Application Progress of Microbial Immobilization Technology Based on Biomass Materials

Zeyu Zhang, Zheng Fan,* Guoliang Zhang, Lei Qin, and Jie Fang

In recent years, microbial degradation technology has shown broad potential in the fields of agriculture, industry, and environmental protection. However, in practical applications the technology still encounters many problems, such as low bacterial survivability during dynamic operations, the need to remove bacterial liquid, and low tolerance in high-toxic environments, among other issues. Immobilization technology has been developed to overcome such limitations. Microbial strains have been prepared for a specific range of activities utilizing self-fixation or exosome fixation. Immobilization can significantly improve strain density, toxicity tolerance, and bacterial liquid removal. This review first presents the advantages and disadvantages of the current microbial immobilization technologies and then summarizes the properties and characteristics of various carrier materials. The review focuses on how biomass-derived materials have been used as the carriers in new microbial immobilization technologies. The excellent biocompatibility, unique physical structure, and diversified modification methods of biomass-derived materials have shown excellent prospects in the field of microbial immobilization. Finally, microbial immobilization technologies' potential applications in agriculture, industry, and environmental applications are considered.

Keywords: Biodegradation; Immobilization technology; Biomass material; Biocompatibility

*Center for Membrane and Water Science &Technology, Institute of Oceanic and Environmental Chemical Engineering, State Key Lab Breeding Base of Green Chemical Synthesis Technology, Zhejiang University of Technology, Hangzhou 310014, China; *Corresponding author: fanzh@zjut.edu.cn*

INTRODUCTION

Biodegradation can be defined as a green process that uses microorganisms to adsorb and digest pollutants and finally enzymatically degrade them. Biodegradation exhibits many advantages compared with the high-cost physical degradation (Ebrahimpour *et al*. 2017). Physical degradation has limited purification capacity. On the other hand chemical degradation has high energy consumption and is prone to secondary pollution (Ding *et al*. 2000). By contrast, biodegradation has a low operating cost, a thorough purification effect, environmental friendliness, and high adaptability to complex environments. However, microbial biodegradation is still hindered by many factors, including low bacterial survivability, the problematic separation of bacterial cultures, and the reduced microbial activity in highly toxic environments (He *et al*. 2018; Wu *et al*. 2019). The development of immobilization has become an important research topic in pollutant treatment (Gao *et al*. 2012). Immobilization technology can effectively offer the solution to overcome these problems by encapsulating and protecting bacteria from the external polluted conditions (Roy *et al*. 2018).

With the advancement of microbial immobilization technologies, suitable materials and fixation selections have been the most critical factors in the immobilization process

(Girigan and Kumar 2019). Various materials and fixation procedures will have distinctive influences upon activity, tolerance, and adhesion of bacteria. In theory, a sound immobilization system can offer a rapid exchange, high adhesion, and efficient toxic substance exclusion. The excellent carrier material needs to be non-toxic and harmless to microbial cells during the fixing process, and the fixing process is required not to hinder the microbial activities. The encapsulation materials are required to be economically sustainable, abundant, and environmentally friendly. Biomass-derived materials can have all of required characteristics listed above, including widely available raw material in nature, low cost, wide variety, high biocompatibility, and strong plasticity. Biomass can promote the further advancement of carrier materials in microbial immobilization technology. Based on the recent biodegradation research of the authors' research group (Fan *et al*. 2018; Li *et al*. 2018); this review considers the characteristics of pollutant degradation and different materials and fixation procedures to analyze the selection of materials and methods in the immobilization technology. In particular, the selection and modification of biomass materials are scrutinized in-depth, offering reference points for research in immobilization technology.

Classification of The Microbial Immobilization Technologies

Methods to immobilize bacteria can be placed into different categories: self-immobilization, which is known as the self-attachment of microorganisms to the carrier, or self-aggregation, which is known as the flocculation, or foreign aid immobilization, which involves physically adding flocculants, embedding agents, or changing the external conditions of microorganisms, among others. Several fixation methods can generally be divided into physical and chemical methods (Bai *et al*. 2018; Ke *et al*. 2018). The physical method requires the retention of bacteria on the surface of the support material or the bacterial encapsulation inside the fixation material *via* physical forces, such as van der Waals forces and electrostatic interactions. The actual practices require two-step processes: embedding fixation and adsorption fixation (Fig. 1). The chemical grafting method is known to mainly produce chemical bonds *via* chemical grafting to covalently bond materials and bacteria; a further step is done *via* a fixing process, such as an aggregation-crosslinking method.

