Roles of Surfactants in Oriented Immobilization of Cellulase on Nanocarriers and Multiphase Hydrolysis System

Zhiquan Wang1,2,3, Chunzhen Fan1,2,3, Xiangyong Zheng1,2,3*, Zhan Jin1,2,3, Ke Bei1,2,3, Min Zhao1,2,3 and Hainan Kong4

1School of Life and Environmental Science, Wenzhou University, Wenzhou, China, 2State and Local Joint Engineering Research Center for Ecological Treatment Technology of Urban Water Pollution, Wenzhou, China, 3Zhejiang Provincial Key Lab for Water Environment and Marine Biological Resources Protection, Wenzhou, China, 4School of Environmental Science and Engineering, Shanghai Jiao Tong University, Shanghai, China

Surfactants, especially non-ionic surfactants, play an important role in the preparation of nanocarriers and can also promote the enzymatic hydrolysis of lignocellulose. A broad overview of the current status of surfactants on the immobilization of cellulase is provided in this review. In addition, the restricting factors in cellulase immobilization in the complex multiphase hydrolysis system are discussed, including the carrier structure characteristics, solid-solid contact obstacles, external diffusion resistance, limited recycling frequency, and nonproductive combination of enzyme active centers. Furthermore, promising prospects of cellulase-oriented immobilization are proposed, including the hydrophilic-hydrophobic interaction of surfactants and cellulase in the oil-water reaction system, the reversed micelle system of surfactants, and the possible oriented immobilization mechanism.

Keywords: cellulase, surfactants, nanocarriers, reversed micelle system, oriented immobilization

INTRODUCTION

Bioethanol, as a renewable, economically affordable, and environmentally safe energy material, will gradually become a substitute for fossil fuels. It has far-reaching research significance and application value for the development of a sustainable energy strategy (Karimi et al., 2021; Zeng et al., 2021; Ziaei-Rad et al., 2021; Suhartini et al., 2022). Due to competition with food supply in the first generation of bioethanol production, lignocellulose, a non-starch material, has become an important raw material for bioethanol production (Alonso et al., 2019; Maia et al., 2020; Winarni et al., 2020). Adsorption of cellulases onto lignin has been considered as the major factor in retarding enzymatic cellulose degradation of lignocellulosic biomass (Djajadi et al., 2018). Hydrophobic interaction, electrostatic interaction and hydrogen bonding have been regarded as the cause of the nonproductive binding of cellulases to lignin (Djajadi et al., 2018; Li et al., 2020; Song et al., 2020). A natural “biodegradable barrier” of lignin cell walls which are connected in a strong, yet resilient network under the action of covalent and non-covalent bonds render the cellulose inaccessible (Mnich et al., 2020; Chu et al., 2021). Therefore, to reduce the recalcitrance of lignocellulosic biomass to biochemical degradation, pretreatment methods have been developed to break down the lignin-hemicellulose-cellulose matrix and increase the enzyme accessibility of the cellulose scaffold (Jiang et al., 2017a; Jia et al., 2018; Rocha-Martin et al., 2018).
In general, lignin-derived inhibition is the major physical obstacle restricting the enzymatic hydrolysis of cell wall polysaccharides (Leonidas et al., 2019; Zheng et al., 2021). More importantly, the non-specific binding of free cellulase on lignocellulosic substrates may account for the low rate of hydrolysis at the action mechanism level during enzymatic hydrolysis. Some enzymes remain free after the enzymatic hydrolysis of lignocellulosic substrates, while non-specific binding to the residual substrates also prevents the efficient recycling of cellulase (Rahikainen et al., 2011; Kellock et al., 2017; Bhawna et al., 2020). Moreover, the utility of cellulases has been limited due to their low operational stability, high costs, and poor reutilization when used in the native form (Yang et al., 2017).

To overcome these barriers, immobilization is usually used to improve enzyme stability and even activity or selectivity when properly designed, which can also facilitate the reuse of enzymes and effective cost of catalytic processes (Mita and Eldin, 2014; Li et al., 2016; Mehta et al., 2016; Xu et al., 2016; Zhang et al., 2016). The characteristics of various immobilization methods of enzymes is summarized in Table 1. Cellulases represent a large group of enzymes from various organism and with different substrate specificity, biophysical properties, etc. The immobilization behavior is different depending on the enzyme or enzyme mixture investigated. During the immobilization process of cellulase, the structure and properties of carrier materials have significant effects on the performance of the immobilized enzyme (Kalantari et al., 2013; Li et al., 2018). The size of the carriers plays an important role in determining the activity of the immobilized enzyme owing to the inverse relationship between the carrier size and enzyme loading. Thus, large carrier size decreases enzyme activity in general (Valencia et al., 2010), and a reduction in the size of the carriers results in a higher surface area for enzyme binding (Malar et al., 2018; Malar et al., 2020). For the immobilization of cellulase, the smaller size of the surface pore should be kept lower than that of the cellulase macromolecule (6–20 nm), which can further reduce the internal and external diffusion resistance in the heterogeneous system (DiCosimo et al., 2013; Santos et al., 2015). Therefore, nanocarriers are widely used in the immobilization of enzymes because of their unique properties, such as large specific surface area to volume ratio (Cao et al., 2016; Roth et al., 2016; Malar et al., 2020).

