

Immobilization of Tyrosinase and Its Application

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Article history

Received

26 October 2022

Revised

23 February 2023

Accepted

23 February 2023

Published online

25 May 2023

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Abstract

Immobilized enzymes are more robust and resistant to environmental changes than free enzymes in solution. More crucially, the immobilized enzyme systems' heterogeneity enables facile recovery of enzymes and products, multiple reuses, continuous enzymatic processes, quick reaction termination, and a more comprehensive range of bioreactor designs. This paper examines recent findings on enzyme immobilization using diverse approaches for various uses. The information gathered from the reactions catalyzed by the encapsulated tyrosinase provided a good view of hetero-biocatalysts in the phenol biosensor industries. This review proposes an effective method for immobilizing tyrosinase biomolecules into a silica aerogel matrix. Silica matrix has been utilized to encapsulate a wide range of biomolecules, mainly in sol-gel composites. We also discovered that silica aerogel synthesized from sol-gel method retains all the immobilized enzyme activity. The use of a silica matrix for enzyme immobilization, in conjunction with a moderate immobilization method, results in the successful retention of enzyme activity. Future studies should explore practical encapsulating approaches and inventively modified supports to enhance the commercialization of immobilized enzymes and offer fresh perspectives to the industrial sector.

Keywords Enzyme immobilization, sol-gel, tyrosinase, silica matrix

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1.0 INTRODUCTION

Over the last few decades, biochemical and biophysical research has improved enzyme stability and activity through enzyme immobilization. Immobilized enzymes have significantly enhanced industrial processes' technical performance, productivity, and economic efficiency. Recent improvements and unique tactics in enzyme immobilization methods are briefly reviewed in this paper. Thus, it provides essential information for selecting the best immobilization technique and support material to improve the stability and activity of the enzyme.

In enhancing the stability and reusability of the enzyme, enzymes are often immobilized by physical or chemical means to the surface of insoluble supports. An immobilized enzyme can be defined as an enzyme that is not freely soluble and whose movement in space is entirely or partially restricted to a small region [1]. It is also known as imprisoning an enzyme molecule in a distinct phase that allows for exchange but is separated from the bulk phase in which substrate effectors or inhibitor molecules are dispersed [2]. The immobilized enzyme is usually insoluble in water, and the support used to immobilize the enzyme is composed of high molecular weight, the hydrophilic polymer [3].

Immobilization of enzymes on a solid surface places them in a more natural environment and, in many cases, allows them to function more efficiently [4]. When the soluble enzyme is used to catalyze a reaction, the reaction can only be terminated by deactivating the enzyme or changing the environment. The extent of the response can be varied by adjusting the residence time of the reactants or by removing the immobilized enzyme from the reaction solution [5]. Immobilized enzymes also retain their catalytic properties for a more extended period, thus making their use even more economical. Another benefit of immobilized enzymes is inhibiting enzyme activity, which minimizes excess product [6]. Immobilized enzymes can also be used for multi-enzyme systems where several enzymes are placed in the same support, thus catalyzing a sequence reaction. Using immobilized enzymes rather than free enzymes is advantageous [7]. The following are the reasons for the immobilization of enzymes:

- To improve the stability of the enzyme in adverse reaction conditions
- To enhance the strength of the enzyme in the presence of organic solvents
- To separate the enzyme from the product stream
- To allow continuous flow operations and repetitive usage

The application of immobilized enzymes in analytical chemistry is not a new concept. Immobilizations of biological compounds into inorganic support are usually applied in various fields, such as biosensing [8], affinity chromatography [9] and enzyme reactors [10]. The importance of immobilized enzymes as analytical reagents in clinical chemistry [11], food analysis [12], and the pharmaceutical industry [2] has been steadily increasing. To simplify the enzymatic measurement of glucose, the principle of the litmus paper used for pH measurement has been implemented [13]. The first 'enzyme test strip' was obtained by impregnating filter paper with glucose-converting enzymes. It can be regarded as the predecessor of optoelectronic biosensors, which initiated the development and application of 'dry chemistry'.