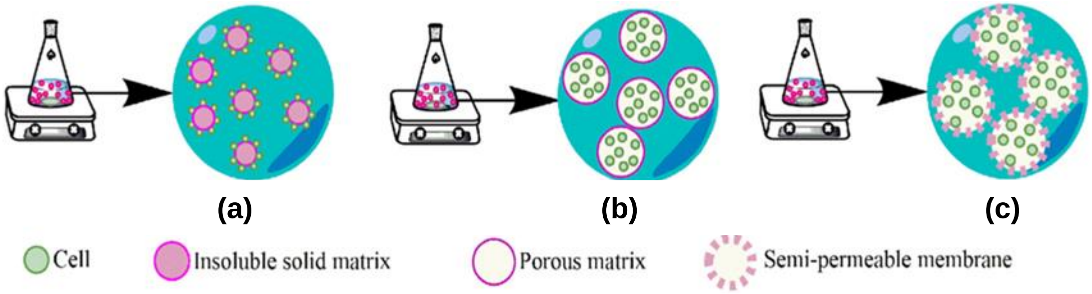

Fig. 1. Schematic diagram showing immobilization technology of adsorption method, embedding method, and encapsulation method. Reprinted from *Science of The Total Environment*, Vol 730, Wang *et al.,* Copyright (2020), with permission from Elsevier

Embedding method

Embedding has been well-established and is commonly used to immobilize bacteria. The embedded bacteria can be protected from direct contact with unfavorable external factors. This beneficial embedding method can also assist the growth, reproduction, and diffusion of the bacteria's metabolic substances, extending the lifespan of immobilized bacteria with the improved activity. Various ways of embedding bacteria can be classified into two primary methods: traditional embedding and microcapsule embedding. Among these methods, the gel method is an exciting method that has attracted researcher attention compared to the traditional embedding method. For example, Zhang *et al.* (2020) utilized the bamboo charcoal-sodium alginate as the support material to embed *Bacillus cereus* WL08 using the gel method to degrade dimethomorph. This immobilization technology was reported to enhance the tolerance and stability of the strain WL08 significantly. Under the optimal conditions of pH 7.0 and temperature of 30 °C, the degradation rate of 50 mg/L dimethomorph by immobilized WL08 within 72 h was found to be 96.88%, while the non-immobilized bacteria was found only to be able to degrade 66.95%.

Furthermore, Qiao *et al*. (2020) proposed the method to fix four strains inside magnetically floating biochar gel beads to form a well-protected microbial consortium. This immobilized microbial consortium was shown to possess better degradation performances against high molecular weight polycyclic aromatic hydrocarbons than a single bacterial strain. The tolerances of pH, temperature, and salinity of this immobilized bacterial consortium were found to be much higher than non-immobilized bacteria. This microbial consortium was found to possess better degradation operations against the high molecular weight polycyclic aromatic hydrocarbons than a single bacterial strain without immobilization. Another advantage was that these synthesized magnetic-floating biochar gel beads could float on seawater's surface. Thus, they were easy to be re-collected using strong magnets. Embedding and immobilized bacteria have been shown to provide continuous and stable processing capability in various environments.

Another interesting point is that the immobilized bacteria can be processed together with other materials such as chemical reagents. For example, Shi *et al*. (2020) proposed embedding enterobacteria inside sodium alginate beads that were crosslinked with calcium hydroxide and ferric chloride. The microbial beads were found to be capable of removing hexavalent chromium and trivalent arsenic simultaneously. The combination of immobilized strains and chemical reagents was demonstrated to remove heavy metals more effectively than non-immobilized bacteria. Apart from common materials such as sodium alginate, Wang *et al*. (2020b) proposed the utilization of polyvinyl alcohol/chitosan gel beads to immobilize anaerobic bacteria. The system was shown to improve the retention capacity of anaerobic microbial biomass effectively. From the above examples, the materials and methods of embedding and the pretreatment of bacteria have been shown to have a significant influence on the final characteristics of immobilized bacteria and pollutant degradation.