Moreover, the immobilization of cellulase has been achieved based on physical adsorption, covalent binding, or affinity interactions (Zang et al., 2014; Hosseini et al., 2018; Zhang and Hay, 2019), including carrier-binding, microemulsion-based organo-gels (MBGs), ultrasonic encapsulation, crosslinking, entrapment, glutathione-labeling, and chelation (Mroczkiewicz et al., 2012; Nicoletti et al., 2015). However, enzymes often display drastically lower activity in organic solvents than in water, and the water layer on the molecular surface of enzymes determines their activity in organic media (Zhang et al., 2012). Therefore, among several approaches to resolve the challenges, one of the most effective methods is immobilization of the enzymes within an aqueous microenvironment in the organic solvents. Microemulsions formed by amphiphilic surfactants have been widely reviewed as effective media for the immobilization of enzymes in hydrophobic solvents (Itabaiana et al., 2014; Rajnish et al., 2021; Savic et al., 2021). The MBGs method based on microemulsions has been used to form matrices for enzyme immobilization to achieve enzymatic catalysis in nonconventional medium as they appear to be rigid and stable for a long time, even within the reaction solution (Zhang et al., 2012). Therefore, the MBGs method has unique advantages of improving the chemical stability of immobilized enzymes and maintaining high catalytic activity (Pavlidis et al., 2010; Itabaiana et al., 2014).

### Table 1 | The characteristics of various immobilization method of enzymes.

| Methods                  | Mechanisms                                                                 | Characteristics                                                                 | References          |
|--------------------------|-----------------------------------------------------------------------------|--------------------------------------------------------------------------------|---------------------|
| Adsorption               | Physical: Adsorbed on the carriers, ionic: Combined with water-insoluble carrier containing ion-exchange group by electrostatic force | Active center of the enzyme is not easily to be destroyed, and not obvious structure change occurs. Structure and amino acids of the active center rarely change, and the higher activity immobilized enzyme can be obtained. | Gao et al. (2009)   |
|                          | Encapsulation: Mixed with polymer monomer and further embedded in the polymer | It is not necessary to combine with amino acid residues of enzyme protein, and rarely change the spatial conformation of enzyme. | Sui et al. (2015)   |
|                          | Covalent binding: Covalently bonded to the water-insoluble carrier          | Enzyme molecules are firmly connected with the carrier, the structure of the enzyme protein is often changed, resulting in the damage of the active center of the enzyme. | Ghasemi et al. (2021) |
|                          | Cross-linking: Bil-functional reagent or multifunctional reagent is used to form covalent bond between enzyme molecules | Combined with adsorption or encapsulation method, the activity of immobilized enzyme can be increased and the reinforcement effect can be achieved. | Ouyang et al. (2020) |
|                          | Cross-linked enzyme aggregates (CLEAs): Covalently bound by cross-linking agent to keep the supramolecular structure and activity | Carrier free immobilization, good stability, low cost, large activity per unit volume, and high space efficiency. | Xu et al. (2020)    |
| Co-immobilization        | Different enzymes are immobilized in the same carrier at the same time        | Several kinds of enzymes and cells with different functions work together in the same system. | Qiu et al. (2021)   |
| Oriented immobilization  | Specific site of enzyme connects with carrier and the active site faces outsid | It is beneficial for the substrates to enter into the active site of the enzyme and can significantly improve the activity of the immobilized enzyme. | Zhou et al. (2021)  |
enzyme loading (Lou et al., 2017; Bao et al., 2019). However, inhibitory effects have been observed with the addition of surfactants (Li, Kawakami, and Hiramatsu, 2003). Although a large dose of original cellulase is added for a higher load of immobilized enzyme to improve the activities of the immobilized enzyme, no significant improvement in enzymatic activity has been observed due to the structural distortion caused by the strong enzyme-support interactions may produce steric hindrances and catalytic cleft blockage (Carlsson et al., 2014; Suárez et al., 2018). Although a large dose of original cellulase is added for a higher load of immobilized enzyme to improve the activities of the immobilized enzyme, no significant improvement in enzymatic activity has been observed due to the random and inhomogeneous combination of the nanocarriers and cellulase molecules (Nakayama et al., 2009). Oriented immobilization, as a specific binding method, can effectively prevent the nonproductive combination of enzymes and nanocarriers, which further improves the immobilization and hydrolysis efficiency. The reversed micelles formed by surfactants have been successfully used in the preparation of oriented-immobilized lipase when their concentration exceeds the critical micelle concentration (CMC) (Fan et al., 2016). To date, few studies have reported the oriented immobilization of cellulase. Therefore, this review mainly focuses on the important roles of surfactants in the immobilization of cellulase, mainly including the preparation of nanocarriers and cellulase hydrolysis. Moreover, a novel insight into the oriented immobilization of cellulase in a surfactant reversed micelle (SRM) system was discussed and found to have promising prospects.

