Function Of Surfactants In Immobilization of Cellulase And Multiphase Hydrolysis: A Review

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Abstract

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 invalid 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.

Highlights

(1) The bridge role of surfactants in enzymatic immobilization and hydrolysis is emphasized.

(2) The restricting factors in cellulase immobilization in multiphase hydrolysis system are discussed.

(3) The oriented immobilization of cellulase based on the surfactant reversed micelle (SRM) system is proposed.

(4) The oriented immobilization mechanism of cellulase in SRM system is discussed.

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 (Adewuyi 2020; Thatoi et al. 2016; Zhao et al. 2017). 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; Balat 2011; Jing et al. 2013; Pirzadah et al. 2014; Winarni et al. 2020). The hydrophobic character of lignocellulose hinders the accessibility of enzymes to cellulose, which is a major obstacle restricting enzymatic hydrolysis (Ferreira et al. 2013; Rahikainen et al. 2011). This is because a natural “biodegradable barrier” of biomass created by the basic framework of plant cell walls under the action of covalent and non-covalent bonds render the cellulose inaccessible and difficult to hydrolyze enzymatically (Mnich et al. 2020; Nakagame et al. 2011). Therefore, lignocellulosic materials must first be pretreated to improve the cellulose fraction content and maximize the cellulase enzymatic hydrolysis efficiency (Jia et al. 2018; Rocha-Martin et al. 2018). Various studies have been conducted to achieve the efficient hydrolysis of lignocellulosic biomass. Systematic hydrolysis methods are shown in **Fig. 1**.

In general, lignin-derived inhibition is the major physical obstacle restricting the enzymatic hydrolysis of cell wall polysaccharides (Lm et al. 2019; Rahikainen et al. 2011; Tu et al. 2010; 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, whilenon-specific binding to the residual substrates also prevents the efficient recycling of cellulase (Kellock et al. 2017; Kuhad et al. 2011; Rahikainen et al. 2011). 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 (Li et al. 2016; Mehta et al. 2016; Mita and Eldin 2014; Xu et al. 2016; Zhang et al. 2016). 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. 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; Malar et al. 2020; Roth et al. 2016). Moreover, the immobilization of cellulase has been achieved based on physical adsorption, covalent binding, or affinity interactions (Hosseini et al. 2018; Zang et al. 2014; 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; Pavlidis et al. 2010; Uskokovi and Drofenik 2007). 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 (Itabaiana et al. 2014; Pavlidis et al. 2010). In addition, surfactants play an important role in the preparation of nanomaterials (Helle et al. 2010; Lou et al. 2017; Seo et al. 2011b). For the preparation of nanocarriers, forming the nano-template by micelles and emulsions of surfactants is a common method that can greatly reduce the surface tension of the solvent and change the interface composition and structure (Bao et al. 2019; Carter and Puig-Sellart 2016;

Nascimento 2014). Desirable nanostructured materials can be produced because of the special nanoreactors formed by surfactant micelles and the oriented alignment characteristics of surfactants in solution, such as the Langmuir-Blodgett (LB) membranes and liposomes (Gutierrez et al. 2016; Lok Kumar et al. 2014). Furthermore, surfactants can significantly enhance cellulose hydrolysis, thus reducing enzyme loading, especially non-ionic surfactants (Lou et al. 2017; Yan et al. 2015). However, inhibitory effects have been observed with the addition of amphoteric, anionic, and cationic surfactants (Lou et al. 2017; Yan et al. 2015). Moreover, the loss of enzyme activity during immobilization is a notable problem; the structural distortion caused by the strong enzyme-support interactions may produce steric hindrances and active site 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 invalid combination of enzymes and nanocarriers, which further improves the immobilization and hydrolysis efficiency. The reversed micelles formed by surfactants when their concentration exceeds the critical micelle concentration (CMC) in nonpolar organic solvents have been successfully used in the preparation of oriented-immobilized lipase (Fan et al. 2016). To date, few studies have reported on 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 made them widely useful in the field of nanotechnology (Yan 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. 2019; Yan et al. 2017).

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; Bhuvnesh Bharti et al. 2012; Huang et al. 2011; Junfang et al. 2018). 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 polylactic acid matrix and to evaluate the effects of surface chemistry on the reinforcement mechanisms (Orellana et al. 2018). Meanwhile, the presence of surfactants can reduce the surface energy of nanocarriers and form a steric hindrance effect, which makes it more difficult to re-agglomerate (Tan et al. 2019; Wang et al. 2013a) because 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 (Begum et al. 2019; Malar et al. 2020; Santos et al. 2015). 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 (Blanco et al. 2004; Brady and Jordaan 2009; 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 (Trevisan et al. 2000); 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; Malar et al. 2020; Santos et al. 2015); 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, 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 (Alftrén et al. 2014; Cao et al. 2016; Cipolatti et al. 2014; Xing et al. 2015). During immobilization of cellulase, magnetic chitosan microspheres (C-MNPs) are used as carriers because of their significant biological (i.e., biodegradable, biocompatible, bioactive) and chemical properties (polycationic, hydrogel, contains reactive groups, such as hydroxyl [OH] and NH_2). 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 main process of immobilizing cellulase molecules on a single magnetic nanocarrier is shown in **Fig. 2.** Chitosan was first coated on the magnetic nanocarriers for further combination with cellulase. Fe_3O_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; Zhang et al. 2014b). 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 (Berlin et al. 2016; Frančič et al. 2016; Hui et al. 2016; Santos et al. 2015; Zhou et al. 2018). The conformational change of the enzyme caused by the chemical properties of carriers during the immobilization process is shown in **Fig. 3.** In this process, the CAGs directly participate in binding with enzyme molecules, but the carrier-bound inert groups 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 active sites 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 site, and ineffective immobilization may lead to a significant loss of enzymatic activity and reduce the accessibility of the substrate to the functional site. Moreover, the partition and mass transport limitations of nanocarriers may cause spatial variation in local reaction rates and further affect enzymatic hydrolysis (Du et al. 2017). The chitosan molecules are mostly used because of the large number of -OH and amino groups (-NH_3), which are easier to co-precipitate with cellulase (Bindhu and Abraham 2010; Mo et al. 2020; Saha et al. 2019; Urrutia et al. 2018). Moreover, surface modification is an important strategy for tuning the properties of nanocarriers. Surface modification can either alter the existing property or introduce new properties onto nanoparticles using various agents, such as organ siloxane, N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), and carbodiimide as well as amino silanes, such as 3-aminopropyltriethoxysilane, aminoethyl aminopropyl polydimethylsiloxane, and silica (Chang et al. 2011; Gokhale et al. 2013; Malar et al. 2018; Malar et al. 2020; Riedel et al. 2017; Zhang et al. 2014a).

