DELIGNIFICATION OF LIGNOCELLULOSIC BIOMASS BY IMMOBILIZED LACCASE: A MINI REVIEW

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ABSTRACT

The production of energy from lignocellulosic biomass has become common interest among the researchers to fully utilize the waste by converting it into valuable energy sources. The main problem is that the lignocellulosic biomass is difficult to hydrolyse due to their recalcitrant and heterogeneous structure. Therefore, a pretreatment or delignification process is required to remove or modify the lignin structure in order to make it more susceptible to the hydrolysis stage. The first part of this paper reviews the current method used in delignification and emphasizes more on the biological treatment which is more eco-friendly and less energy consuming. While the second part focuses on ligninolytic enzymes, laccase which is considered to have great potential in delignification. Due to the limitations of the enzyme in term of operational stability and reusability, the improvements of enzyme after immobilization are discussed briefly. Finally, the future outlooks and challenges on the implementation of immobilized laccase in delignification process are also covered in this review.

Keywords: Delignification; Lignocellulosic biomass; Laccase; Immobilization

1. INTRODUCTION

The development of technology and civilization nowadays leads to an increasing demand in energy source which is mainly dependent on the non-renewable supply of fossil fuels. An alternative supply from a renewable source such as lignocellulosic biomass gives a promising solution in order to fulfill the energy demand in the future. Lignocellulosic biomass is referred as plant biomass that is composed of three major components which are cellulose, hemicellulose and lignin. It is the most abundant organic component on earth which can be used as the feedstock for biofuel and fine chemical productions.

Degradation of lignocellulose material is the major focus prior to its application as the feedstock in biofuels production. Lignocellulose is a complicated natural composite with three main biopolymers which are cellulose, hemicellulose and lignin. The presence of lignin hinders the enzymatic hydrolysis during the production of biofuel. The functions of lignin in lignocelluloses are for structural rigidity, integrity and prevention from swelling. Lignin holds the cellulose and hemicellulose together and it act as a physical barrier which hinders the enzymatic hydrolysis (Alvira *et al.*, 2010). Thus, its presence and distribution in the lignocellulosic biomass is one of the factors which is responsible for the recalcitrance of lignocellulosic biomass. Since, cellulose and hemicellulose are closely associated with lignin, it needs to be modified or removed to allow hydrolysis of hemicellulose and cellulose (Singh *et al.*, 2014).

2. PRETREATMENT OF LIGNOCELLULOSIC BIOMASS

A pretreatment process such as delignification is one of the most important processes in the production of biofuel from the lignocellulose material in order to tackle the limiting factors in enzymatic hydrolysis. There are several factors that hinder the enzymatic action during the hydrolysis such as cellulose crystallinity, cellulose degree of polymerization, available surface area of the substrate, lignin content, hemicellulose content and feedstock particle size (Alvira *et al.*, 2010). Several requirements suggested by Sun and Cheng (2002) on the delignification process are: (i) enhance the formation of sugar during enzymatic hydrolysis; (ii) avoid the degradation of carbohydrate; (iii) avoid the byproducts formation that inhibit hydrolysis and fermentation process; (iv) cost effective.

There are many methods that have been developed and used in the delignification process including physical, chemical and physicochemical methods. However, most of the methods require the usage of harmful chemical and perform in harsh condition which will cause problems to the environment. On the other hand, a promising method which is more environmentally friendly and less energy consumption is being offered by the biological pretreatment. Regardless on its limitations- low efficiency and long residence time (Liguori and Faraco., 2016), this method shows advantages in term of energy, environment and safety. It does not require high amount of energy as the process can be carried out under normal conditions, no toxic material is generated as no harmful and severe substances are used in the process, does not require corrosion-resistant reactors and reduce or no inhibitor to fermentation (Wan and Li, 2012).

In biological pretreatment, two different methods are being used which are fungal pretreatment and enzyme pretreatment. The fungal pretreatment required longer residence times (minimum of 3 to 7 weeks) than enzymatic pretreatment which make it less favorable to be used in the industry. Besides shorter residence times, enzymatic pretreatment also does not require nutrient supplementation and no sugar consumptions (Liguori and Faraco, 2016). The advantages offer by enzymatic pretreatment shows that it is a great alternative way to be used in biological pretreatment.