**EFFECTS OF SURFACTANTS ON NANOCARRIERS**

**Preparation of Nanocarriers Based on Surfactants**

The basic physical and chemical properties of surfactants, such as micelle formation, dispersing, emulsifying, and solubilizing, have

---

**Table 2: Applications of surfactants in preparing nanomaterials.**

| Applications | Types                     | Characteristics                                                                                     | References                          |
|-------------|---------------------------|------------------------------------------------------------------------------------------------------|-------------------------------------|
| Nanomaterials | Metallic nanoparticles     | It is usually prepared in the reversed micelles and microemulsions system                           | Kawasaki, (2013)                    |
|             | Semiconductor nanoparticles| It is prepared in the reversed microemulsions system, including the oxides, sulfides, and selenides etc. | Anjum et al. (2019)                |
|             | Organic nanoparticles      | It includes organic drug nanoparticles and polymer nanoparticles, which can be prepared in microemulsions system | Li, Kawasaki, and Hiramatsu, 2003   |
|             | Nanowires                  | It can be prepared by the templates from nanocarriers, liquid crystals, vesicles formed by the surfactants | Xu et al. (2010)                    |
|             | Porous nano-materials      | Surfactants can be the structure directing agent of mesoporous materials                           | Camillo et al. (2011)               |
|             | Nano-films                | It mainly includes Langmuir-Blodgett (LB) film and Molecular-Deposition (MD) film                  | Shih et al. (2017); Lai et al. (2020) |
|             | Nanocomposites            | Organic polymer was encapsulated in inorganic nanoparticles in inverse microemulsion system         | Al-Shemmari et al. (2014)           |
| Methods     | Template-directed synthesis| The electrostatic attraction, hydrogen bond and Van der Waals force between surfactant molecules and nano materials are used for the formation of special micelle structures, which can further used as the synthesis templates of nanomaterials | Xu et al. (2010); Kaythomayun et al. (2020) |
|             | Microemulsion method       | When the amount of surfactant and polar organic matter is large, the microemulsion can be obtained, which can be used as a microreactor for synthesizing nanomaterials | Anjum et al. (2019); Cui et al. (2019) |
|             | Hydrothermal synthesis     | Surfactants are mainly used as auxiliary materials                                                  | Cui et al. (2019)                   |
|             | Sol-gel method             | The transparent sol is formed by hydrolysis and condensation reaction, and gradually gelatinization. After drying and heat treatment, nanomaterials can be obtained | Hassanzadeh-Tabrizi et al. (2016)    |
| Surface modification | Physical and chemical properties | Surface adsorption and chemical reactivity of surfactants can modify the surface of nanoparticles | Chaudhary et al. (2014)             |
|             | Interfacial modification of nanofilms | Hydrophilicity or lipophilicity of surfactants can be used to modify the interface of nanofilms | Kovalchuk, (2015)                   |
| Effects     | Dispersion of nanoparticles in media | Prevent particle agglomeration                                                                  | Fiorati et al. (2021)               |
|             | Functional effects on nanoparticles | Improve the compatibility and affinity between polymer materials and inorganic materials     | You et al. (2019)                   |
made them widely useful in the field of nanotechnology (Yang et al., 2017). Several ordered aggregations formed by the surfactants are used as nano-templates for the preparation of nanocarriers, such as micelles and reversed micelles. The process can greatly reduce the surface tension of the solvent and change the interface composition and structure (Bao et al., 2019). For the preparation of nanocarriers, surfactant micelles are the microreactors of nanocarriers during the preparation process, and the morphology of microreactors is controllable because of the amphiphilic characteristics of surfactants, which have been used for the preparation of desirable nanostructured carriers (Yiamsawas et al., 2017). For instance, hydrophilic surfactants are often used for the preparation of spherical nanocarriers because of their dispersibility in water (Luan and Ramos, 2010). Similarly, the reversed micelles of surfactants can effectively define the particle size and reaction microenvironment in the water, providing a nanoscale reaction space. It has been widely used because the aggregates self-assembled by surfactant molecules can be used to synthesize ordered mesoporous materials with a simpler operation and more uniform channel distribution (Bao et al., 2017; Bao et al., 2019).

**Surface Modification of Nanocarriers in the Surfactant System**

Surfactants can also change the surface properties of nanocarriers, such as their morphology, magnetic properties, dispersion, and catalytic performances (Asghar et al., 2016; Wei et al., 2018; Lopes et al., 2019; Alexander et al., 2020). This modification may result in a new structure with new surface activity due to the combination of hydrophilic groups of surfactants and surface groups of nanocarriers. For example, the use of surfactants of decylamine and cetyltrimethylammonium bromide can provide an easy and effective way to change the functionality of cellulose nanocrystals with a hydrophobic polyactic acid matrix and to evaluate the effects of surface chemistry on the reinforcement mechanisms (Orellana et al., 2018). Meanwhile, the presence of surfactants can make nanocarriers more difficult to re-agglomerate by reducing the surface energy and form a steric hindrance effect (Wang M. et al., 2013; Tan et al., 2019), the surfactants are coated on the surface of the nanocarriers to form a space barrier layer, the hydrophilic group faces outward and the hydrophobic group faces inward, so that the agglomeration of the particles is avoided.

**EFFECTS OF NANOCARRIERS ON IMMobilIZATION OF CELLULASE**

The structure and properties of carrier materials have great influence on the properties of immobilized cellulase, such as internal geometry (e.g., flat surfaces or thin fibers), specific surface area, superficial activation degree, mechanical resistance, and pore diameter (Santos et al., 2015; Begum et al., 2019; Malar et al., 2020). Meanwhile, partitioning and mass transport limitations may yield spatial variations in local reaction rates in porous materials (Neira and Herr, 2017). Therefore, to improve the stability and catalytic activity of immobilized cellulase, various materials, such as chitin, chitosan, nylon, and polyvinyl alcohol, have been widely used as carriers (Cherian et al., 2015; Priydarshani et al., 2018).