Roles Of Surfactants On Cellulase Hydrolysis

Some hydrophilic ionic liquids can accelerate the dissolution of enzyme molecules and 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 (Predvoditelev et al. 2003; Zuev et al. 2003). However, in a pure hydrophobic ionic liquid medium, immiscible nonpolar hydrophobic solvents do not cause the dehydration of biocatalysts, such as heptane, octane, and benzene. 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 (Levashov and Klyachko 2001; Muginova et al. 2010). Non-ionic surfactants can significantly accelerate the enzymatic hydrolysis of lignocellulose (Eckard et al. 2012; Qing et al. 2010; Seo et al. 2011b; Yiamsawas et al. 2017). For instance, Tween-20 can enhance the specific adsorption of cellulase, and the conversion efficiency of cellulose increased from 9–21% 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 (Lou et al. 2017; Sipos et al. 2010). The adsorption of cellulase onto lignin substrates is mostly irreversible, and non-ionic surfactants can render lignin surfaces more hydrophilic by increasing their polar surface energy component, which can reduce the enzyme adsorption (Jiang et al. 2017), thereby promoting the enzymatic hydrolysis of lignocellulose. Non-ionic surfactants can reduce the non-productive adsorption of cellulases onto lignocellulosic substrates; this change plays an important role in preventing the ineffective combination with enzymes (Jiang et al. 2017). However, for the anionic surfactant-cellulase 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 (Dyk and Pletschke 2012; 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 (Boraston et al. 2004; Liu et al. 2011; Tomme et al. 2015). 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 an invalid combination. 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 et al. 2013b; Yan et al. 2015). However, promotion effect has been observed in the oil-water micelle system formed by the low concentration of sodium lignosulfonate and cellulase because the oil-water micelles can improve the adsorption of enzymes on cellulose (Lou et al. 2014).

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; Marhuendaegea 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, -NH_2, and -SO_3H) 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; Xiaodong 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 cracks, which provide a different microenvironment (Zhang et al. 2015) because the structures of cellulase active centers are mainly composed of eight kinds of amino acids (tryptophan, tyrosine, histidine, phenylalanine, aspartic acid, glutamic acid, and arginine), most of which are hydrophobic (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 effects of cellulase on the W/O interface, and the existence of a crosslinking agent promotes the covalent crosslinking of enzyme molecules (Hyemin et al. 2012). 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, which can 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 **Fig. 4.** 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 (**Fig. 5**). 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 active sites increase the effective attachment of immobilized cellulase to solid substrates, which further promotes enzymatic hydrolysis. Therefore, the oriented immobilization of enzymes was obtained in the SRM system, which can prevent invalid 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 invalid 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, which further promotes enzymatic hydrolysis.

Declarations

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Conflicts of interest

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

Availability of data and material

Not applicable

Code availability

Not applicable

Author contributions

Zhiquan Wang: conceptualization, investigation, methodology, experiment, software, formal analysis and writing (original draft preparation).

Deyi Wu: methodology, formal analysis and investigation.

Jimeng Feng: formal analysis and investigation.

Xinze Wang: methodology, formal analysis and investigation.

Yan Lin: methodology, writing (review and editing), visualization, supervision and funding acquisition.

Ethics approval

Not applicable

Consent to participate

Not applicable

Consent for publication

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References

1. Adewuyi A (2020) Challenges and prospects of renewable energy in Nigeria: A case of bioethanol and biodiesel production. Energy Rep 6:77–88. https//doi.org/10.1016/j.egyr.2019.12.002
2. Alftrén J, Hobley TJ, Prins W, Overend R (2014) Immobilization of cellulase mixtures on magnetic particles for hydrolysis of lignocellulose and ease of recycling. Biomass Bioenerg 65:72–78. https//doi.org/10.1016/j.biombioe.2014.03.009
3. Alonso LA, Heredia E, Sernalogaldivar SO, Belloccérez LA (2019) Whole unripe plantain (Musa paradisiaca L.) as raw material for bioethanol production. J Sci Food Agric 99:1–8. https//doi.org/10.1002/jsfa.9847
4. Asghar K, Qasim M, Nelabhotla DM, Das D (2016) Effect of surfactant and electrolyte on surface modification of c-plane GaN substrate using chemical mechanical planarization (CMP) process. Colloids Surface A 497:133–145. https//doi.org/10.1016/j.colsurfa.2016.02.035
5. Balat M (2011) Production of bioethanol from lignocellulosic materials via the biochemical pathway: A review. Energ Convers Manage 52:858–875. https//doi.org/10.1016/j.enconman.2010.08.013
6. Bao Y, Liu P, Guo J (2019) Research progress on the preparation of nanomaterials and mesoporous materials using gemini surfactants. Mater Rep 33:3678–3685. https//doi.org/10.11896/cldb.18050316
7. Barbosa O, Ortiz C, Berenguer-Murcia Á, Torres R, Rodrigues RC, Fernandez-Lafuente R (2015) Strategies for the one-step immobilization–purification of enzymes as industrial biocatalysts. Biotechnol Adv 33:435–456. https//doi.org/10.1016/j.biotechadv.2015.03.006
8. Begum G, Oschatz C, Oschatz M, Kaskel S, Brunner E, Kroeger N (2019) Influence of silica architecture on the catalytic activity of immobilized glucose oxidase. Bioinspir Biomim Nan 8:72–80. https//doi.org/10.1680/jbibn.18.00002

9. Berlin Z, Jun R, Li X, Lingyun J (2016) Direct site-specific immobilization of protein A via aldehyde-hydrazide conjugation. J Chromatogr B 1008:132–138. https//doi.org/10.1016/j.jchromb.2015.11.019
10. Bhuvnesh B, Jens M, Urs G, Findenegg GH (2012) Surfactant adsorption and aggregate structure at silica nanoparticles: Effects of particle size and surface modification. Soft Matter 8:6573–6581. https//doi.org/10.1039/c2sm25648g
11. Bindhu LV, Abraham ET (2010) Immobilization of horseradish peroxidase on chitosan for use in nonaqueous media. J Appl Polym Sci 88:1456–1464. https//doi.org/10.1002/app.11815
12. Blanco RM, Terreros P, Fernández-Pérez M, Otero C, DíAz-González G (2004) Functionalization of mesoporous silica for lipase immobilization: Characterization of the support and the catalysts. J Mol Catal B-Enzym 30:83–93. https//doi.org/10.1016/j.molcatb.2004.03.012
13. Boraston AB, Bolam DN, Gilbert HJ, Davies GJ (2004) Carbohydrate-binding modules: fine-tuning polysaccharide recognition. Biochem J 382:769–781. https//doi.org/10.1042/BJ20040892
14. Brady D, Jordaan J (2009) Advances in enzyme immobilisation. Biotechnol Lett 31:1639–1650. https//doi.org/10.1016/S0378-7788(00)00086-4
15. Cao S, Xu P, Ma Y, Yao X, Yao Y, Zong M, Li X, Lou W (2016) Recent advances in immobilized enzymes on nanocarriers. Chinese J Catal 37:1814–1823. https//doi.org/10.1016/S1872-2067(16)62528-7
16. Carlsson N, Gustafsson H, Thorn C, Olsson L, Holmberg K, Åkermana BR (2014) Enzymes immobilized in mesoporous silica: A physical–chemical perspective. Adv Colloid Interfac 205:339–360. https//doi.org/10.1016/j.cis.2013.08.010
17. Carter KC, Puig-Sellart M (2016) Nanocarriers made from non-ionic surfactants or natural polymers for pulmonary drug delivery. Curr Pharm Design 22:3324–3331. https//doi.org/10.2174/1381612822666160418121700
18. Chang HY, Jang J, Wu CW (2011) Cellulase immobilized mesoporous silica nanocatalysts for efficient cellulose-to-glucose conversion. Green Chem 13:2844–2850. https//doi.org/10.1039/c1gc15563f
19. Chen LF, Gong CS, Tsao GT (2010) Immobilized glucose isomerase on deae cellulose beads. Starch Stärke 33:58–63. https//doi.org/10.1002/star.19810330207
20. Cherian E, Dharmendirakumar M, Baskar G (2015) Immobilization of cellulase onto MnO_2 nanoparticles for bioethanol production by enhanced hydrolysis of agricultural waste. Chinese J Catal 36:1223–1229. https//doi.org/10.1016/S1872-2067(15)60906-8
21. Cipolatti EP, Silva MJA, Klein M, Feddern V, Feltes MMC, Oliveira JV, Ninow JL, Oliveira DD (2014) Current status and trends in enzymatic nanoimmobilization. J Mol Catal B-Enzym 99:56–67. https//doi.org/10.1016/j.molcatb.2013.10.019
22. Cristina GG, Ángel BM, Roberto FL, Rafael C, Rodrigues RC (2011) Potential of different enzyme immobilization strategies to improve enzyme performance. Adv Synth Catal 353:2885–2904. https//doi.org/10.1002/adsc.201100534