The appropriate matrix or support for the immobilization of enzymes is chosen based on several different properties which affect the production process [3]. One of the properties is that the materials need a high surface area, particularly up to 100 m²/g, for high enzyme loadings and porosity to access the substrate. The immobilization matrix must also be resistant to chemical degradations and mechanical stability. Microbial resistance of the matrix is also an important property that needs to be considered since a significant concern to any immobilized enzyme process is the presence of microbes. Furthermore, the durability of the carrier is often determined by its resistance to microbial degradation [14]. The overview of the support materials, immobilization methods, and their applications is summarized in Table 1.

Table 1: Types of enzyme immobilization and example of their applications.

Technique	Support Material	Enzyme	Application	Reference
Adsorption	Polydopamine coating	Lipase	Wastewater treatment	[15]
Covalent bonding	Pinewood nanobiochar	Crude laccase	Wastewater treatment	[16]
Crosslinking	Polyhydroxybutyrate	Lipase	Acylglycerol production	[17]
Entrapment	Poly(ethylene glycol) diacrylate hydrogel	β -Galactosidase	Lactose-free dairy product	[18]
Encapsulation	Silica Aerogel	Tyrosinase	Phenol removal	[19]

Tyrosinase is one of the enzymes being extensively studied for enzyme-based biosensors. As a biocatalyst, it is excellent for oxidizing many phenolic compounds of interest in foods, medicine, pharmacy, and other disciplines. Tyrosinase is a family of phenoloxidases that catalyzes the conversion of several phenolic and non-phenolic aromatic chemicals. The use of free and immobilized tyrosinase in many practical fields is well-covered in the literature. Nevertheless, there is still a need for a thorough study of the core components of immobilized tyrosinase. As such, this paper aims to provide an overview of the current knowledge in this subject area.

2.0 IMMOBILIZED TYROSINASE FOR THE REMOVAL OF PHENOLIC POLLUTANTS

Aromatic compounds, including phenols and aromatic amines, constitute one of the major pollutants in many countries [20]. Some industrial areas that discharge phenol include oil refineries, coke and coal conversion plants, plastics and petrochemical companies, dyes, textiles, and paper industries. Almost all phenols are toxic. Furthermore, phenol and many of its derivatives are considered hazardous pollutants. Due to its toxicity and hazard, a phenol concentration greater than 50 ppb harms some aquatic species and ingesting 1 g phenol may be fatal to humans [21].

Consequently, this may affect the ecosystem of water sources where phenols are discharged. Thus, the removal of phenol in water is essential. Table 2 summarizes various methods used to remove the phenol from wastewater.

Table 2 Overview of methods used for phenol removal.

Material	Technique	Efficiency	Reference
Carbon nanosphere-based surface molecularly imprinted polymer	Adsorption	Adsorption capacity 85.72 mg/g	[22]
Fly ash-loaded titanium TiO ₂ -Fe ³⁺ particles	Ultrasound-assisted electrochemical treatment	High removal at ultrasonic frequency 45 kHz, power 200 W, the current 1.2 A, pH 5	[23]
Magnetic palm kernel biochar	Adsorption	Adsorption capacity 10 mg/g	[24]
Room temperature ionic liquid (RTIL) encapsulated polymer microcapsules (MC)	Extraction with chemical reaction	Adsorption capacity 9.07 mg/g	[25]
<i>M. peregrina</i> seed extract	Electrocoagulation	Optimum removal capacities achieved with coagulant dose 0.1-0.3 ml/L, pH 5	[26]

Conventional methods for removing phenol and aromatic compounds from industrial waste are solvent extractions, microbial degradation, adsorption of activated carbon and chemical oxidation [27, 28]. Although these methods are practical and valuable for removing phenol in water, they suffer from serious drawbacks such as high cost, incompleteness of purification, formation of hazardous by-products and applicability to only a limited phenol concentration range. Many researchers have proposed enzymatic treatment as a convenient method for removing phenol [22, 23]. Enzymes are highly selective and can effectively treat phenol even in dilute wastes [29]. Moreover, enzymes operate over a broad aromatic concentration range and require low retention times concerning other treatment methods [30].

Tyrosinase was demonstrated to remove phenols and aromatic amines from phenolic industrial effluents [31]. Figure 1 shows the Field Emission Scanning Electron Microscope (FESEM) image of tyrosinase.