The physical effects of nanocarriers on immobilized cellulase are as follows: 1) The pore size and effective surface area of the nanocarriers. Not all porous carriers can be used for immobilization of cellulase due to the limitation of pore size, which should be larger than or equal to that of the cellulase to reduce steric hindrance. The effective surface area occupied by the enzyme determines the maximum load of the immobilized cellulase (Santos et al., 2015). When a stable surface area is maintained, the amount of immobilized or absorbed cellulases is related to the pore size because the pore diameter determines the size of the protein that can be immobilized on that carrier (Teresa et al., 2009; Webster et al., 2015); 2) the number of carrier-bound active groups (CAGs) is another key factor controlling the enzyme-carrier multi-interaction (Cristina et al., 2011; Santos et al., 2015); 3) the size of carriers plays a very important role in the preparation of immobilized cellulase, in that a smaller carrier size with larger specific surface area will be better for the cellulase immobilization load, and the higher surface porosity of the carriers providing numerous binding sites for cellulase is one of the most important factors influencing the activity of immobilized cellulase (Chen et al., 2010; Santos et al., 2015; Malar et al., 2020); 4) the mechanical properties of the carriers need to be controlled considering the final configuration of the reactor. If the reactor is a fixed-bed reactor, such as inorganic supports like porous glass, silicates, it should possess very high rigidity to withstand high pressures without pressure problems, but the situation is different if a stirred-tank reactor is used (Cristina et al., 2011; Santos et al., 2015); 5) after the cellulase penetrates the carriers, the internal morphology of carriers will determine the possibility of obtaining a very intense or very limited enzyme-carrier interaction (Santos et al., 2015). When the diameter of the carriers is smaller than that of the enzyme, it is difficult to obtain an intense enzyme-carrier interaction (Cristina et al., 2011), but if the carriers have sufficiently large internal surfaces, it is possible to get an intense interaction with a similar flat surface (e.g., agarose beads, porous glass, or silicates) (Malar et al., 2018).

In particular, the special superparamagnetism of magnetic nanocarriers has attracted increasing interest as they allow easy recycling and separation of catalysts and biomolecules from high-viscosity liqueurs and high-solid-content broths. This unique characteristic has been well-applied to immobilization of cellulase, and a better hydrolysis efficiency and recycling feasibility have been observed (Alfré (e.t al., 2014; Cao et al., 2016; Cipolatti et al., 2014; Xing et al., 2015). During immobilization of cellulase, magnetic chitosan microspheres (C-MNPs) are frequently used as carriers because of their significant biological (i.e., biodegradable, biocompatible, bioactive) and chemical properties (polycationic, hydrogel, contains reactive groups, such as -OH and -NH₂). Moreover, the hydrophilic properties of the C-MNPs play an important role in the preparation of oriented-immobilized cellulase based on the
SRM system. The conventional immobilization of cellulase molecules on a single magnetic nanocarrier is simple, the chitosan was usually first coated on the magnetic nanocarriers for further combination with cellulase (Figure 1). Subsequently, the combined material based on Fe$_3$O$_4$ nanocarriers have received extensive attention in cellulase immobilization to improve enzyme activity, loading, and stability because of their low toxicity, biocompatibility, and easy synthesis (Jordan et al., 2011). Magnetite nanocarriers coated with silica and modified by organic-silanes, biocompatible, and with hydrophilic properties, are promising for cellulase immobilization.

The binding sites of enzymes on the surfaces of carriers depend on the chemical properties of the carriers. For non-covalent immobilization, the chemical structure of the skeleton and surface determines the applicability of carriers. The functional groups play a key role in the activity, stability, and selectivity of the enzyme, and the size, charge, polarity, and hydrophilicity/hydrophobicity of groups can affect their binding functions (Watanabe et al., 2010). Different properties of the ionic groups on the surfaces of carriers may result in different enzyme activities and further determine the structure of immobilized cellulase (Santos et al., 2015; Berlin et al., 2016; Frančič et al., 2016; Hui et al., 2016; Zhou et al., 2018). The chemistry properties of enzyme and carrier cause the oriented distribution of catalytic domain of enzyme from dispersion layer to diffusion layer during the immobilization process is shown in Figure 2. In this process, the CAGs directly participate in binding with enzyme molecules, but the carrier-bound inert groups (CIGs) are not directly involved. This interaction inevitably disturbs the maintenance of the natural conformation of the enzyme, leading to structural and functional changes in the enzyme molecules. No obvious stability change has been observed when the newly formed conformation is similar to that of the natural enzyme.