23. Dong XY, Feng XD, Sun Y (2010) His-tagged protein purification by metal-chelate affinity extraction with nickel-chelate reverse micelles. Biotechnol Prog 26:1088–1094. https//doi.org/10.1002/btpr.428
24. Du J, Cao Y, Liu G, Zhao J, Li X, Qu Y (2017) Identifying and overcoming the effect of mass transfer limitation on decreased yield in enzymatic hydrolysis of lignocellulose at high solid concentrations. Bioresource Technolo 229:88–95. https//doi.org/10.1016/j.biortech.2017.01.011
25. Dyk JSV, Pletschke BI (2012) A review of lignocellulose bioconversion using enzymatic hydrolysis and synergistic cooperation between enzymes-Factors affecting enzymes, conversion and synergy. Biotechnolo Adv 30:1458–1480. https//doi.org/10.1016/j.biotechadv.2012.03.002
26. Eckard AD, Muthukumarappan K, Gibbons W (2012) Pretreatment of extruded corn stover with polyethylene glycol to enhance enzymatic hydrolysis: Optimization, kinetics, and mechanism of action. Bioenerg Res 5:424–438. https//doi.org/10.1007/s12155-011-9162-2
27. Fan Y, Wu G, Su F, Li K, Xu L, Han X, Yan Y (2016) Lipase oriented-immobilized on dendrimer-coated magnetic multi-walled carbon nanotubes toward catalyzing biodiesel production from waste vegetable oil. Fuel 178:172–178. https//doi.org/10.1016/j.fuel.2016.03.071
28. Ferreira SDS, Nishiyama MY, Paterson AH, Souza GM (2013) Biofuel and energy crops: high-yield Saccharinae take center stage in the post-genomics era. Genome Biol 14:210. https//doi.org/10.1186/gb-2013-14-6-210
29. Frančič N, Bellino MG, Solerillia GJ, Lobnik A (2016) Mesoporous titania thin films as efficient enzyme carriers for paraoxon determination/detoxification: effects of enzyme binding and pore hierarchy on the biocatalyst activity and reusability. Analyst 141:4235. https//doi.org/10.1039/c6an90029a
30. Fujita K, Ohno H (2010) Enzymatic activity and thermal stability of metallo proteins in hydrated ionic liquids. Biopolymers 93:1093–1099. https//doi.org/10.1002/bip.21526
31. Gokhale AA, Lu J, Lee I (2013) Immobilization of cellulase on magnetoresponsive graphene nano-supports. J Mol Catal B-Enzym 90:76–86. https//doi.org/10.1016/j.molcatb.2013.01.025
32. Gutierrez J, Cruz J, Rondón-Villarreal P, Jones N, Ortiz C (2016) Small gold nanocomposites obtained in reverse micelles as nanoreactors. Effect of surfactant, optical properties and activity against Pseudomona aeruginosa. New J Chem 40:10432–10439. https//doi.org/10.1039/C6NJ02259F
33. Hasegawa M, Kitano H, Nishida R, Kobashi T (2010) Macroporous and hydrophilic polymer resins modified with isothiocyanate groups for immobilization of enzymes. Biotechnol Bioeng 36:219–223. . 10.1002/bit.260360302
34. Helle SS, Duff SJB, Cooper DG (2010) Effect of surfactants on cellulose hydrolysis. Biotechnol Bioeng 42:611–617. https//doi.org/10.1002/bit.260420509
35. Hernandez K, Fernandez-Lafuente R (2011) Control of protein immobilization: Coupling immobilization and site-directed mutagenesis to improve biocatalyst or biosensor performance. Enzyme Microb Technol 48:107–122. https//doi.org/10.1016/j.enzmictec.2010.10.003
36. Hosseini SH, Hosseini SA, Zohreh N, Yaghoubi M, Pourjavadi A (2018) Covalent immobilization of cellulase using magnetic poly(ionic liquid) support: Improvement of the enzyme activity and stability.

J Agr Food Chem 66:789. https//doi.org/10.1021/acs.jafc.7b03922

37. Huang Y, Jihuai WU, Huang ML, Lin JM, Huang YF (2011) Influence of surfactants on the morphology and photocatalytic activity of Bi_2WO_6 by hydrothermal synthesis. Sci China Chem 54:211–216. https//doi.org/10.1007/s11426-010-4166-x
38. Hui J, Yingwu W, Yan B, Rong L, Renjun G (2016) Site-specific, covalent immobilization of dehalogenase ST2570 catalyzed by formylglycine-generating enzymes and its application in batch and semi-continuous flow reactors. Molecules 21:895. https//doi.org/10.3390/molecules21070895
39. Hyemin P, Jungoh A, Juwhan L, Hyeokwon L, Chunsuk K (2012) Expression, immobilization and enzymatic properties of glutamate decarboxylase fused to a cellulose-binding domain. Int J Mol Sci 13:358–368. https//doi.org/10.3390/ijms13010358
40. Itabaiana I, GonçAlves KM, Zoumpanioti M, Leal ICR, Miranda LSME, Xenakis A, De-Souza ROMA (2014) Microemulsion-based organogels as an efficient support for lipase-catalyzed reactions under continuous-flow conditions. Org Process Res Dev 18:1372. https//doi.org/10.1021/op500136c
41. Jia W, Collins SRA, Adam E, Nikolaus W, Jo D, Roberts IN, Waldron KW (2018) Release of cell wall phenolic esters during hydrothermal pretreatment of rice husk and rice straw. Biotechnol Biofuels 11:162. https//doi.org/10.1186/s13068-018-1157-1
42. Jiang F, Qian C, Esker AR, Roman M (2017) Effect of non-ionic surfactants on dispersion and polar interactions in the adsorption of cellulases onto lignin. J Phys Chem B 121:9607–9620. https//doi.org/10.1021/acs.jpcb.7b07716
43. Jing Z, Quan CS, Fan SD (2013) Role of lignin in bio-ethanol production from lignocellulosic biomass. J Biobased Mater Bio 7:533–540. https//doi.org/10.1166/jbmb.2013.1382
44. Jordan J, Kumar CSSR, Theegala C (2011) Preparation and characterization of cellulase-bound magnetite nanoparticles. J Mol Catal B-Enzym 68:139–146. https//doi.org/10.1016/j.molcatb.2010.09.010
45. Junfang W, Xiaojing W, Fang Z (2018) Influence of surfactant on the morphology and photocatalytic activity of anatase TiO_2 by solvothermal synthesis. J Nano 2018:1–7. https//doi.org/10.1155/2018/3086269
46. Kalantari M, Kazemeini M, Arpanaei A (2013) Evaluation of biodiesel production using lipase immobilized on magnetic silica nanocomposite particles of various structures. Biochem Eng J 79:267–273. https//doi.org/10.1016/j.bej.2013.09.001
47. Kellock M, Rahikainen J, Marjamaa K, Kruus K (2017) Lignin-derived inhibition of monocomponent cellulases and a xylanase in the hydrolysis of lignocellulosics. Bioresource Technolo 232:183–191. https//doi.org/10.1016/j.biortech.2017.01.072
48. Kuhad RC, Gupta R, Khasa YP, Singh A, Zhang YHP (2011) Bioethanol production from pentose sugars: Current status and future prospects. Renew sust energ Rev 15:4950–4962. https//doi.org/10.1016/j.rser.2011.07.058
49. Kumar R, Wyman CE (2010) Effect of additives on the digestibility of corn stover solids following pretreatment by leading technologies. Biotechnol Bioeng 102:1544–1557.