The microencapsulation method is another type of embedding method, different from the traditional embedding process, and it exhibits more advantages. Recently, the microencapsulation methods have received much attention from researchers. Microencapsulation is the complete encapsulation of dispersed solid, liquid, or gaseous substances in a semi-permeable membrane to form fine particles. The fundamental principle is to form a polyelectrolyte complex. Two polyelectrolytes with different active groups are dissolved in two mutually incompatible solvents. Mixing two solutions allows a composite film to form at the interface of the two solutions. Compared with traditional embedding methods, the liquid environment of a microcapsule is more suitable for bacteria to absorb nutrients and release metabolic waste. Due to the mild reaction conditions, the loss of biologically active substances or microbial activities in the microcapsules during the preparation process is minimal. Ding (2008) designed a sodium alginate-chitosan-activated carbon microcapsules system, which possesses a higher bacterial load and less loss during the degradation process. The encapsulated bacteria were found to have better performance in degrading phenolic substances, with a removal rate of 91.7% compared to the non-encapsulated bacteria. In another study, Chen *et al*. (2006) applied sodium cellulose sulfate to immobilize *Klebsiella* into microencapsulation to produce 1,3-propanediol. The reuse rate of encapsulated bacteria was found to increase; the fermentation time was found to be lower; and the production efficiency was found to be improved. These advantageous characteristics benefit the continuous operation to produce and solve the problem of subsequent separation. Compared with traditional embedding, microencapsulation can resolve the substrate inhibition problem during the microbial fermentation process.

Aggregation-crosslinking

The aggregation-crosslinking method is a chemical fixation method mainly composed of two steps: aggregation and crosslinking. Firstly, bacteria aggregate with the same strains or other bacteria *via* physical and chemical means. The common method involves centrifugal aggregation or flocculation aggregation. After aggregation, a multifunctional group crosslinking agent is added to mix with aggregated bacteria. The crosslinking agent can covalently bond with the groups on the bacterial surfaces, such as amino and carboxyl groups. The structures are reinforced with further crosslinking among these bacteria. After detoxification and activation, aggregated bacteria can be obtained. A suitable crosslinking agent is a crucial step for successful immobilization, improving the efficacy of bacterial processes. The polymer matrix's crosslinking can help bacteria successfully attach and prevent their loss (Sheldon and van Pelt 2013). In the degradation catalyzed by bacteria, choosing an appropriate crosslinking agent will preactivate the polymer to successfully connect the enzyme molecule or a given microbial cell (Barbosa *et al*. 2015). The formation of durable bonds promotes the microbial attachment, demonstrating improved stability, activity, and resistance compared to thermal and chemical degradation methods. Facilitating the use of microbial encapsulation allows the bioremediation to remove toxic compounds such as nitriles. Glutaraldehyde is another crosslinking agent, which has been widely used in many immobilization studies (Barbosa *et al*. 2015). Glutaraldehyde is a functional reagent due to two aldehyde groups, which can be used to bond both bacteria and carriers. For example, Ortega *et al*. (2009) used glutaraldehyde as a crosslinking agent to immobilize *Neutrase* on alginate beads. It was found that the thermal stability and pollution resistance of *Neutrase* have been enhanced. Singh *et al*. (2020) proposed the immobilization of pyridine bacteria and *Rhodococcus* on chitosan using the crosslinking technology promoted by N,N'-methylenebisacrylamide. After immobilization, the bacterial activity was significantly improved even at 4 °C for 30 days, and the activity was found to reach 80%.

Selection and Characteristics of Carrier Materials in the Microbial Immobilization Technology

Choosing suitable carrier materials is an essential factor for achieving successful microbial immobilization technology. The most used materials are known to be divided into the inorganic, synthetic polymers and biomass-derived materials. The materials can be used to encapsulate bacteria via simple crosslinking. Alternatively, the materials can be blended with bacteria; then the mixtures can be crosslinked to immobilize microbial cells inside the gel matrix.

Inorganic materials

The inorganic carrier materials exhibit advantages, such as high mechanical strength, high-temperature resistance, abrasion resistance, non-toxicity to microorganisms, and no secondary pollution. Some common inorganic carriers include ceramics, functional glass, activated carbon, and diatomaceous earth. Chen *et al*. (2015) modified bamboo charcoal as a carrier to immobilize *Acinetobacter venetianus*, which was used to remove diesel. These immobilized bacteria were reported to remove 86.35% of diesel, and the removal rate was significantly boosted in comparison non-immobilized bacteria. Bamboo charcoal is known to act as an excellent carrier to load strains, and it can also adsorb diesel to enhance the removal efficiency. This study also demonstrated that adsorption is the main driving mechanism to immobilize microbial cells into the inorganic material carriers. However, the binding force was reported to vary between different carriers and bacteria. This problem can cause the fall-off of microbial cells.