The covalent binding between carriers and catalytic cleft of the enzyme not only causes pore plugging of the surface, but also leads to the drag increment of in-diffusion. Although an initial high dosage of cellulase is added, the inhomogeneous distribution of the carrier surface structure results in the uncontrollable immobilization sites, and ineffective immobilization may lead to a significant loss of enzymatic activity and reduce the accessibility of the substrate to the functional sites. Moreover, the partition and mass transport limitations of nanocarriers may cause spatial variation in local reaction rates and further affect...
The lignocellulosic substrate was added (Seo et al., 2011a). The cellulose increased from 9 to 21% within 72 h when a high adsorption of cellulase, and the conversion efficiency of cellulase increased (Seo et al., 2011b; Eckard et al., 2012; Yiamsawas et al., 2020). Moreover, surfactant modification is an important strategy for tuning the properties of nanocarriers. Surface modification can either alter the existing property or introduce new properties onto nanoparticle surfaces using various agents, such as organ siloxane, N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), and carbodiimide, as well as amino silanes, such as 3-amino propyl-triethoxysilane, aminoethylethoxysilane, and silica (Chang et al., 2011; Gokhale et al., 2013; Riedel et al., 2017; Malar et al., 2018; Malar et al., 2020).

**ROLES OF SURFACTANTS ON CELULASE HYDROLYSIS**

In most reports about hydrophobic ionic liquids, the enzymes are not dissolved but merely in a dispersed state and therefore regarded as a heterogeneous catalyst. Some hydrophilic ionic liquids can accelerate the dissolution of enzyme molecules, but cause the destruction of the protein secondary structure, leading to the inactivation of the enzyme (Fujita and Ohno, 2010; Moniruzzaman et al., 2010). In pure hydrophilic ionic liquids the enzymes can be dispersed at the monomolecular level. The hydrophilic proteins in almost anhydrous nonpolar solvents form suspensions, whereas proteins with extended hydrophobic surface segments form microemulsions in the same media, greatly reducing the catalytic efficiency of the enzyme (Zuev et al., 2003; Predvoditelev et al., 2010). Nonpolar hydrophobic solvents, such as heptane, octane, and benzene, do not cause the dehydration of biocatalysts. Therefore, the enzyme can maintain its catalytic activity (Muginova et al., 2010). Similarly, the catalytic activity of enzymes can be retained in the surfactant micelle system due to the water-oil amphiphilicity of surfactants (Muginova et al., 2010). Non-ionic surfactants can significantly accelerate the enzymatic hydrolysis of lignocellulose (Qing et al., 2010; Seo et al., 2011b; Eckard et al., 2012; Yiamsawas et al., 2017). For instance, Tween-20 can enhance the specific adsorption of cellulase, and the conversion efficiency of cellulose increased from 9 to 21% within 72 h when a high lignocellulosic substrate was added (Seo et al., 2011a). The prevention of non-productive enzyme adsorption onto lignin is the most widely investigated mechanism for this enhancement (Sipos et al., 2010; Lou et al., 2017). Recently, Dijadji et al. (2018) has proved that the adsorption of cellulases onto lignin substrates is reversible by nature, the reversible adsorption-desorption is existing in the process. But the non-productive adsorption caused by the ineffective combination will occupy large number of catalytic clefts of enzyme molecule which greatly hinder the enzymatic hydrolysis. Non-ionic surfactants can render lignin surfaces more hydrophilic by increasing their polar surface energy component, which can reduce the non-productive adsorption of cellulases onto lignocellulosic substrates caused by the ineffective combination between catalytic clefts of enzymes and lignin substrates (Jiang et al., 2017b), thereby promoting the enzymatic hydrolysis of lignocellulose. However, for the anionic surfactant-cellulose system, the adsorbed surfactants on the surface of cellulase cause a lower negative charge area, which further leads to negative catalytic activity due to the presence of sulfonic acid groups with a higher ionization degree (Yu and Zhang, 2016). Furthermore, the effect of surfactants on cellulase hydrolysis is related to the concentration of surfactants (Zhou et al., 2015). In the enzymatic hydrolysis process, cellulose molecules are specifically adsorbed by the cellulose-binding domain (carbohydrate-binding module, CBM) and exert a driving force on the enzyme during the hydrolysis of cellulose (Liu et al., 2011; Tomme et al., 2015; Arslan et al., 2016). The adsorption of CBM can increase the cellulase concentration of the substrate surface by promoting the association of enzymes and substrates, but the non-covalent interactions (e.g., hydrogen bonds, electrostatic, and hydrophobic interactions) may lead to a nonproductive combination, because the random combination will occupy the active center of enzyme, resulting in the loss of catalytic activity. Ineffective adsorption can be reduced in the presence of surfactants due to the hydrophobic structure of surfactants, which can interact with the hydrophobic lignocellulosic substrates and form a coating (Kumar and Wyman, 2010; Li et al., 2012). However, contrasting results were obtained when different concentrations of surfactants were added to the enzymatic hydrolysis system. Some studies have suggested that a high concentration of surfactants can inhibit cellulase activity because strong hydrophobic interaction between the surfactant and cellulase can further reduce the effective adsorption of enzymes on cellulose (Wang Z. et al., 2013; Bao et al., 2019). However, the promotion effect of surfactant in enzymatic saccharification was observed in low concentration of lignosulfonate with low molecular weight and good sulfonation, which can be explained that the lignosulfonate can prevent the nonproductive binding of cellulase to lignin substrate, and the formed lignosulfonate-cellulase aggregate can also stabilize and enhance the binding of cellulase to lignin substrate (Lou et al., 2014).