https//doi.org/10.1002/bit.22203

50. Levashov AV, Klyachko NL (2001) Micellar enzymology: methodology and technique. Russ Chem B + 50:1718–1732. https//doi.org/10.1023/A:1014361508512
51. Li FH, Tang N, Wang YQ, Zhang L, Du W, Xiang J, Cheng PG (2018) Synthesis and characterization of magnetic carriers based on immobilized enzyme. Iop Conference 359: 012044. https//doi.org/10.1088/1757-899X/359/1/012044
52. Li J, Li S, Fan C, Yan Z (2012) The mechanism of poly(ethylene glycol) 4000 effect on enzymatic hydrolysis of lignocellulose. Colloid Surface B 89:203–210. https//doi.org/10.1016/j.colsurfb.2011.09.019
53. Li M, Yue Y, Zhang ZJ, Wang ZY, Tan TW, Fan LH (2016) Site-specific and high-loading immobilization of proteins by using cohesin-dockerin and CBM-cellulose interactions. Bioconj Chem 27:1579–1583. https//doi.org/10.1021/acs.bioconjchem.6b00282
54. Liu J, Shi J, Li J, Yuan X (2011) Characterization of the interaction between surfactants and enzymes by fluorescence probe. Enzyme Microb Tech 49:360–365. https//doi.org/10.1016/j.enzmictec.2011.06.014
55. Lok Kumar S, Rekha Goswami S, Neus V, Carlos RA, Katsuhiko A (2014) In-situ formation of silver nanoparticles using nonionic surfactant reverse micelles as nanoreactors. J Nanosci Nanotechno 14:2238–2244. https//doi.org/info:doi/10.1166/jnn.2014.8548
56. Lou H, Zeng M, Hu Q, Cai C, Lin X, Qiu X, Yang D, Pang Y (2017) Nonionic surfactants enhanced enzymatic hydrolysis of cellulose by reducing cellulase deactivation caused by shear force and air-liquid interface. Bioresour Technol 249:1–8. https//doi.org/10.1016/j.biortech.2017.07.066
57. Lou H, Zhou H, Li X, Wang M, Qiu X (2014) Understanding the effects of lignosulfonate on enzymatic saccharification of pure cellulose. Cellulose 21:1351–1359. https//doi.org/10.1007/s10570-014-0237-z
58. Luan Y, Ramos L (2010) Role of the preparation procedure in the formation of spherical and monodisperse surfactant/polyelectrolyte complexes. Chem-Eur J 13:6108–6114. https//doi.org/10.1002/chem.200601422
59. Malar CG, Seenuvasan M, Kumar KS (2018) Prominent study on surface properties and diffusion coefficient of urease-conjugated magnetite nanoparticles. Appl Biochem Biotechnol 186:174–185. https//doi.org/10.1007/s12010-018-2719-1
60. Malar CG, Seenuvasan M, Kumar KS, Kumar A, Parthiban R (2020) Review on surface modification of nanocarriers to overcome diffusion limitations: An enzyme immobilization aspect. Biochem Eng J 158:107574. https//doi.org/10.1016/j.bej.2020.107574
61. Marhuendaegea FC, Pieravelázquez S, Cadenas C, Cadenas E (2015) Reverse micelles in organic solvents: a medium for the biotechnological use of extreme halophilic enzymes at low salt concentration. Archaea 1:105. https//doi.org/10.1155/2002/626457
62. Mehta J, Bhardwaj N, Bhardwaj SK, Kim KH, Deep A (2016) Recent advances in enzyme immobilization techniques: Metal-organic frameworks as novel substrates. Coordin Chem Rev

322:30–40. https//doi.org/10.1016/j.ccr.2016.05.007

63. Mita DG, Eldin MSM (2014) Immobilized enzymes: Strategies for overcoming the substrate diffusion-limitation problem. Curr Biotechno 3:207–217. https//doi.org/10.2174/2211550103031409181147 37
64. Mnich E, Bjarnholt N, Eudes A, Harholt J, Ulvskov P (2020) Phenolic cross-links: building and de-constructing the plant cell wall. Nat Prod Rep 37:1–43. https//doi.org/10.1039/c9np00028c
65. Mo H, Qiu J, Yang C, Zang L, Chen J (2020) Porous biochar/chitosan composites for high performance cellulase immobilization by glutaraldehyde. Enzyme Microb Technol 138:109561. https//doi.org/10.1016/j.enzmictec.2020.109561
66. Moniruzzaman M, Kamiya N, Goto M (2010) Activation and stabilization of enzymes in ionic liquids. Org Biomol Chem 8:2887–2899. https//doi.org/10.1039/b926130c
67. Mroczkiewicz M, Bronowska A, Pietrzak M, Malinowska E (2012) Different methods of acid phosphatase immobilization for its application in FIA systems with potentiometric detection. Procedia Eng 47:265–268. https//doi.org/10.1016/j.proeng.2012.09.134
68. Muginova SV, Galimova AZ, Polyakov AE, Shekhovtsova TN (2010) Ionic liquids in enzymatic catalysis and biochemical methods of analysis: Capabilities and prospects. J Anal Chem + 65:331–351. https//doi.org/10.1134/S1061934810040027
69. Nakagame S, Chandra RP, Kadla JF, Saddler JN (2011) Enhancing the enzymatic hydrolysis of lignocellulosic biomass by increasing the carboxylic acid content of the associated lignin. Biotechnol Bioeng 108:538–548. https//doi.org/10.1002/bit.22981
70. Nakayama RI, Imai M, Suzuki I (2009) Enzymatic cellulose degradation using suitable combination of cellulase and enhancement of reaction rate with the aid of ultrasonic pretreatment. J Biosci Bioeng 108:S89. https//doi.org/10.1016/j.jbiosc.2009.08.261
71. Nascimento RSV (2014) Surfactants nanocarriers to enhanced oil recovery (EOR). TechConnect Briefs 3:505–508
72. Neira HD, Herr AE (2017) Kinetic analysis of enzymes immobilized in porous film arrays. Anal Chem 89:10311–10320. https//doi.org/10.1021/acs.analchem.7b02075
73. Nicoletti G, Cipolatti EP, Valério A, Carbonera NG, Soares NS, Theilacker E, Ninow JL, Oliveira DD (2015) Evaluation of different methods for immobilization of Candida antarctica lipase B (CalB lipase) in polyurethane foam and its application in the production of geranyl propionate. Bioprocess Biosyst Eng 38:1739–1748. https//doi.org/10.1007/s00449-015-1415-6
74. Orellana JL, Wichhart D, Kitchens CL (2018) Mechanical and optical properties of polylactic acid films containing surfactant-modified cellulose nanocrystals. J Nano 2018:1–12. https//doi.org/10.1155/2018/7124260
75. Pavlidis IV, Tzafestas K, Stamatis H (2010) Water-in-ionic liquid microemulsion-based organogels as novel matrices for enzyme immobilization. Biotechnol J 5:805–812. https//doi.org/10.1002/biot.201000052