Synthetic polymer materials

Synthetic materials have the advantage that their pore size, specific surface area, and mass transfer performance can be controlled artificially, so that they can have a better match with microorganisms. Therefore, synthetic polymer materials have been widely used as carriers to immobilize microbial cells. The common synthetic polymer-carriers are plastic, rubber, fibre, and foam. Lobakova *et al*. (2016) designed a new biological hybrid material (BHM), which is based on methyl methacrylate-acrylonitrile (MA) to immobilize bacteria. The specific method is to add phytoplankton biomass and derived biomass cell structure carrier materials to the polymer fibers. The surface of the polymer is porous and has protrusions due to the incorporated plant structure, so the specific surface area is correspondingly increased. Anchorages are available to attach and fix microorganisms. The fixed bacteria on the BHM can also float on the water surface. In actual practices, the immobilized bacteria can degrade up to 93% to 97% of the n-alkanes in the artificial seawater within 25 days, achieving a high treatment effect. However, synthetic polymer materials still possess limitations such as temperature resistance and strength, recycling rate problems, and recycling feasibility.

Biomass material

Materials originating from organisms such as animals, plants, and microorganisms are referred to as biomass materials. In comparison to inorganic and synthetic polymer materials, biomass-derived materials exhibit better performances, such as excellent biocompatibility and unique physical structure. Also, biomass has the conditions required for diversified modification. It also has other advantages, such as good mass transfer performance, low cost, non-toxicity, and simple and easy immobilization operation. Through adsorption, covalent bonding, and matrix trapping, the biomass carrier binds to microorganisms. Because of its distinct benefits, it will have no effect on the activity of the loaded microorganisms while improving their survival in a variety of severe conditions (lyase, high temperature, ultraviolet light, *etc*.). Additionally, there are routes for the interchange of gaseous and liquid nutrients, which explains why fixed bacteria outperform free microorganisms in biodegradation (Jiang *et al*. 2018). Geng *et al*. (2019) utilized mesoporous silica nanoparticles (MSN-$B(OH)_2$) containing $B(OH)_2$ to encapsulate yeast cells, which increases their survivability in hostile conditions. Encapsulated yeast cells are very resistant to high temperatures, UV radiation, and lytic enzymes, and their activity is enhanced.

1. Biocompatibility

Biocompatibility is known to involve reactions between microorganisms and inert materials. This characteristic generally refers to the compatibility between the carrier and the immobilized microorganisms. After bacteria and enzymes are included inside the biomass material, these two materials can influence and interact with each other. Moreover, this interaction cycle can continue until the equilibrium is reached. After bacteria are fixed, the materials cannot adversely influence the fixed bacteria; thus the fixation is beneficial for the higher bacterial survivability and growth. Kandylis *et al*. (2012) proposed the work where yeast cells can adhere to barley grains; this process will prepare a new biocatalyst to produce wine. The biomass material was used in barley grain, which will not cause irritation and teratogenicity to yeast cells. During the fermentation process, the physical properties were reported to improve the available area for immobilization (Fig. 2).

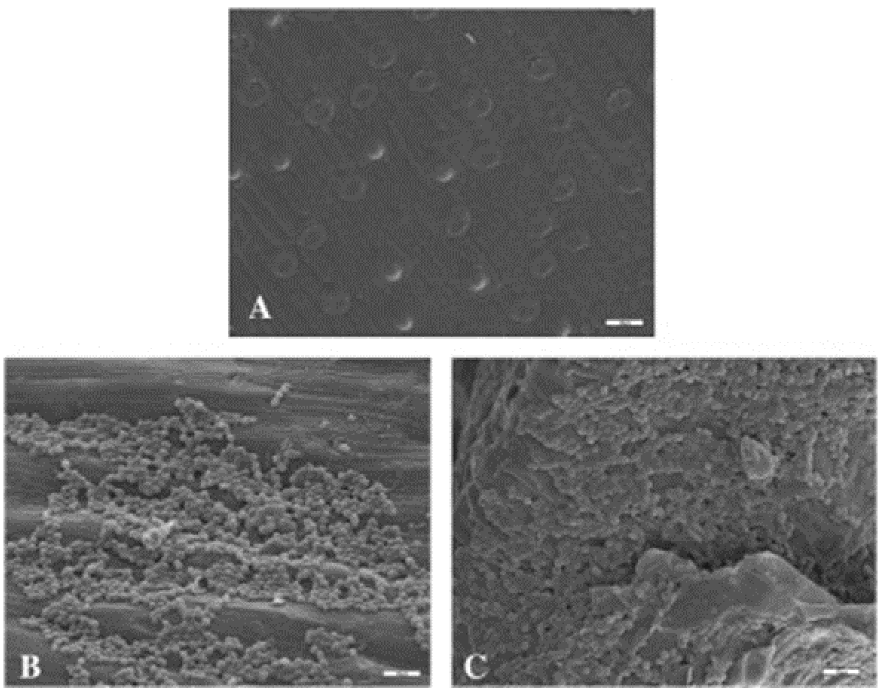

Fig. 2. SEM schematic diagram of the bacterial load of barley grains. A. Surface condition of barley grains before bacterial fixation; B. Surface condition of barley grains after bacterial fixation; C. Surface and internal conditions of barley grains after bacterial fixation. Reprinted from *Food Chemistry*, Vol 130, 425-431, Kandylis *et al.,* Copyright (2012), with permission from Elsevier