**THE ORIENTED IMMobilIZATION OF CELLULASE IN THE SRM SYSTEM**

The oriented immobilization of proteins on a solid support can effectively avoid its denaturation and keep its catalytic clefts fully exposed to solution, thus maximally preserving the bioaffinity or bioactivity. Liu and Yu (2016) has summarized the recent advances in oriented immobilization of proteins with a particular focus on antibodies and enzymes. However, the orientated immobilization of enzymes at the solvent interface is never involved. Thereby, the follow-up content will propose a novel method to achieve the oriented immobilization of cellulase in the SRM system.
Construction of the SRM System
The SRM system has been widely used in the preparation of immobilized enzymes (Dong et al., 2010; Marhuenda et al., 2015). The special structure of surfactant molecules caused a water-oil amphipathy with a hydrophobic nonpolar hydrocarbon chain (alkyl) and a hydrophilic polar group (such as -OH, -COOH, -NH₂, and -SO₃H) distributed at different ends. In the water-oil (W/O) system, the surfactants are dissolved in the nonpolar organic solvent when a trace of water is provided, and the reversed micelles are formed when the concentration exceeds the CMC (Takagi et al., 2019; Chi et al., 2018). In reversed micelles, the nonpolar groups of the surfactants are exposed to the nonpolar organic solvents, while the polar groups are arranged inside. Therefore, a polar core with the ability to dissolve polar substances in the microreactors is formed. The SRMs are nanoscale aggregates that are formed spontaneously, and the W/O microemulsion with low water content provides a stable thermodynamic system (Tao et al., 2013). According to the hydrophilic-hydrophobic interaction of surfactants and cellulase in the oil-water reaction system, the large number of oil-water interfaces in the system provides a good environment for the catalytic reaction of enzyme molecules (Brady and Jordaan, 2009).

Mechanism of Oriented-Immobilized Cellulase in the SRM System
Multipoint covalent attachment is likely the most effective strategy for immobilization, but it is difficult to allow the immobilization of enzymes at a well-defined position since the proteins are usually attached to the solid surface by uncontrolled chemical bonds (Barbosa et al., 2015; Hernandez and Fernandez-Lafuente, 2011; Li et al., 2016). The uncontrolled conformational changes were caused by random immobilization, which may lead to a significant loss of enzyme activity, and the disordered enzyme orientation may also reduce the accessibility of the substrate to functional sites (Orellana et al., 2018; Steen Redeker et al., 2013; Yu et al., 2012). However, the hydrophilic cellulase will be dissolved in the SRM system due to the existence of surfactants, which can maintain the activity of the enzyme and prevent the toxic effects of organic solvents (Tao et al., 2013). The active centers of cellulase molecules are usually clefts, which provide a different microenvironment (Orellana et al., 2018; Steen Redeker et al., 2013; Yu et al., 2012). However, the hydrophilic cellulase will be dissolved in the SRM system due to the existence of surfactants, which can maintain the activity of the enzyme and prevent the toxic effects of organic solvents (Tao et al., 2013). The active centers of cellulase molecules are usually clefts, which provide a different microenvironment (Orellana et al., 2018; Steen Redeker et al., 2013; Yu et al., 2012). However, the hydrophilic cellulase will be dissolved in the SRM system due to the existence of surfactants, which can maintain the activity of the enzyme and prevent the toxic effects of organic solvents (Tao et al., 2013). The active centers of cellulase molecules are usually clefts, which provide a different microenvironment (Orellana et al., 2018; Steen Redeker et al., 2013; Yu et al., 2012). However, the hydrophilic cellulase will be dissolved in the SRM system due to the existence of surfactants, which can maintain the activity of the enzyme and prevent the toxic effects of organic solvents (Tao et al., 2013). The active centers of cellulase molecules are usually clefts, which provide a different microenvironment (Orellana et al., 2018; Steen Redeker et al., 2013; Yu et al., 2012).
the tryptophan which has the highest content and plays an important role in the recognition and binding of enzyme molecules and substrate (Zhang et al., 2015). Hydrophobic active centers are conducive to the combination of catalyzed groups of cellulase and substrates. When the specific substrate is close to the active centers, a change in the conformation of the cellulase molecule can be induced so that the reaction groups of the enzyme active centers and substrate are aligned correctly. Meanwhile, the molecular orbitals between the reaction groups of the active centers are strictly located in the right direction for easier enzymatic reactions. Therefore, cellulase is distributed in the W/O interface, and the catalytic active center is toward the organic solvent and the other side toward the “pool”. Moreover, the addition of surfactants can enhance the aggregation effect of cellulase on the W/O interface, and the existence of a crosslinking agent promotes the covalent crosslinking of enzyme molecules. The catalytic activity centers of the cross-linked microspheres are distributed uniformly and toward the outside, which solves the challenge of the uncontrollable attachment sites of the cellulase molecules in the immobilization process (Li et al., 2016; Steen Redeker et al., 2013; Yu et al., 2012). In the SRM system, the hydrophobic active molecules are exposed to the outside, which is beneficial for the further combination of immobilized cellulase and lignocellulosic substrates. However, the immobilized sites of cellulase molecules remain stochastic and heterogeneous, which may lead to covalent binding between the carriers and the active center of the enzyme and further cause ineffective immobilization and enzymatic reactions (Li et al., 2016). Therefore, to achieve oriented immobilization and improve the recycling times of cellulase, C-MNPs can be used as carriers as shown in Figure 3. This method can effectively prevent the ineffectiveness of cellulase immobilization. In this process, glutaraldehyde is used as the crosslinking agent, and EDC and N-hydroxysuccinimide are the coupling agents (Figure 4). In the W/O system, the free carboxyl group (-COOH) in the adsorption zone of the cellulase molecules can realize covalent binding with a large number of amino terminal catalytic residues of chitosan molecules (Fan et al., 2016). The process cannot destroy the catalytic center of cellulase, and the exposed catalytic clefts increase the effective attachment of immobilized cellulase to solid substrates, which further promotes enzymatic hydrolysis. Therefore, the oriented immobilization of enzymes is obtained in the SRM system, which can prevent nonproductive combinations effectively and further promote enzymatic hydrolysis.