76. Pirzadah TB, Malik B, Kumar M, Rehman RU (2014) Lignocellulosic biomass: As future alternative for bioethanol production. In: Hakeem K, Jawaid M, Rashid U (eds) Springer, Cham. pp 145–163. https://doi.org/10.1007/978-3-319-07578-5_8
77. Predvoditelev DA, Kosarev GV, Nifant'Ev EE (2003) New type of cationic glycerophospholipids. Russ J Gen Chem + 73:1522–1529. https://doi.org/10.1023/B:RUGC.0000016014.90042.3b
78. Priydarshani S, Mustafa M, Yuan G, Robinson AJ, Ilias K (2018) Immobilization and stabilization of alcohol dehydrogenase on polyvinyl alcohol fibre. Biotechnol Rep 19:e00260. https//doi.org/10.1016/j.btre.2018.e00260
79. Qing Q, Yang B, Wyman CE (2010) Impact of surfactants on pretreatment of corn stover. Bioresour Technol 101:5941–5951. https//doi.org/10.1016/j.biortech.2010.03.003
80. Rahikainen J, Mikander S, Marjamaa K, Tamminen T, Lappas A, Viikari L, Kruus K (2011) Inhibition of enzymatic hydrolysis by residual lignins from softwood-study of enzyme binding and inactivation on lignin-rich surface. Biotechnol Bioeng 108:2823–2834. https//doi.org/10.1002/bit.23242
81. Riedel M, Sabir N, Scheller FW, Parak WJ, Lisdat F (2017) Connecting quantum dots with enzymes: mediator-based approaches for the light-directed read-out of glucose and fructose oxidation. Nanoscale 9:2814–2823. https//doi.org/10.1039/C7NR00091J
82. Rocha-Martin J, Martinez-Bernal C, Zamorano LS, Manuel RSF, Diez GB (2018) Inhibition of enzymatic hydrolysis of pretreated corn stover and sugar cane straw by laccases. Process Biochem 67:88–91. https//doi.org/10.1016/j.procbio.2018.01.021
83. Roth H, Schwaminger SP, Peng F, Berensmeier S (2016) Immobilization of cellulase on magnetic nanocarriers. Chem open 5:183–187. https//doi.org/10.1002/open.201600028
84. Saha K, Verma P, Sikder J, Chakraborty S, Curcio S (2019) Synthesis of chitosan-cellulase nanohybrid and immobilization on alginate beads for hydrolysis of ionic liquid pretreated sugarcane bagasse. Renew Energ 133:66–76. https//doi.org/10.1016/j.renene.2018.10.014
85. Santos JCSD, Barbosa O, Ortiz C, Berenguer MA, Rodrigues RC, Fernandez-Lafuente R (2015) Importance of the support properties for immobilization or purification of enzymes. Chemcatchem 7:2413–2432. https//doi.org/10.1002/cctc.201500310
86. Seo DJ, Fujita H, Sakoda A (2011a) Effects of a non-ionic surfactant, Tween 20, on adsorption/desorption of saccharification enzymes onto/from lignocelluloses and saccharification rate. Adsorption 17:813–822. https//doi.org/10.1007/s10450-011-9340-8
87. Seo DJ, Fujita H, Sakoda A (2011b) Structural changes of lignocelluloses by a nonionic surfactant, Tween 20, and their effects on cellulase adsorption and saccharification. Bioresource Technol 102:9605–9612. https//doi.org/10.1016/j.biortech.2011.07.034
88. Sipos B, Dienes D, Schleicher Á, Perazzini R, Crestini C, Siika-aho M, Réczey K (2010) Hydrolysis efficiency and enzyme adsorption on steam-pretreated spruce in the presence of poly(ethylene glycol). Enzyme Microb Technol 47:84–90. https//doi.org/10.1016/j.enzmictec.2010.05.010
89. Steen RE, Ta DT, Cortens D, Billen B, Guedens W, Adriaensens P (2013) Protein engineering for directed immobilization. Bioconjug Chem 24:1761–1777. https//doi.org/10.1021/bc4002823

90. Suárez S, Guerrero C, Vera C, Illanes A (2018) Effect of particle size and enzyme load on the simultaneous reactions of lactose hydrolysis and transgalactosylation with glyoxyl-agarose immobilized β-galactosidase from Aspergillus oryzae. Process Biochem 73:56–64. https//doi.org/10.1016/j.procbio.2018.08.016
91. Takagi S, Arakawa K, Shimada T, Inoue H (2019) Reversed micelles formed by polyfluorinated surfactant II; the properties of core water phase in reversed micelle. B Chem Soc Jpn 92:1200–1204. https//doi.org/10.1246/bcsj.20190086
92. Tan J, Xiong X, He Z, Cao F, Sun D (2019) Aggregation behavior of polyether based siloxane surfactants in aqueous solutions: Effect of alkyl groups and steric hindrance. J Phys Chem B 123:1390–1399. https//doi.org/10.1021/acs.jpcb.8b10727
93. Tao L, Zhao Y, Wang X, Xiang L, Yan Y (2013) A novel oriented immobilized lipase on magnetic nanoparticles in reverse micelles system and its application in the enrichment of polyunsaturated fatty acids. Bioresource Technol 132:99–102. https//doi.org/10.1016/j.biortech.2012.12.191
94. Thatoi H, Dash PK, Mohapatra S, Swain MR (2016) Bioethanol production from tuber crops using fermentation technology: a review. Int J Sol Energ 35:443–468. https//doi.org/10.1080/14786451.2014.918616
95. Tomme P, Creagh AL, Kilburn DG, Haynes CA (2015) Interaction of polysaccharides with the N-terminal cellulose-binding domain of Cellulomonas fimi CenC. 1- Binding specificity and calorimetric analysis. Biochemistry 35:13885–13894. https//doi.org/10.1021/bi961185i
96. Tu M, Chandra RP, Saddler JN (2010) Recycling cellulases during the hydrolysis of steam exploded and ethanol pretreated lodgepole pine. Biotechnol Prog 23:1130–1137. https//doi.org/10.1021/bp070129d
97. Urrutia P, Bernal C, Wilson L, Illanes A (2018) Use of chitosan heterofunctionality for enzyme immobilization: β-galactosidase immobilization for galacto-oligosaccharide synthesis. Int J Biol Macromol 116:182–193. https//doi.org/10.1016/j.ijbiomac.2018.04.112
98. Uskokovi V, Drofenik M (2007) Reverse micelles: inert nano-reactors or physico-chemically active guides of the capped reactions. Adv Colloid Interfac 133:23–34. https//doi.org/10.1016/j.cis.2007.02.002
99. Valencia P, Wilson L, Aguirre C, Illanes A (2010) Evaluation of the incidence of diffusional restrictions on the enzymatic reactions of hydrolysis of penicillin G and synthesis of cephalexin. Enzyme Microb Tech 47:268–276. https//doi.org/10.1016/j.enzmictec.2010.07.010
100. Wang M, Wang Y, Yu D, Han Y, Wang Y (2013a) Salt effects on the aggregation behavior of tripolar zwitterionic surfactants with different inter-charge spacers in aqueous solution. Colloid Polym Sci 291:1613–1621. https//doi.org/10.1007/s00396-013-2895-z
101. Wang ZJ, Lan TQ, Zhu JY (2013b) Lignosulfonate and elevated pH can enhance enzymatic saccharification of lignocelluloses. Biotechnol Biofuels 6:9. https//doi.org/10.1186/1754-6834-6-9
102. Watanabe T, Mori T, Tosa T, Chibata I (2010) Immobilization of aminoacylase by adsorption to tannin immobilized on aminohexyl cellulose. Biotechnol Bioeng 21:477–486.