The number of immobilized cells was analyzed to exceed 7.1×10^7 cells/g. This high number is attributable to its lipophilic characteristics. These immobilized yeast cells were found to produce 25 types of esters during the wine fermentation process, while only 20 types of esters were produced by non-immobilized cells. The result was better quality brewed wine from immobilized yeast cells. In comparison with non-biomass materials, biomass provides excellent biocompatibility, which can significantly enhance the activity of immobilized microorganisms. Farag *et al*. (2010) used porous dust and wax as carriers to fix two *Candida* strains AQ1 and AQ2. The authors found that the fixed bacteria with sawdust performed much better than those fixed with wax in terms of degrading crude oil.

When wood chips were used as the carrier, the crude oil degradation extents of the two immobilized strains were demonstrated to reach 90% and 73%, respectively. However, two strains with wax fixing only achieved 70% and 62%, respectively. Compared with wax, porous wood chips do not generate toxic low-molecular substances, which are secondary toxic compounds to bacteria during the biodegradation process. Therefore, inside porous wood chips, the quantity per unit volume and bacterial activity of *Candida* were found to have a considerable advantage over wax as a carrier, demonstrating the excellent biocompatibility of biomass materials.

2. Suitable physical structure

Natural biomass materials possess unique surface or internal physical structures, such as porosity, high surface roughness, large specific surface area and pore volume, or they may contain natural cellulose structures such as spirals and filaments. These advantageous features will provide optimum fixing shelters for bacteria and other microbes. For example, Liu *et al*. (2020) proposed fixing bacteria on corn stalk biochar to remediate heavy metals from the contaminated soils. SEM images showed that the corn stalk biochar exhibited a rough surface with the uniform distribution of voids. These features are favorable for bacterial adhesion. Deng *et al*. (2016) utilized chemical methods to alkalize the peanut hull powder. Alkali etching was found to reduce the crystallinity of the biomass due to the loss of the benzene ring structure between the cellulose, resulting in the formation of cylindrical roll structures from fibre surfaces. The chemically treated fibres were found to considerably improve bacterial survival rate and metabolic activity even in toxic substances.