CONCLUSION

Cellulase plays an important role in the production of fuel ethanol by the enzymatic hydrolysis of lignocellulose, and the immobilization of cellulase on the nanocarriers is an effective way to improve hydrolysis efficiency. However, the nanocarrier structure characteristics, solid–solid contact obstacles, external diffusion resistance, limited recycling frequency of nanocarriers, and nonproductive combination of enzyme active centers restricted the further improvement of hydrolysis efficiency in the complex multiphase system. Surfactants can promote the enzymatic hydrolysis of lignocellulose and play an important role in the preparation of nanocarriers. The special SRM system caused by the amphiphilicity in the oil-water reaction system can provide effective protection to obtain the immobilization of single-layer cellulase, which can effectively prevent the immobilization of cellulase and increase the effective attachment of immobilized cellulase and solid substrates.

AUTHOR CONTRIBUTIONS

ZW: conceptualization, investigation, methodology, and writing (original draft preparation). CF: methodology and article revision. XZ: data collection and writing (review and editing). ZJ: methodology and investigation. KB: formal analysis and investigation. MZ: data collection, article revision and funding acquisition. HK: methodology, writing (review and editing) and supervision.

FUNDING

This work was supported by the major projects of study on National Social Science Foundation of China and the spirit explanation of the Fifth Plenary Session of the 19th CPC Central Committee (21ZDA028) “Construction of ecological friendly water environment management system in new rural areas of socialist modernization pilot area”. And this work was also supported by the Major Project of Science and Technology in Zhejiang Province (No. 2020C02010-002).

REFERENCES

Al-Shemmari, F. H. J., Al-Mulla, E. A. J., and Rabah, A. A. (2014). A Comparative Study of Different Surfactants for Natural Rubber clay Nanocomposite Preparation. Rend. Fis. Acc. Lincei 25 (3), 409–413. doi:10.1007/s12210-014-0307-z

Alexander, K., Gajghate, S., Katarkar, A. S., Majumder, A., and Bhauumik, S. (2020). Role of Nanomaterials and Surfactants for the Preparation of Graphene Nanofluid: A Review. Mater. Today 44, 1136–1143. doi:10.1016/j.mattod.2020.11.231

Alfrén, J., Hobley, T. J., Prins, W., and Overend, R. (2014). Immobilization of Cellulase Mixtures on Magnetic Particles for Hydrolysis of Lignocellulose and nonproductive combinations effectively and further promote enzymatic hydrolysis.

Ease of Recycling. Biomass Bioenerg. 65 (3), 72–78. doi:10.1016/j.biombioe.2014.03.009

Alonso-Gómez, L. A., Heredia-Olea, E., Serna-Saldívar, S. O., and Bello-Pérez, L. A. (2019). Whole Unripe Plantain (Musa Paradisiaca L.) as Raw Material for Bioethanol Production. J. Sci. Food Agric. 99 (13), 5784–5791. doi:10.1002/jsfa.9847

Anjum, S., Shaheen, S., Awan, M. S., and Zia, R. (2019). Effect of Various Surfactants on Optical and Electrical Properties of Cu+2-Doped ZnS Semiconductor Nanoparticles. Appl. Phys. A. 125 (4), 273. doi:10.1007/s00339-019-2538-0

Arslan, B., Colpun, M., Ju, X., Zhang, X., Kostyukova, A., and Abu-Lail, N. I. (2016). The Effects of Noncellulosic Compounds on the Nanoscale Interaction Forces Measured between Carbohydrate-Binding Module and Lignocellulosic
Valencia, P., Wilson, L., Aguirre, C., and Illanes, A. (2010). Evaluation of the Incidence of Diffusional Restrictions on the Enzymatic Reactions of Hydrolysis of Penicillin G and Synthesis of Cephalaxin. Enzyme Microb. Tech. 47 (6), 268–276. doi:10.1016/j.enzmicro.2010.07.010

Wang, M., Wang, Y., Yu, D., Han, Y., and Wang, Y. (2013a). Salt Effects on the Aggregation Behavior of Triplet Zwitterionic Surfactants with Different Inter-charge Spacers in Aqueous Solution. Colloid Polym. Sci. 291 (7), 1613–1621. doi:10.1007/s00399-013-2895-2

Wang, Z., Lan, T., and Zhu, J. (2013b). Lignosulfonate and Elevated pH Can Enhance Enzymatic Saccharification of Lignocellulosics. Biotechnol. Biofuels 6 (1), 9. doi:10.1186/1754-6834-6-9

