what and how, Chem. Soc. Rev. 42 (15) (2013) 6223–6235, https://doi.org/10.1039/C3CS60075K.
[204] A. Berezhetskyy, O. Sosovska, C. Durrieu, J.-M. Chovelon, S. Dzyadevych, C. Tran-Minh, Alkaline phosphatase conductometric biosensor for heavy-metal ions determination, Irbm 29 (2-3) (2008) 136–140, https://doi.org/10.1016/j.rbmret.2007.12.007.
[205] E. Younes Jomma, S.-N. Ding, Recent advances on electrochemical enzyme biosensors, Curr. Anal. Chem. 12 (1) (2016) 5–21, https://doi.org/10.1021/acs.analchem.6b04281.
[206] M.Y. Arica, S. şenel, N.G. Alaeddinoğlu, S. Patir, A. Denizli, Invertase immobilized on spacer-arm attached poly (hydroxyethyl methacrylate) membrane: preparation and properties, J. Appl. Polym. Sci. 75 (14) (2000) 1685–1692, https://doi.org/10.1002/(sici)1097-4628(20000401)75:14%3C1685::aid-app1%3E3.0.co;2-6.
[207] S. Dalal, A. Sharma, M.N. Gupta, A multipurpose immobilized biocatalyst with pectinase, xylanase and cellulase activities, Chem. Cent. J. 1 (1) (2007) 16, https://doi.org/10.1186/1752-153X-1-16.
[208] D. Brady, J. Jordaan, Advances in enzyme immobilisation, Biotechnol. Lett. 31 (11) (2009) 1639, https://doi.org/10.1007/s10529-009-0076-4.
[209] E. Górecka, M. Jastrzębska, Immobilization techniques and biopolymer carriers–A review, Appl. Biotechnol. Food Sci. Policy 75 (2011) 27–34, https://doi.org/10.34658/bfs.2011.75.1.65-86.
[210] N. Munjal, S.K. Sawhney, Stability and properties of mushroom tyrosinase entrapped in alginate, polyacrylamide and gelatin gels, Enzyme Microb. Technol. 30 (5) (2002) 613–619, https://doi.org/10.1016/S0141-0229(02)00019-4.
[211] A.E. Danks, S.R. Hall, Z. Schnepp, The evolution of 'sol–gel'chemistry as a technique for materials synthesis, Mater. Horiz. 3 (2) (2016) 91–112, https://doi.org/10.1039/c5mh00260e.
[212] A. Pierre, The sol-gel encapsulation of enzymes, Biocatal. Biotransformation 22 (3) (2004) 145–170, https://doi.org/10.1080/10242420412331283314.
[213] R. Sui, P. Charpentier, Synthesis of metal oxide nanostructures by direct sol–gel chemistry in supercritical fluids, Chem. Rev. 112 (6) (2012) 3057–3082, https://doi.org/10.1021/cr2000465.
[214] G.J. Owens, et al., Sol–gel based materials for biomedical applications, Prog. Mater. Sci. 77 (2016) 1–79, https://doi.org/10.1016/j.pmatsci.2015.12.001.
[215] M. Fernández-Fernández, M.Á. Sanromán, D. Moldes, Recent developments and applications of immobilized laccase, Biotechnol. Adv. 31 (8) (2013) 1808–1825, https://doi.org/10.1016/j.biotechadv.2012.02.013.
[216] M. Kubota, K. Yoshimoto, X. Yuan, Y. Nagasaki, Improvement of the thermal stability of streptavidin immobilized on magnetic beads by the construction of a mixed poly (ethylene glycol) tethered-chain layer, Polym. J. 43 (5) (2011) 493–496, https://doi.org/10.1038/pj.2011.5.
[217] E. Magner, Immobilisation of enzymes on mesoporous silicate materials, Chem. Soc. Rev. 42 (15) (2013) 6213–6222, https://doi.org/10.1039/c2cs35450k.