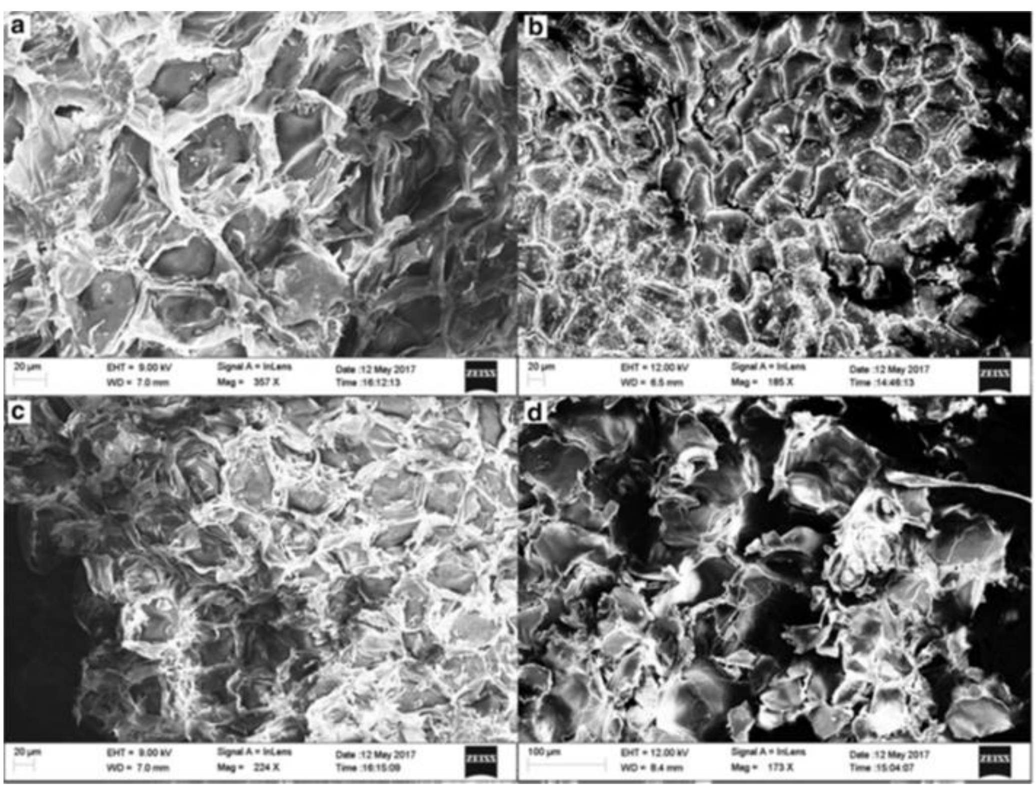

Fig. 3. SEM analysis of bark. a. Bark morphology; b. Bark morphology after microbial fixation; c. Modified bark morphology; d. Modified bark morphology after microbial fixation (He *et al*. 2019)

The fixed bacteria can maintain high degradation efficiency even in the environment with a high concentration of pyrene. He *et al*. (2019) initially utilized acid aldehyde to etch the pine bark and corn stalks' surface chemically. Further, the treated fibres were coated with a sodium alginate solution. The gaps at the polygonal stacks of the fibre structures were found to become loosened and cracked to a certain extent, with the formation of small holes. At the same time a channel with a diameter of about 40 microns is formed (Fig. 3). These structures provide favorable shelters for microbial cells with more effective adhesion and nutrient substance transfer channels for such substances as inorganic salts and vitamins, resulting in the better efficiency of bacteria in wastewater degradation.

3. Diversified modification capabilities

Biomass materials are easy to chemically or physically modify due to the abundant functional groups on the surface or inside and their highly plastic physical form. These characteristics can provide better immobilization and compatibility with microorganisms to improve the degradability in wastewater or the adaptability in the complex environments. Xu *et al*. (2018) proposed the method of pretreating the corn stalks by drying them. Then, under the closed vacuum condition, the stalks were further modified with high-temperature treatment. After modification at different temperatures, the thermal curvature of the fibres was found to instigate significant changes inside the pores between the corn stalk fibres. Also, the thermal curvature was found to enlarge the pores with a higher temperature.

In another study involving petroleum degradation, *Pseudomonas aeruginosa* was immobilized inside heat-modified corn stalks. It was found that due to the improved porosity of the straw material, the diffusion of substrates and metabolites was increased, and the required oxygen and space levels raised the extent of bacterial proliferation, resulting in the higher degradation efficiency of petroleum. The bacterial load was estimated to achieve 18.25×10^{10} CFU/g within 48 hours, and the degradation rate was found to improve significantly. After 5 days, the degradation rate of diesel fuel still was maintained at 59.69%.

In terms of chemical modification, Dhiman *et al*. (2019) modified sodium alginate with β-cyclodextrin by adding sodium alginate and β-cyclodextrin to sodium hydroxide solution. The mixture was further mixed under constant temperature in a water bath. After cooling, the prepared mixture was dropped into the calcium chloride solution to form gel beads. After successful grafting, it was found that the immobilization rate of mannan bacteria was improved to 91.5%. Also, the pH and temperature adaptability were enhanced, and it was able to preserve 70% of the activity after 15 times repeated use. After 30 days of storage at 4 °C, the immobilized bacteria inside the bead still maintained around 60% activity. This method demonstrated the excellent resistance against high temperature, ease of operation, and storage stability, which will benefit future industrial applications. The surface of chitosan was modified by grafting 3-aminopropyltriethoxysilane (APTES) and glutaraldehyde (GLA) (Patel *et al*. 2020).

The process is shown in Fig. 4. The study showed that chemical grafting could significantly improve bacteria and carrier compatibility through electrochemical interaction. In the application of biogas production, the methanol output of immobilized bacteria on the modified chitosan was found to be significantly higher than non-immobilized bacteria.