https//doi.org/10.1002/bit.260210309

103. Winarni I, Bardant T, Hendra D (2020) Enhancement the added value of sengon wood waste pulp as bioenergy raw material for bioethanol production. IOP Conference Series Earth and Environmental Science 415:012012. https//doi.org/10.1088/1755-1315/415/1/012012
104. Xiaodong C, Gretchen P, Chandler B, Vincent L (2018) Controlling structure beyond the initial coordination sphere: Complexation-induced reversed micelle formation in calix[4]pyrrole-containing diblock copolymers. J Am Chem Soc 140:13219–13222. https//doi.org/10.1021/jacs.8b09620
105. Xing Z, Su Y, Zhang Q, Ruan X, Lin Y, Wang X, Kong H (2015) Research progress on cellulase immobilized by magnetic nanoparticles as carriers. Biotechnol Bullet 8:59–65. https//doi.org/10.13560/j.cnki.biotech.bull.1985.2015.08.009
106. Xu G, Jiang Y, Tao R, Wang S, Zeng H, Yang S (2016) A recyclable biotransformation system for L-2-aminobutyric acid production based on immobilized enzyme technology. Biotechnol Lett 38:123–129. https//doi.org/10.1007/s10529-015-1957-3
107. Yan B, Jiajia G, Jianzhong M, Pan L, Qiaoling K (2017) Cationic silicon-based gemini surfactants: Effect of hydrophobic chains on surface activity, physic-chemical properties and aggregation behaviors. J Ind Eng Chem 53:51–61. https//doi.org/10.1016/j.jiec.2017.03.045
108. Yan Z, Hongmei C, Feng Qi, Xuebing Z, Dehua L (2015) Non-ionic surfactants do not consistently improve the enzymatic hydrolysis of pure cellulose. Bioresour Technol 182:136–143. https//doi.org/10.1016/j.biortech.2015.01.137
109. Yang Q, Wang B, Zhang Z, Lou D, Tan J, Zhu L (2017) The effects of macromolecular crowding and surface charge on the properties of an immobilized enzyme: activity, thermal stability, catalytic efficiency and reusability. Rsc Adv 7:38028–38036. https//doi.org/10.1039/C7RA06544B
110. Yiamsawas D, Beckers S, Lu H, Landfester K, Wurm FR (2017) Morphology-controlled synthesis of lignin nanocarriers for drug delivery and carbon materials. ACS Biomater-Sci Eng 3:1–26. https//doi.org/10.1021/acsbiomaterials.7b00278
111. Yu CC, Kuo YY, Liang CF, Chien WT, Wu HT, Chang TC, Jan FD, Lin CC (2012) Site-specific immobilization of enzymes on magnetic nanoparticles and their use in organic synthesis. Bioconj Chem 23:714. https//doi.org/10.1021/bc200396r
112. Yu Y, Zhang J (2016) Interaction of cellulase with surfactants and their application in detergent. CIESC J 67:3023–3031. https//doi.org/10.11949/j.issn.0438-1157.20151989
113. Zang L, Qiu J, Wu X, Zhang W, Sakai E, Wei Y (2014) Preparation of magnetic chitosan nanoparticles as support for cellulase immobilization. Ind Eng Chem Res 53:3448–3454. https//doi.org/10.1021/ie404072s
114. Zhang B, Li P, Zhang H, Wang H, Li X, Tian L, Ali N, Ali Z, Zhang Q (2016) Preparation of lipase/$Zn_3(PO_4)_2$ hybrid nanoflower and its catalytic performance as an immobilized enzyme. Chem Eng J 291:287–297. https//doi.org/10.1016/j.cej.2016.01.104
115. Zhang B, Yang T, Hu X, Zhang J (2014a) Characteristic of the cellulose immobilized on aminated SiO2. Acta Sci Natur Univ Pekinensis 50:965–968. https//doi.org/10.13209/j.0479-8023.2014.126

116. Zhang H, Hay AG (2019) Magnetic biochar derived from biosolids via hydrothermal carbonization: Enzyme immobilization, immobilized-enzyme kinetics, environmental toxicity. J Hazard Mater 384:121272. https//doi.org/10.1016/j.jhazmat.2019.121272
117. Zhang WW, Wang N, Zhou YJ, He T, Yu XQ (2012) Enhancement of activity and stability of lipase by microemulsion-based organogels (MBGs) immobilization and application for synthesis of arylethyl acetate. J Mol Catal B-Enzym 78:65–71. https//doi.org/10.1016/j.molcatb.2012.02.005
118. Zhang WJ, Qiu JH, Feng HX, Wu XL, Zang LM, Yi W, Eiichi S (2014b) Preparation and characterization of functionalized magnetic silica nanospheres with the immobilized cellulase. Appl Mechan Mater 543–547:3892–3895. https//doi.org/10.4028/www.scientific.net/AMM.543-547.3892
119. Zhang X, Wang S, Wu X, Liu S, Li D, Xu H, Gao P, Chen G, Wang L (2015) Subsite-specific contributions of different aromatic residues in the active site architecture of glycoside hydrolase family 12. Sci Rep-UK 5:18357. https//doi.org/10.1038/srep18357
120. Zhao J, He C, Cui B, Xiong J, Jiang H, Ao J, Xiang G (2017) Recyclable magnetic carboxymethyl chitosan/calcium alginate–cellulase bioconjugates for corn stalk hydrolysis. Carbohyd Polym 166:358–364. https//doi.org/10.1016/j.carbpol.2017.03.003
121. Zhou Y, Chen H, Qi F, Zhao X, Liu D (2015) Non-ionic surfactants do not consistently improve the enzymatic hydrolysis of pure cellulose. Bioresour Technol 182:136–143. https//doi.org/10.1016/j.biortech.2015.01.137
122. Zhou Y, Zhang L, Tao S (2018) Porous TiO_2 with large surface area is an efficient catalyst carrier for the recovery of wastewater containing an ultrahigh concentration of dye. Rsc Adv 8:3433–3442. https//doi.org/10.1039/C7RA11985B
123. Zuev YF, Vylegzhanina NN, Zakhartchenko NL (2003) Effects of protein solubilization on the structure of the surfactant shell of reverse micelles. Appl Magn Reson 25:29–42. https//doi.org/10.1007/BF03166964