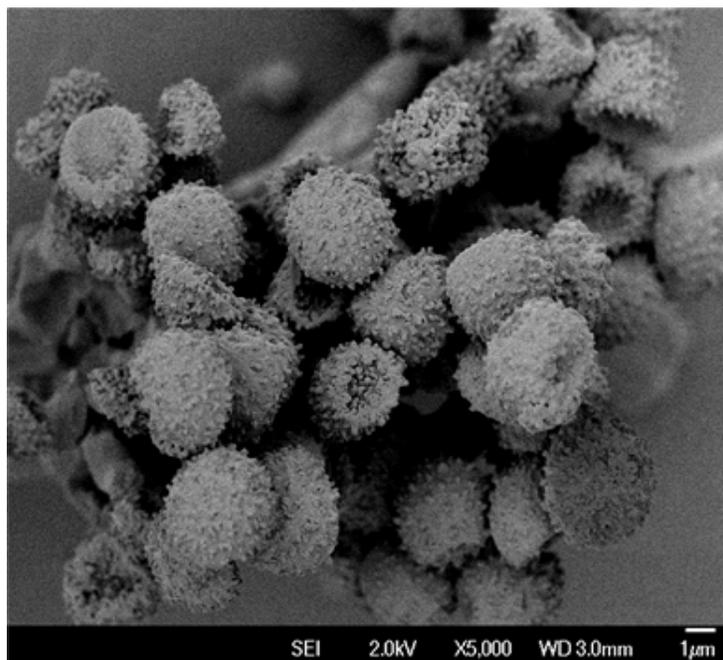


Figure 1 FESEM micrograph showing the surface morphology of free tyrosinase.

Atlow and his group [32] reported successfully applying soluble tyrosinase to "cleanse" pollutants. In the presence of a proton, the oxidation process generates phenoxy radicals that diffuse from the active centre into the solution to react with

phenol and form substances much less water-soluble, known as quinones. These quinones are reactive and undergo non-enzymatic oligomerization reactions that yield a high molecular weight of insoluble polyphenolics [33]. Consequently, it inactivated the tyrosinase for further development reaction. The oxidation of phenol catalyzed by tyrosinase is shown in Figure 2.

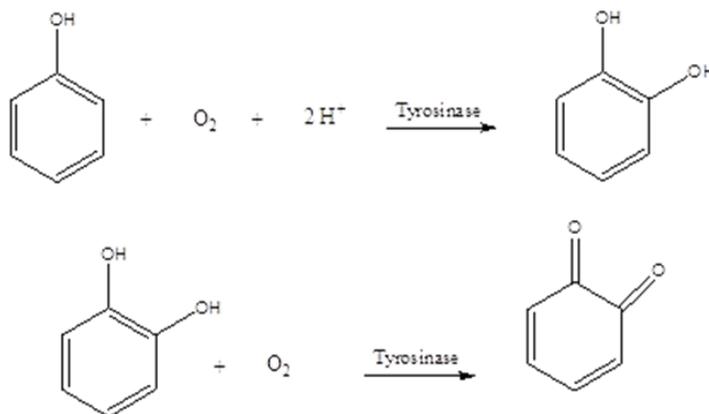


Figure 2 Oxidation of phenol catalyzed by tyrosinase.

Like most other enzymes, tyrosinase is expensive; thus, using the soluble enzyme is impractical [31]. Therefore, tyrosinase encapsulation efficiently exploits its catalytic properties and improves cost-effectiveness [13]. Table 3 summarizes various supports used to immobilize tyrosinase for phenol detection.

Table 3 Immobilization of tyrosinase on various materials.

Material	Technique	Purpose	Reference
Al ₂ O ₃ membrane	Adsorption	Detection of phenol in wastewater	[34]
Al ₂ O ₃ sol-gel	Adsorption	Detection of bisphenol A in food or drinking packages	[35]
Carbon nanotube (SWCNT) and polyaniline (PANI) on the surface of a vitreous carbon electrode (GCE)	Incorporation	Detection of catechol and hydroxycinnamic acid in food	[36]
AuNPs-modified screen-printed electrode (AuNPs-SPCE)	Cross-linking with glutaraldehyde	Detection of caffeic acid in beers	[37]
Au nanoparticles and polymer-modified glassy carbon	Polymerization nanocomposite	Determining tyramine in fermented food and beverages	[38]
Carbon nanofiber modified with gold nanoparticles (CNF-GNP/SPE)	Casting technique followed by cross-linking with glutaraldehyde	Detection of ferulic acid in cosmetics	[39]
3,4-ethylene dioxythiophene) - tyrosinase/Sonogel-carbon (PEDOT-Tyr/SNGC)	Sinusoidal electrodeposition (SC)	Biosensor and analyze beer and wine products	[40]
Polyhydroxyalkanoate (PHA) biopolymer beads (PHA-BmTyr)	Plasmid construction	Detection of bisphenol in wastewater	[41]