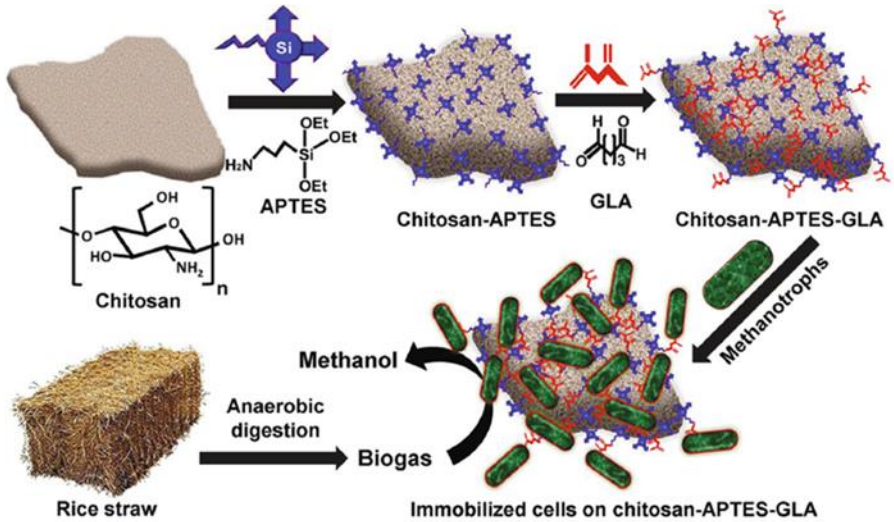

Fig. 4. Chemical modification of chitosan with APTES, and then grafting APTES and GLA on the surface of chitosan by solvent method and immobilizing methanotrophic bacteria
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Application of Microbial Immobilization Technology

Immobilized bacteria technology can provide many advantages, including prolonging the growth cycle of bacteria, easier storage, and better resistance to environmental pressure. The immobilized microbial cells can reduce practical hindrance by creating a more favorable microbial environment in the support matrix. The cells inside the matrix can survive better in a wider pH range and on various complex substrates. Additionally, immobilized bacteria possess the better metabolic activity and promote the coexistence of different types of microbial species (Willaert and Baron 1996). These characteristics are conducive to the immobilization applications in many fields such as agriculture, industry and environmental applications. (Lu *et al*. 2014)

Application in agriculture

Microbial immobilization technology has been found in many applications used in farmland planting, water aquaculture, and other fields to improve the output of crops and aquaculture or alleviate soil pollution (Cesari *et al*. 2020). For example, in peanut planting, Cesari *et al*. (2020) successfully fixed *Rhizobium* and *Azospirillum* in sodium alginate microspheres. The sodium alginate matrix was shown to interact with bacteria and possess the capability to release bacteria continuously. This action can improve their viability and ability to interact with peanuts. The bacteria fix the nitrogen in the atmosphere, which not only increases the yield of peanuts, but it also reduces the use of chemical fertilizers. At present, rice, beans and other crops grown in large quantities in Asian countries such as China, Malaysia, Thailand, and others, after the use of bacterial immobilization technology, can significantly improve economic benefits and develop environmentally friendly agriculture. The problem of eutrophication in water bodies in aquaculture was resolved via the denitrification of immobilized microbes. Yu and Yang (2004) proposed the use of zeolite powder and sand, which were used together to immobilize *Bacillus*; the final immobilized mixtures were left in in the bottom mud of the water body. The immobilized microbial treatment agents can maintain good biological effects to remove and maintain nitrite levels in shrimp ponds.

Application in industry

Currently, microbial immobilization technology has been found in the fields of food brewing and fermentation, such as wine brewing, meat processing and preservation, antioxidants, and flavor products production (Kosseva 2011; Genisheva *et al*. 2014). In food processing, natural biomass materials have been widely used due to their non-toxicity, good biocompatibility, low price, and easy availability; examples include cellulose (Ikonomopoulou *et al*. 2003), wheat grains (Kandylis *et al* 2010), potato chips (Kandylis *et al*. 2008), and corn starch gel (Kandylis *et al*. 2008). Kandylis and Koutinas (2008) immobilized yeast cells into potato fragments, enabling it to make wine at low temperatures. The produced wine was found to exhibit better fragrance than wine produced by non-immobilized cells. In medicine, immobilization technology has been used mostly as biosensors to diagnose the condition of patients. Immobilized settings designed by Zaitsev *et al*. (2019) immobilized lipase on polysaccharides (mainly chitin and chitosan) showed high activity and stability. The material was placed on the biosensor platinum electrode, and the amperometric method was used to measure the triglyceride acid level in the serum of healthy and sick patients. Immobilization technology can also be applied to other industrial fields, such as the synthesis of useful biological products, such as bioethanol and lactic acid (Arias *et al*. 2018) and nitrification reactors using cell immobilization technology (Kunapongkiti *et al*. 2020).