Figures

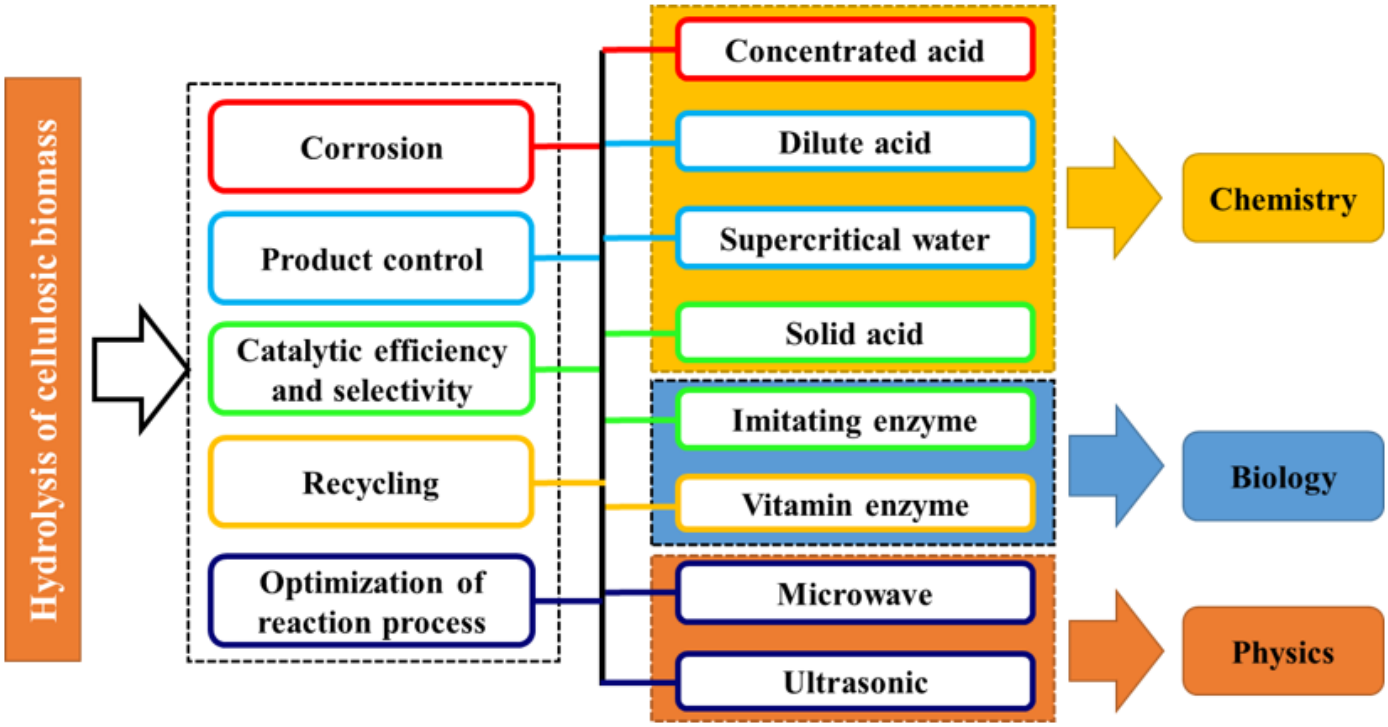

Figure 1

Hydrolysis methods of cellulosic biomass

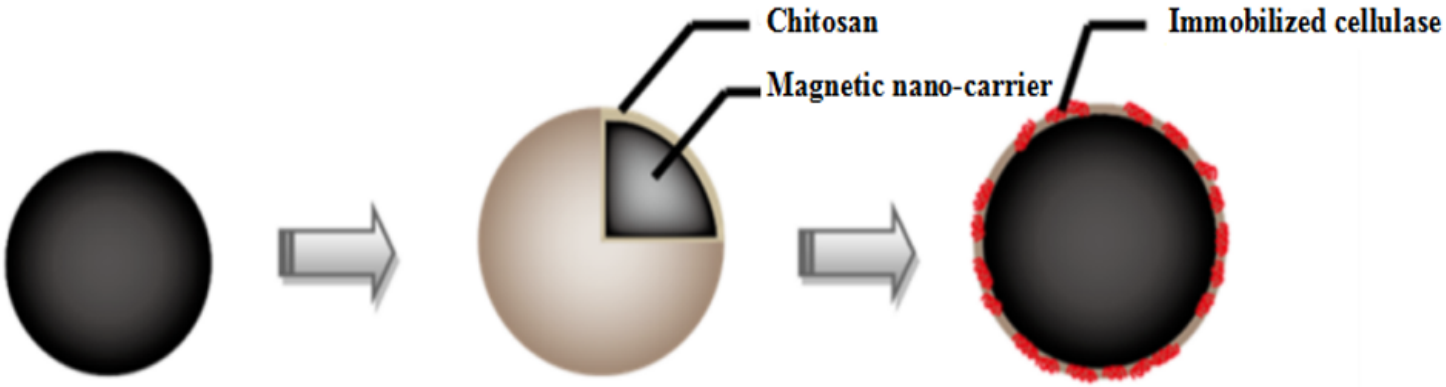

Figure 2

Schematic diagram of immobilized cellulase on a magnetic nanocarrier

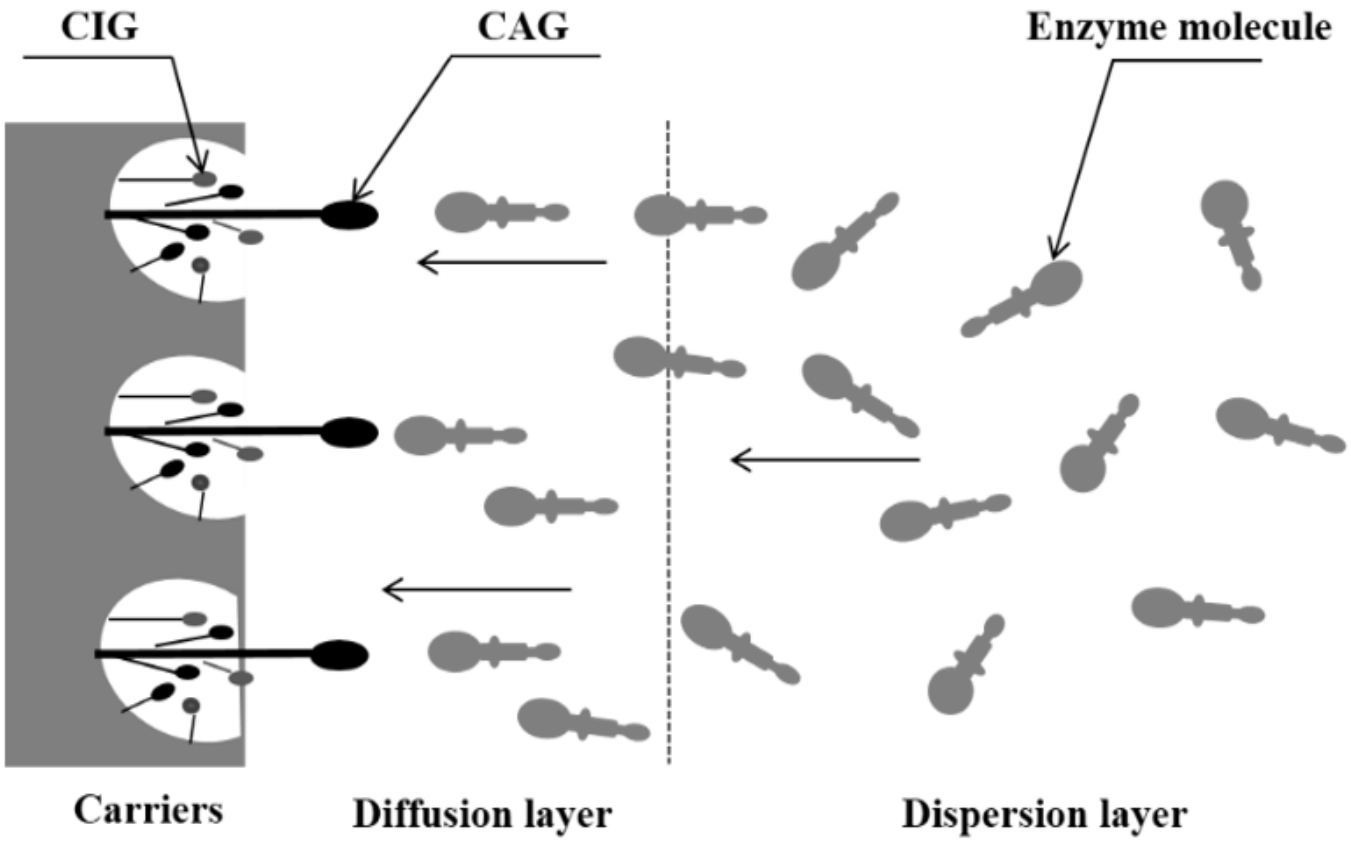

Figure 3

Schematic diagram of the conformational change of the enzyme caused by carrier chemistry during the immobilization process