The improved stability of the encapsulated enzyme allows it to be highly reusable. Several techniques based on immobilized tyrosinase have been developed to treat phenolic industrial wastewater [42]. The best and most practical support can stabilize the enzyme activity and increase its half-life. The enzyme's attachment method should not disturb the active enzyme centre. The immobilization process must be cost-effective, and only small amounts of enzymes need to be used [43].

If these requirements are met, effectively immobilized tyrosinase for phenol degradation may be achieved [8, 44]. Aside from using a sophisticated biocatalytic system, significant efforts are being made to widen the spectrum of biosensors through the immobilization of tyrosinase in silica host matrixes.

3.0 IMMOBILIZATION OF TYROSINASE BY SOL-GEL METHOD

In the last decade, a new approach has been developed to immobilize enzymes using silica-based inorganic polymers. This method was pioneered by Avnir and his co-workers [45] based on the so-called sol-gel process. Mild processing conditions, excellent thermostability and chemical inertness of the support are the significant characteristics of this method. Silica host matrixes made by the sol-gel process have emerged as a promising platform for immobilizing enzymes [7].

Silica has been extensively used as an enzyme carrier [4]. One reason is its high mechanical strength at a wide range of operating pressures, as proven by its use in High-Performance Liquid Chromatography (HPLC) [46]. Additionally, silica has relatively higher thermal and chemical stabilities and is resistant to microbial degradation. Since then, various biologically active species of interest, such as enzymes, proteins, antibodies, viruses, and bacteria, have been immobilized via a sol-gel process in different silica supports [47, 48]. Besides being inexpensive, silica matrix has been considered a potential support material in enzyme immobilization for phenol removal because of its chemical inertness, high mechanical strength, hydrophilicity, biocompatibility, and biodegradability [42, 49]. It can also be prepared under ambient conditions and exhibit tunable porosity, high thermal stability and minor swelling in both aqueous and non-aqueous solutions [3, 7].

The numerous reports on such stabilization described mainly success rather than failure. For instance, Seetharam and his teams [6] suggested using immobilized tyrosinase for phenol degradation. Tyrosinase that immobilized on calcium aluminosilicate (CaA), and sodium aluminosilicate (NaA), successfully removed between 15% and 60% of the phenol from the solution. It depended upon the initial phenol concentration, enzyme loading, pH, and reaction duration. It could be reused repeatedly without any decrease in performance. In addition, it is advantageous to use porous support such as aerogel to spread the enzyme on a large surface area [4]. The advantages of their usage in enzyme immobilization include the following:

- The high surface area of the silica matrix provides the possibility of high enzyme loadings in the matrix.
- The silica matrix consists of surface hydroxyl groups that can be readily attached by enzymes.
- The open-pore morphology of the silica matrix allows substrates to move into the interior regions of the particle quickly.
- Solvents used in the processing of silica materials are environmentally benign, thus avoiding the denaturation of the enzyme.

Numerous techniques such as physical adsorption, covalent attachment, entrapment and encapsulation in polymer and inorganic matrixes have been explored over the years to achieve a high-yield, reproducible, and preserve the biological molecules' activity [9, 10, 29]. Enzymes find a more stable environment upon encapsulation in a silica host because the polymeric framework grows around the biomolecules, creating a cage, thus protecting the enzyme from aggregation and unfolding or microbial attack [50]. Encapsulations protect the enzyme against deterioration by the hydrophilic solvent if the proper gel is selected [51]. Therefore, the encapsulated biomolecules often retain sufficient activity and functionality, presumably because of adequate retention of their native state conformations. Moreover, the matrix pores allow the diffusion of reactant molecules and their reaction with the encapsulated biomolecules. Eventually, the encapsulated enzyme can even improve the activity and storage stability of the enzymes and will be easier to be used because they can quickly be recovered and washed [7].