Table 1. Comparing Various Aspects of Different Immobilization Technologies

Characteristic	Crosslinking	Adsorption	Chelation	Covalent binding	Entrapment
Preparation	Intermediate	Simple	Simple	Difficult	Intermediate
Binding force	Strong	Weak	Intermediate	Strong	Intermediate
Retention of activity	Low	High	Intermediate	Low	Intermediate
Regeneration of carrier	Impossible	Possible	Possible	Rare	Impossible
Cost of immobilization	Intermediate	Low	Low	High	Low
Stability	High	Low	Intermediate	High	High
Applicability in food technology	No	Yes	No	No	Yes
Protection from microbial attack	No	Yes	No	No	Yes
Viability	No	Yes	No	No	Yes

Application in the field of environmental protection

Immobilization technology has been developed and applied for environmental engineering as early as the 1980s. The microorganisms strongly bind in the immobilization carrier, and immobilized bacteria can resist and tolerate the harsh environmental conditions. These characteristics render the immobilized bacteria suitable to be used in environmental remediation. The volatile organic compounds (VOC), such as toluene and acetone caused by petroleum, coal chemical, and fuel combustion can easily cause atmospheric environmental problems such as haze and photochemical smog. Typically, a biological trickling filter (BTF) has been used to treat VOC. Liu *et al*. (2019) used the bamboo-derived activated carbon beads, which consist of activated carbon and fixed bacteria packing into BTF. The carbon beads have been known to play the dual role in the adsorption of pollutants and the promoting of attached bacterial growth, thereby improving the removal efficiency of pollutants. The removal rate of toluene was found to reach more than 99%. In terms of absorbing heavy metals, Youngwilai *et al*. (2020) modified sawdust biochar with hydrogen peroxide to immobilize manganese-oxidizing bacteria SBP1. The highest removal efficiency reached 78%.

Moreover, one of the main advantages of immobilization technology is to increase the toxicity-intolerance of bacteria. At present, pollution in the coastal waters of China is relatively serious, and organic compounds are one of the main sources of pollution. Their long durability and high toxicity make it a difficult point in environmental governance. Using immobilization technology efficiently removes many toxic compounds and other compounds with low biodegradability, such as PAH, phenol, PPCP, and endocrine-disrupting compounds. Namane *et al*. (2020) proposed how to fix bacteria in calcium alginate beads to degrade phenol. Under optimal conditions, the degradation performance of phenol was found to reach 800 mg/L.

SUMMARY AND OUTLOOK

Microbial immobilization technologies, as a new class of biological treatment technologies having great potential, have gradually been demonstrating broad application potential in many fields. The immobilization technologies exhibit a lot of advantages, such as increased bacterial load, activity, and protecting microbes from unfavorable external factors. In particular, in agriculture, industry, and environmental protection, the technology has been widely utilized. With the continuous enhancement of sustainable development, future microbial immobilization technology will advance better environmental bioremediation prospects, food safety, biosensing, medical medicine, and ecological construction. Especially in emerging fields, such as sustainable energy production, biotechnology employing immobilized cells as a biological production platform is seen as a sustainable way of chemical and electrical energy generation. Immobilized cells can be utilized as catalysts for green chemical processes in the field of green catalysis. Cell therapy, as a new technology in medicine, is critical for medication delivery and transplantation. Additionally, it may be used to screen and find novel medicines in the laboratory as an in vitro reagent that can be employed in place of animal studies. These applications provide significant economic benefits, demonstrating that microbial immobilization has a bright future (Michelini and Roda 2012; Geng *et al*. 2018). The improvement of the immobilization methods is continuously required. The continuous innovation and progress of immobilized carrier materials and establishing a complete evaluation system of immobilized microbial technology have become the focus of future research and application. In-depth research should be conducted from the following aspects: (1) developing new methods of microbial immobilization, optimizing the preparation process, and highly disperse microbes in porous biological carrier materials to obtain high-quality immobilized microbial carriers; (2) in-depth exploring of the influence of biomass material carriers with different structures and functions on the growth rate and activity of microorganisms to realize the controllable preparation of high-performance biological fillers; and (3) synthesizing new biomass materials, improving the strength and flexibility of the pore structure, enriching the surface characteristics, and using them for environmental pollution control and resource reuse, and expanding their industrial applications.

ACKNOWLEDGEMENTS

Thanks to the National Natural Science Foundation of China (Grant No.21736009), Zhejiang Public Welfare Research Project (Grant No. LGF19B070005, 2016C32022) and Minjiang Scholarship from Fujian Provincial Government for supporting this work.

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Article submitted: January 24, 2021; Peer review completed: July 11, 2021; Revised version received and accepted: July 20, 2021; Published: September 17, 2021.
DOI: 10.15376/biores.16.4.Zhang