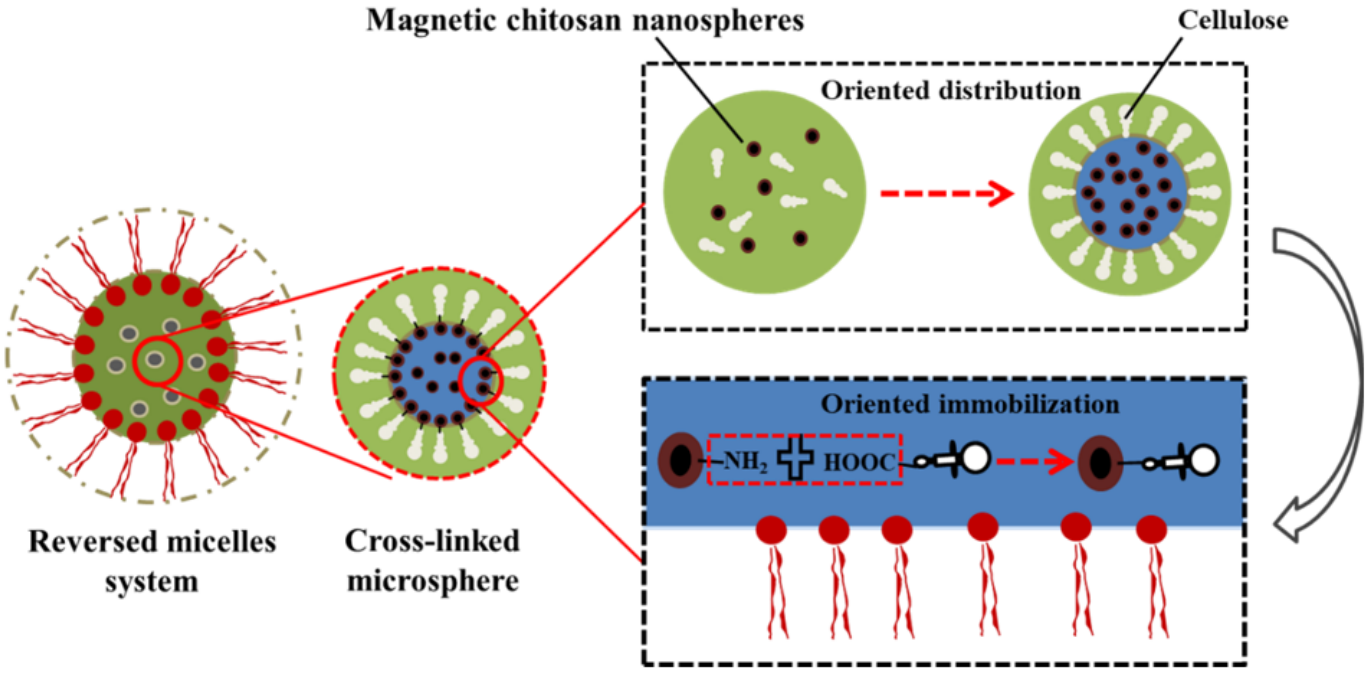

Figure 4

The oriented immobilization diagrammatic sketch of single-layer cellulase in the surfactant reversed micelles system

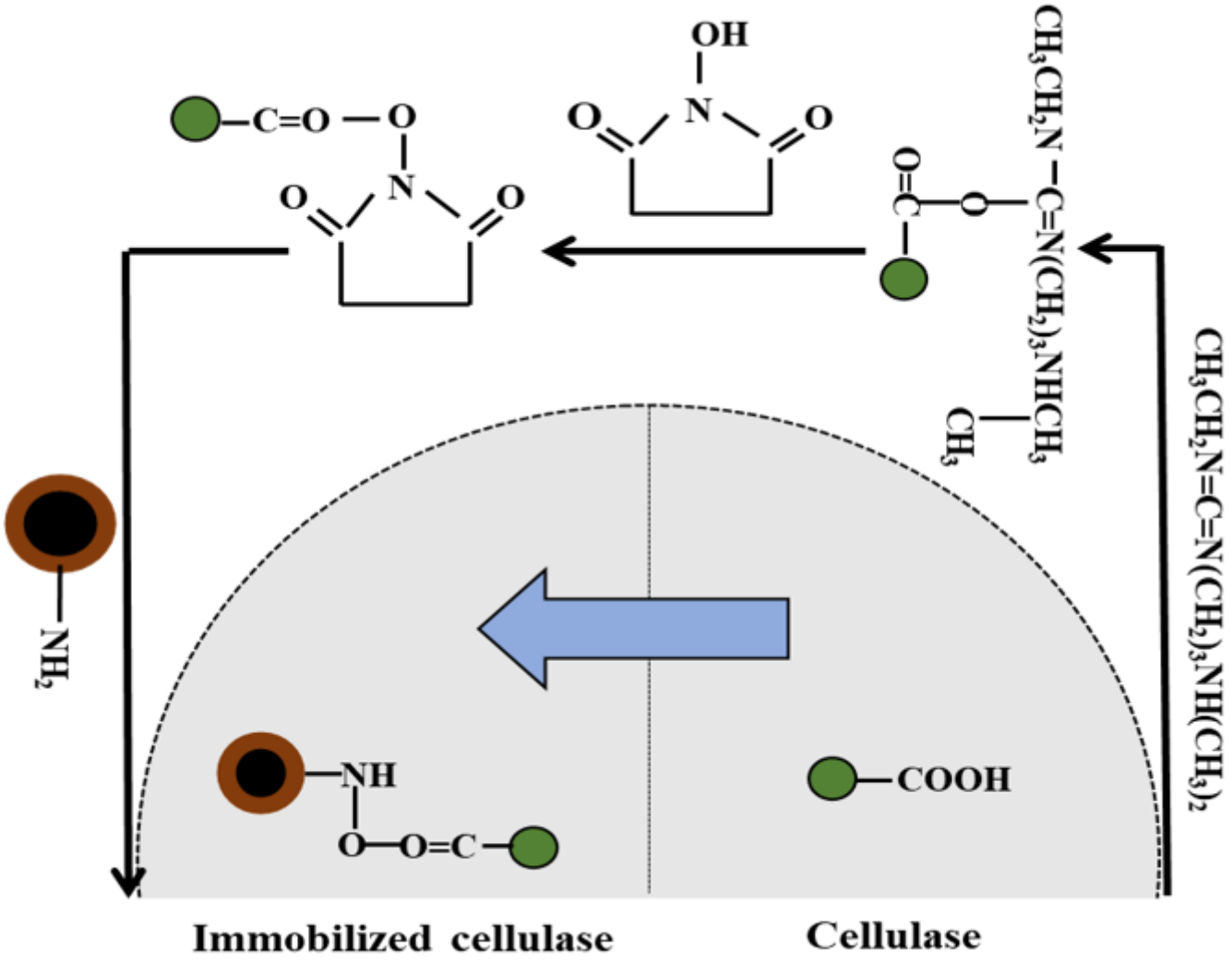

Figure 5

The oriented immobilization of cellulase on magnetic nanoparticles