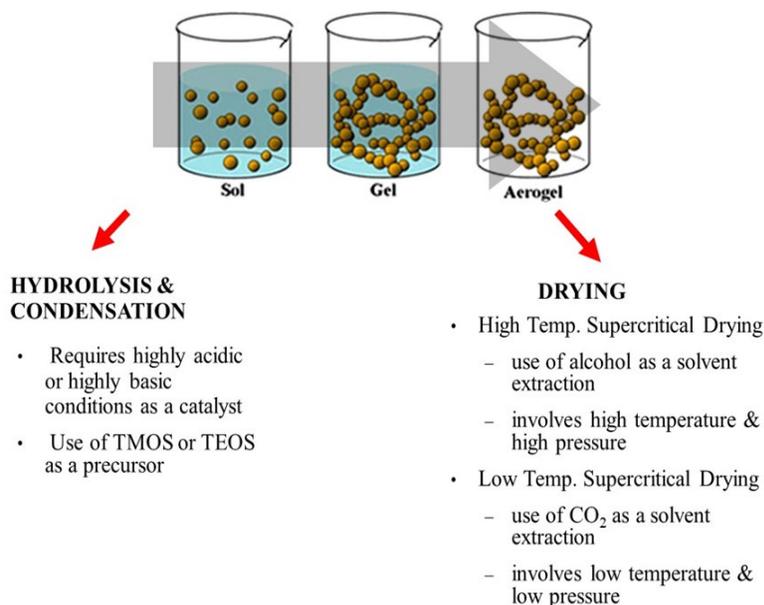


Figure 3 The standard sol-gel technique in the preparation of silica aerogel.

The immobilization of enzymes in the inorganic porous network can be developed using the sol-gel method. Figure 3 illustrates the standard sol-gel technique in the preparation of silica aerogel. Inorganic polymeric networks can be created even in settings with a low ambient temperature, which is advantageous for biocatalyst entrapment. Enzymes remain catalytically active, according to advancements in the enzyme encapsulation method. There are various appealing benefits of sol-gel encapsulation. The matrix's hydrophilicity can be easily modified. Because of the inorganic matrix's excellent mechanical and chemical durability, the enzyme can be physically enclosed in a rigid glass framework [52]. The inorganic matrix's fixed structure stabilizes enzyme interactions and prevents leaching issues. The denaturing phenomena are diminished due to biomolecules' high degree of stiffness. In addition, the surface area of support synthesized from the sol-gel method is adjustable and capable of withstanding a wide temperature range [19]. Thus, encapsulated enzymes are more resistant to thermal and chemical denaturation, which increases enzyme storage and operational stability.

However, the sol-gel procedure has a drawback in that the capillary tensions during drying might significantly change the gel's texture [53]. A protein chain folded over itself, making it susceptible to drying conditions. The conventional sol-gel technique has considerably used the silica alkoxide precursor. However, the production of alcohol as a by-product from tetramethoxysilane (TMOS) or tetraethoxysilane (TEOS) as the starting material can harm the activity of proteins. Therefore, it is crucial to design an alcohol-free sol-gel technique by encapsulating the biological components in a transparent matrix. The particular encapsulating method of enzyme on silica matrix with various drying procedures must be highlighted to produce a more effective biocatalyst with increased activity, high storage stability, and protection against degradation by the hydrophilic solvent.

4.0 TYROSINASE ENCAPSULATED SILICA AEROGEL

Tyrosinase encapsulated silica aerogel (TESA) was successfully prepared via an alcohol-free aqueous colloidal sol-gel route by Sani et al. [19]. The encapsulation process was carried out at room temperature and neutral pH to minimize enzymes' denaturation. Sodium silicate from rice husk ash (RHA), an agricultural waste, was used as a silica source since RHA contained much amorphous silica (more than 95%). TESA and silica aerogel was prepared by drying the samples via ambient conditions. Figure 4 illustrates the encapsulation of tyrosinase in silica aerogel networks.