Watanabe, T., Mori, T., Tosa, T., and Chibata, I. (2010). Immobilization of Aminocyclase by Adsorption to Tannin Immobilized on Aminohexyl Cellulose. Biotechnol. Bioeng. 21 (3), 477–486. doi:10.1002/bit.260210309

Webster, J. A., Schwarz, C. E., and Bates, F. S. (2015). Using the Rotational Masking Concept to Enhance Substrate Inhibited Reaction Rates: Controlled Pore Supports for Enzyme Immobilization. Enzyme Microb. Technol. 7 (6), 266–274. doi:10.1016/j.enzymtec.2013.12.005

Wei, J., Wen, X., and Zhu, F. (2018). Inhibition of Activity and Stability of Lipase by Microemulsion-Based Organogels (MBGs) and Their Use in Organic Synthesis. Biotechnol. Biofuels 11 (1), 9. doi:10.1186/s13068-017-1021/bc200396r

Xing, Z., Su, Y., Zhang, Q., Ruan, X., Lin, Y., Wang, X., et al. (2015). Research on the Reusability of Cross-Linked Enzyme Aggregates (CLEAs) of β-galactosidase from Lactobacillus Leichmannii 313. Food Bioproducts Process. 124, 82–96. doi:10.1016/j.fbp.2020.08.004

Xu, G., Jiang, Y., Tao, R., Wang, S., Zeng, H., and Yang, S. (2016). A Recyclable Biotransformation System for L-2-Aminobutyric Acid Production Based on Immobilized Enzyme Technology. Biotechnol. Lett. 38 (1), 123–129. doi:10.1007/s10529-015-1957-3

Xu, M., Li, D., Deng, Y., and Agrei, D. (2020). Preparation and Assessment of Cross-Linked Enzyme Aggregates (CLEAs) of α-amylase from Phanerochaete chrysosporium. J. Biotechnol. 325 (3), 124691. doi:10.1016/j.jbiotec.2021.124691

Zhang, W.-W., Wang, N., Zhou, Y.-J., He, T., and Yu, X.-Q. (2012). Enhancement of Activity and Stability of Lipase by Microemulsion-Based Organogels (MBGs) and Application for Synthesis of Arylethyl Acetate. J. Mol. Catal. B: Enzymatic 78, 65–71. doi:10.1016/j.molcatb.2012.02.005

Zhang, X., Wang, S., Wu, X., Liu, S., Li, D., Xu, H., et al. (2015). Subsite-specific Contributions of Different Aromatic Residues in the Active Site Architecture of Glycoside Hydrolase Family 12. Sci. Rep. 5, 18537. doi:10.1038/srep18537

Zeng, Q., Chang, S., Wang, M., Li, M., Deng, Q., Xiong, Z., et al. (2021). Highly-active, Metal-free, Carbon-Based Orr Cathode for Efficient Organics Removal and Electricity Generation in a Pile System. Chem. Chem. Lett. 32 (7), 2212–2216. doi:10.1002/clct.2020.12.062

Zhang, B., Li, P., Zhang, H., Wang, H., Li, X., Tian, L., et al. (2016). Preparation of lipase/Zn3(PO4)2 Hybrid Nanoflower and its Catalytic Performance as an Immobilized Enzyme. Chem. Eng. J. 291, 287–297. doi:10.1016/j.cej.2016.01.104

Zhang, H., and Hay, A. G. (2019). Magnetic Biochar Derived From Biosolids via Hydrothermal Carbonization: Enzyme Immobilization, Immobilized-Enzyme Kinetics, Environmental Toxicity. J. Hazard. Mater. 384, 121272. doi:10.1016/j.jhazmat.2019.121272

Zhang, W.-W., Wang, N., Zhou, Y.-J., He, T., and Yu, X.-Q. (2012). Enhancement of Activity and Stability of Lipase by Microemulsion-Based Organogels (MBGs) and Application in Synthesis of Arylethyl Acetate. J. Mol. Catal. B: Enzymatic 78, 65–71. doi:10.1016/j.molcatb.2012.02.005

Zou, J., Chen, H., Qi, F., Zhao, X., and Liu, D. (2015). Non-ionic Surfactants Do Not Consistently Improve the Enzymatic Hydrolysis of Pure Cellulose. Bioreosur. Tech. 182, 136–145. doi:10.1016/j.biortech.2015.01.137

Zhou, Y., Zhang, L., and Tao, S. (2018). Porous TiO2with Large Surface Area Is an Efficient Catalyst Carrier for the Recovery of Wastewater Containing an Ultrahigh Concentration of Dye. RSC Adv. 8 (7), 3433–3442. doi:10.1039/c7ra11985b

Ziaei-Rad, Z., Fooladi, J., Pazouki, M., and Gummadi, S. N. (2021). Lignocellulosic Biomass Pre-treatment Using Low-Cost Ionic Liquid for Bioethanol Production: An Economically Viable Method for Wheat Straw Fractionation. Biomass and Bioenergy 151, 106140. doi:10.1016/j.biombioe.2021.106140

Zuev, Y. F., Vyleghahnina, N. N., and Zakhartchenko, N. L. (2003). Effects of Protein Solubilization on the Structure of the Surfactant Shell of Reverse Micelles. Appl. Magn. Reson. 25 (1), 29–42. doi:10.1007/s00335-006-9044-6

Conflicts of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.