[218] Z.-G. Wang, L.-S. Wan, Z.-M. Liu, X.-J. Huang, Z.-K. Xu, Enzyme immobilization on electrospun polymer nanofibers: an overview, J. Mol. Catal., B Enzym. 56 (4) (2009) 189–195, https://doi.org/10.1016/j.molcatb.2008.05.005.
[219] G. Ren, et al., Electrospun poly (vinyl alcohol)/glucose oxidase biocomposite membranes for biosensor applications, React. Funct. Polym. 66 (12) (2006) 1559–1564, https://doi.org/10.1016/j.reactfunctpolym.2006.05.005.
[220] A.C. Patel, S. Li, J.-M. Yuan, Y. Wei, In situ encapsulation of horseradish peroxidase in electrospun porous silica fibers for potential biosensor applications, Nano Lett. 6 (5) (2006) 1042–1046, https://doi.org/10.1021/nl0604560.
[221] K. Hisamatsu, et al., α-Amylase immobilization capacities of mesoporous silicas with different morphologies and surface properties, J. Porous Mater. 19 (1) (2012) 95–102, https://doi.org/10.1007/s10934-011-9452-2.
[222] H. Ikemoto, Q. Chi, J. Ulstrup, Stability and catalytic kinetics of horseradish peroxidase confined in nanoporous SBA-15, J. Phys. Chem. C 114 (39) (2010) 16174–16180, https://doi.org/10.1021/jp103137e.
[223] L. Cui, J. Wu, H. Ju, Electrochemical sensing of heavy metal ions with inorganic, organic and bio-materials, Biosens. Bioelectron. 63 (2015) 276–286, https://doi.org/10.1016/j.bios.2014.07.052.
[224] A. Popat, S.B. Hartono, F. Stahr, J. Liu, S.Z. Qiao, G.Q.M. Lu, Mesoporous silica nanoparticles for bioadsorption, enzyme immobilisation, and delivery carriers, Nanoscale 3 (7) (2011) 2801–2818, https://doi.org/10.1039/c1nr10224a.
[225] G. Zhou, Y. Chen, S. Yang, Comparative studies on catalytic properties of immobilized Candida rugosa lipase in ordered mesoporous rod-like silica and vesicle-like silica, Microporous Mesoporous Mater. 119 (1–3) (2009) 223–229, https://doi.org/10.1016/j.micromeso.2008.10.013.
[226] J. Lei, et al., Immobilization of enzymes in mesoporous materials: controlling the entrance to nanospace, Microporous Mesoporous Mater. 73 (3) (2004) 121–128, https://doi.org/10.1016/j.micromeso.2004.05.004.
[227] N. Miletić, A. Nastasović, K. Loos, Immobilization of biocatalysts for enzymatic polymerizations: possibilities, advantages, applications, Bioresour. Technol. 115 (2012) 126–135, https://doi.org/10.1016/j.biortech.2011.11.054.
[228] F. Hoffmann, M. Cornelius, J. Morell, M. Fröba, Silica-based mesoporous organic–inorganic hybrid materials, Angew. Chemie Int. Ed. 45 (20) (2006) 3216–3251, https://doi.org/10.1002/anie.200503075.
[229] H. Alhmoud, et al., Porous silicon nanodiscs for targeted drug delivery, Adv. Funct. Mater. 25 (7) (2015) 1137–1145, https://doi.org/10.1002/adfm.201403414.
[230] S.P. Low, N.H. Voelcker, L.T. Canham, K.A. Williams, The biocompatibility of porous silicon in tissues of the eye, Biomaterials 30 (15) (2009) 2873–2880, https://doi.org/10.1016/J.BIOMATERIALS.2009.02.008.
[231] C. RoyChaudhuri, A review on porous silicon based electrochemical biosensors: beyond surface area enhancement factor, Sens. Actuators B Chem. 210 (2015) 310–323, https://doi.org/10.1016/j.snb.2014.12.089.