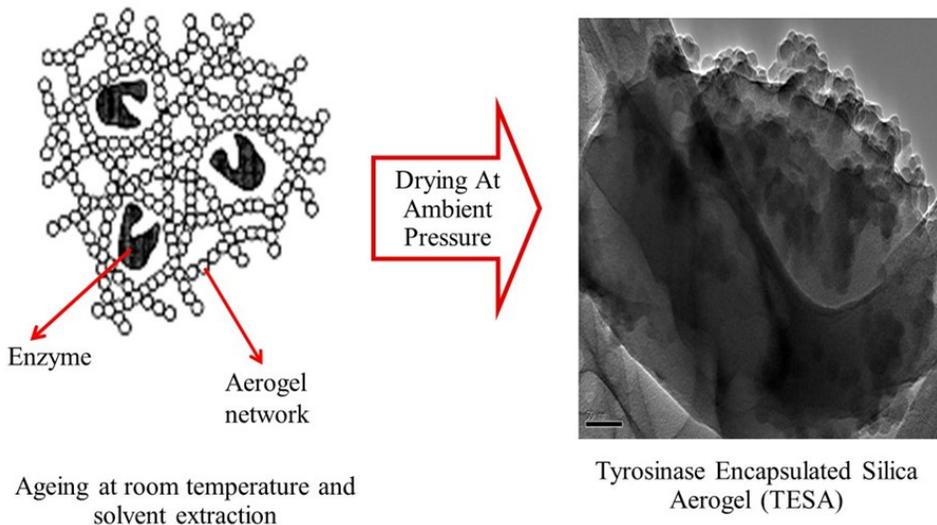


Figure 4 Encapsulation of tyrosinase in silica aerogel networks.

The morphological studies proved the presence of tyrosinase molecules in the spherical particle networks of silica aerogel, thus indicating the encapsulation process's achievement. As a result, it verifies the encapsulation theory proposed in this study, in which the silica aerogel formed a network of nanoparticles and a cage around the tyrosinase molecules.

The efficiency of TESA in the removal of phenol is remarkable. TESA removed phenol up to 90%, 10% lower than free tyrosinase after 3 hours of contact time. The successful degradation of phenol at pH 7 using TESA with 10.00 mg/mL of enzyme loading, aged for 2 days. According to the reports, encapsulated tyrosinase is remarkably stable and reusable since it can remove phenol up to ten times with little to no degradation. After five reuses, the elimination of the phenol profile consistently levelled out due to product inhibition. This study has shown that a possible biosensor based on tyrosinase-encapsulated silica aerogel was created. A wide variety of phenol concentrations can be detected using TESA. It divides the contaminated water into two layers to detect the presence of phenol. The second colorless layer is made up of phenol-free water, while the first brownish layer is made up of phenolic chemicals. The phenolic chemicals can be eliminated with a simple filtration method. With a better understanding of the fundamental reaction, the degree of response can be increased, leading to a potential biosensor with enhanced thermal stability and improved enzymatic performance.

5.0 CONCLUSION

The information gathered from the reactions catalyzed by the encapsulated tyrosinase provided a good view of hetero biocatalysts in the phenol biosensor industries. This review proposes to develop an effective method for immobilizing tyrosinase biomolecules into a silica aerogel matrix. Unrevealing the interactions of the encapsulation of enzymes in the silica aerogel matrix will better understand various chemical and biochemical processes that occur when different synthesis conditions are applied. The fundamental understanding of the enzyme-silica support interactions can help improve the fabrication of enzyme-encapsulated silica aerogel as a potential biosensor with enhanced thermal stability and enzymatic performance. Hence, this review enables new materials for biosensors to be developed. A potential biosensor with excellent thermal stability and enzymatic performance can be produced by increasing the degree of response with a better understanding of the underlying process. Understanding the interactions between the encapsulated enzymes and the silica aerogel matrix would help researchers understand the chemical and biological processes that occur under various synthesis circumstances. A possible biosensor with improved thermal stability and enzymatic performance can be made using enzyme-encapsulated silica aerogel with the help of a basic understanding of the interactions between enzymes and silicon supports.

Acknowledgement

The authors thank the Ministry of Education, Malaysia, and Universiti Teknologi Malaysia (UTM) for financially supporting this research work under the Fundamental Research Grant Scheme (FRGS, Vot No: 4F514).

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