[232] M. Gimon-Kinsel, V. Jimenez, L. Washmon, K. Balkus Jr., Mesoporous molecular sieve immobilized enzymes, Studies in Surface Science and Catalysis vol. 117, Elsevier, 1998, pp. 373–380, https://doi.org/10.1016/s0167-2991(98)81014-8.
[233] S.B. Sigurdardóttir, et al., Enzyme immobilization on inorganic surfaces for membrane reactor applications: mass transfer challenges, enzyme leakage and reuse of materials, Adv. Synth. Catal. 360 (14) (2018) 2578–2607, https://doi.org/10.1002/adsc.201800307.
[234] S. Arshavsky-Graham, N. Massad-Ivanir, F. Paratore, T. Scheper, M. Bercovici, E. Segal, On chip protein pre-concentration for enhancing the sensitivity of porous silicon biosensors, ACS Sens. 2 (12) (2017) 1767–1773, https://doi.org/10.1021/acssensors.7b00692.
[235] F.A. Harraz, Porous silicon chemical sensors and biosensors: a review, Sens. Actuators B Chem. 202 (2014) 897–912, https://doi.org/10.1016/j.snb.2014.06.048.
[236] M. Manzano, et al., Studies on MCM-41 mesoporous silica for drug delivery: effect of particle morphology and amine functionalization, Chem. Eng. J. 137 (1) (2008) 30–37, https://doi.org/10.1016/j.cej.2007.07.078.
[237] M. Arroyo-Hernandez, R. Martın-Palma, J. Perez-Rigueiro, J. Garcıa-Ruiz, J. Garcıa-Fierro, J. Martínez-Duart, Biofunctionalization of surfaces of nanostructured porous silicon, Mater. Sci. Eng. C 23 (6–8) (2003) 697–701, https://doi.org/10.1016/j.msec.2003.09.159.

[238] D.L. Ramasamy, S. Khan, E. Repo, M. Sillanpää, Synthesis of mesoporous and microporous amine and non-amine functionalized silica gels for the application of rare earth elements (REE) recovery from the waste water-understanding the role of pH, temperature, calcination and mechanism in Light REE and Heavy REE separation, Chem. Eng. J. 322 (2017) 56–65, https://doi.org/10.1016/j.cej.2017.03.152.
[239] F.O. Gaslain, C. Delacôte, A. Walcarius, B. Lebeau, One-step preparation of thiol-modified mesoporous silica spheres with various functionalization levels and different pore structures, J. Solgel Sci. Technol. 49 (1) (2009) 112–124, https://doi.org/10.1007/s10971-008-1845-6.
[240] W. Mullins, B. Averbach, Surface properties of silicon and aluminum oxide powders, Surf. Sci. 206 (1–2) (1988) 41–51, https://doi.org/10.1016/0039-6028(88)90013-1.
[241] M. Waseem, S. Mustafa, A. Naeem, G. Koper, Physiochemical properties of mixed oxides of iron and silicon, J. Non. Solids 356 (50–51) (2010) 2704–2708, https://doi.org/10.1016/j.jnoncrysol.2010.09.055.
[242] L. Tiago, et al., Ion exchange of β-galactosidase: the effect of the immobilization pH on enzyme stability, Process. Biochem. 51 (7) (2016) 875–880, https://doi.org/10.1016/j.procbio.2016.03.014.
[243] J. Gómez, M. Romero, T. Fernández, E. Díez, Immobilization of β-glucosidase in fixed bed reactor and evaluation of the enzymatic activity, Bioprocess Biosyst. Eng. 35 (8) (2012) 1399–1405, https://doi.org/10.1007/s00449-012-0728-y.
[244] L.M. Pandey, S.K. Pattanayek, Hybrid surface from self-assembled layer and its effect on protein adsorption, Appl. Surf. Sci. 257 (10) (2011) 4731–4737, https://doi.org/10.1016/j.apsusc.2010.12.148.
[245] M. Park, S.S. Park, M. Selvaraj, D. Zhao, C.-S. Ha, Hydrophobic mesoporous materials for immobilization of enzymes, Microporous Mesoporous Mater. 124 (1–3) (2009) 76–83, https://doi.org/10.1016/j.micromeso.2009.04.032.
[246] F.P. Mathew, E.C. Alocilja, Porous silicon-based biosensor for pathogen detection, Biosens. Bioelectron. 20 (8) (2005) 1656–1661, https://doi.org/10.1016/j.bios.2004.08.006.
[247] L. Canham, T. Cox, A. Loni, A. Simons, Progress towards silicon optoelectronics using porous silicon technology, Appl. Surf. Sci. 102 (1996) 436–441, https://doi.org/10.1016/0169-4332(96)00094-3.
[248] H. Koyama, M. Araki, Y. Yamamoto, N. Koshida, Visible photoluminescence of porous Si and its related optical properties, J. Appl. Phys. 30 (12S) (1991) 3606 http://iopscience.iop.org/article/10.1143/JJAP.30.3606/pdf.
[249] V.S.-Y. Lin, K. Motesharei, K.-P.S. Dancil, M.J. Sailor, M.R. Ghadiri, A porous silicon-based optical interferometric biosensor, Science 278 (5339) (1997) 840–843, https://doi.org/10.1126/science.278.5339.840.
[250] A. Benilov, I. Gavrilchenko, I. Benilova, V. Skryshevsky, M. Cabrera, Influence of pH solution on photoluminescence of porous silicon, Sens. Actuators A Phys. 137 (2) (2007) 345–349, https://doi.org/10.1016/j.sna.2007.02.032.
[251] A. Cullis, L.T. Canham, P. Calcott, The structural and luminescence properties of porous silicon, J. Appl. Phys. 82 (3) (1998) 909, https://doi.org/10.1063/1.366536.
[252] T. Unagami, Oxidation of porous silicon and properties of its oxide film, J. Appl. Phys. 19 (2) (1980) 231, https://doi.org/10.1143/JJAP.19.231.
[253] D. Bellet, G. Dolino, X-ray diffraction studies of porous silicon, Thin Solid Films 276 (1-2) (1996) 1–6, https://doi.org/10.1016/0040-6090(95)08035-X.
[254] M. Hory, et al., Fourier transform IR monitoring of porous silicon passivation during post-treatments such as anodic oxidation and contact with organic solvents, Thin Solid Films 255 (1-2) (1995) 200–203, https://doi.org/10.1016/0040-6090(94)05654-V.
[255] S.V. Rao, K.W. Anderson, L.G. Bachas, Oriented immobilization of proteins, Microchim. Ichnoanal. Acta 128 (3–4) (1998) 127–143, https://doi.org/10.1007/BF01243043.
[256] W.H. Scouten, J.H. Luong, R.S. Brown, Enzyme or protein immobilization techniques for applications in biosensor design, Trends Biotechnol. 13 (5) (1995) 178–185, https://doi.org/10.1016/S0167-7799(00)88935-0.
[257] Y.-J. Han, J.T. Watson, G.D. Stucky, A. Butler, Catalytic activity of mesoporous silicate-immobilized chloroperoxidase, J. Mol. Catal., B Enzym. 17 (1) (2002) 1–8, https://doi.org/10.1016/s1381-1177(01)00072-8.
[258] H.R. Luckarift, J.C. Spain, R.R. Naik, M.O. Stone, Enzyme immobilization in a biomimetic silica support, Nat. Biotechnol. 22 (2) (2004) 211, https://doi.org/10.1038/nbt931.
[259] C. Mateo, J.M. Palomo, G. Fernandez-Lorente, J.M. Guisan, R. Fernandez-Lafuente, Improvement of enzyme activity, stability and selectivity via immobilization techniques, Enzyme Microb. Technol. 40 (6) (2007) 1451–1463, https://doi.org/10.1016/j.enzmictec.2007.01.018.
[260] A.L. Paiva, V.M. Balcao, F.X. Malcata, Kinetics and mechanisms of reactions catalyzed by immobilized lipases★, Enzyme Microb. Technol. 27 (3–5) (2000) 187–204, https://doi.org/10.1016/S0141-0229(00)00206-4.
[261] A. Amine, H. Mohammadi, I. Bourais, G. Palleschi, Enzyme inhibition-based biosensors for food safety and environmental monitoring, Biosens. Bioelectron. 21 (8) (2006) 1405–1423, https://doi.org/10.1016/j.bios.2005.07.012.
[262] J. Li, S.N. Tan, H. Ge, Silica sol-gel immobilized amperometric biosensor for hydrogen peroxide, Anal. Chim. Acta 335 (1–2) (1996) 137–145, https://doi.org/10.1016/S0003-2670(96)00337-6.
[263] A. Chaubey, B. Malhotra, Mediated biosensors, Biosens. Bioelectron. 17 (6–7) (2002) 441–456, https://doi.org/10.1016/s0956-5663(01)00313-x.
[264] K.A. Kilian, T. Böcking, J.J. Gooding, The importance of surface chemistry in mesoporous materials: lessons from porous silicon biosensors, Chem. Commun. (6) (2009) 630–640, https://doi.org/10.1039/b815449j.
[265] M.-J. Song, D.-H. Yun, J.-H. Jin, N.-K. Min, S.-I. Hong, Comparison of effective working electrode areas on planar and porous silicon substrates for cholesterol biosensor, J. Appl. Phys. 45 (9R) (2006) 7197, https://doi.org/10.1143/jjap.45.7197.
[266] L.A. DeLouise, P.M. Kou, B.L. Miller, Cross-correlation of optical microcavity biosensor response with immobilized enzyme activity. Insights into biosensor sensitivity, Anal. Chem. 77 (10) (2005) 3222–3230, https://doi.org/10.1021/ac048144+.
[267] L. Soleymani, F. Li, Mechanistic challenges and advantages of biosensor miniaturization into the nanoscale, ACS Sens. 2 (4) (2017) 458–467, https://doi.org/10.1021/acssensors.7b00069.
[268] S. Setzu, M. Monduzzi, G. Mula, A. Salis, Porous silicon-based electrochemical biosensors, Biosensors-Emerging Materials and Applications, IntechOpen, 2011.
[269] G. Rong, J.D. Ryckman, R.L. Mernaugh, S.M. Weiss, Label-free porous silicon membrane waveguide for DNA sensing, Appl. Phys. Lett. 93 (16) (2008) 161109, https://doi.org/10.1063/1.3005620.
[270] G. Korotcenkov, B. Cho, Silicon porosification: state of the art, Crit. Rev. Solid State Mater. Sci. 35 (3) (2010) 153–260, https://doi.org/10.1080/10408436.2010.495446.
[271] P. Kumar, et al., Hybrid porous thin films: opportunities and challenges for sensing applications, Biosensors and Bioelectronics, (2018), https://doi.org/10.1016/J.BIOS.2018.01.006.
[272] A. Jane, R. Dronov, A. Hodges, N.H. Voelcker, Porous silicon biosensors on the advance, Trends Biotechnol. 27 (4) (2009) 230–239, https://doi.org/10.1016/j.tibtech.2008.12.004.
[273] K. Hernandez, R. Fernandez-Lafuente, Control of protein immobilization: coupling immobilization and site-directed mutagenesis to improve biocatalyst or biosensor performance, Enzyme Microb. Technol. 48 (2) (2011) 107–122, https://doi.org/10.1016/j.enzmictec.2010.10.003.