3. LACCASE

According to Bilal *et al.*, (2017), there are three main ligninolytic enzymes that are usually found in lignin degrading rot fungi which are lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase microorganisms. Among those rot fungi, white rot fungi is the most effective to be used in delignification due to their unique ligninolytic system in producing various extracellular ligninolytic enzymes mainly peroxidase and laccase (Meehnian et al., 2017). Even though there are three main enzymes involved in the modifying or degrading lignin, not all white rot fungi produce all three enzymes (Pinto *et al.*,2012). It shows that every ligninolytic enzyme have their own ways in degrading the lignin structure. The advantage of laccase over peroxidase is that it only requires oxygen as it co-substrate which is present in the surroundings (Arora and Sharma, 2010). However due to its low redox potential, laccase cannot directly attack the substrate with a large size or high redox potential such as non-phenolic lignin. The presence of redox mediators in the process has been proven to provide a key to solve the limitation of laccase by expanding its catalytic activity towards high redox substrates (Fillat *et al.*, 2017). .

4. IMMOBILIZATION OF ENZYME

The application of laccase in delignification process shows a positive alternative as it offers green technology with low energy consumption and little or no waste generation. However, enzyme is sensitive to denaturant agent and difficult to recycle which restricts its application in the industry. Therefore, enzyme immobilization technology helps to enhance the characteristics of the enzyme by holding it in a distinct support or matrix using method as shown in Figure 1. The purpose of immobilization is to make the enzyme immobile during the reaction which contributes to a more efficient process and easy separation from the product so that it is reusable. Finally, immobilization also improves the thermal stability of the enzyme, and it resistance to degradation, denaturation and aggregation (Bilal *et al.*, 2017).

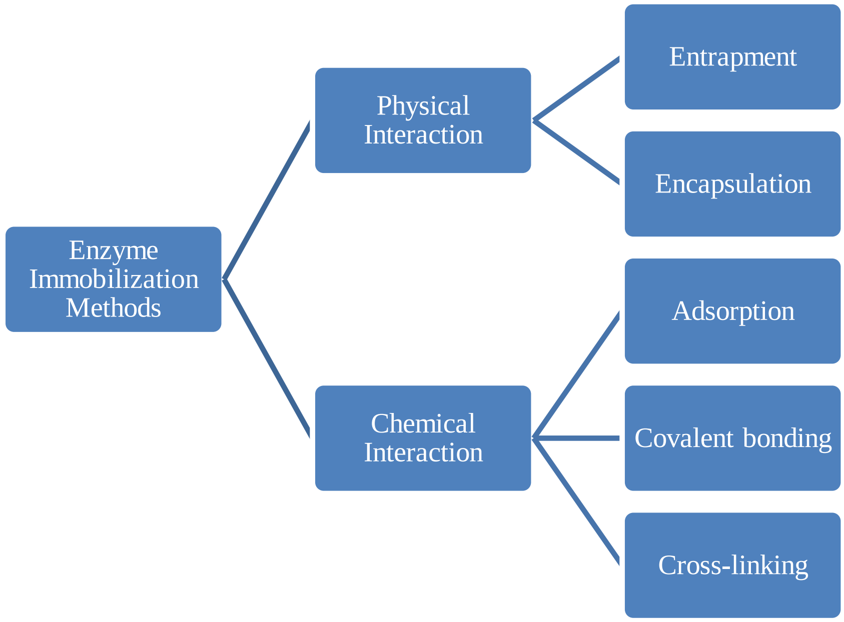

Figure 1. Enzyme Immobilization Methods (Adapted from: Fernández-Fernández *et al.*, 2013)

5. FUTURE DIRECTION AND CHALLENGES OF IMMOBILIZED LACCASE FOR THE APPLICATION IN LIGNOCELLULOSIC BIOMASS DELIGNIFICATION

The enhancement of laccase after immobilization had opened up its usage to be used widely in the industry as enzyme-based process offers notable features such as mild reaction condition, less power and energy consumption and environmentally friendly process. However, there were only a few researches conducted to study on the application of immobilized laccase in delignification of lignocellulosic biomass. Since not all types of support and immobilization techniques are suitable for each industrial application, the study on immobilized laccase which focused on this application should be conducted in depth to create an efficient system.

Besides, laccase is only able to catalyze the oxidation of phenolic subtrates due to it relatively low redox potential. The presence of redox mediators in the system enhances the oxidation capabilities of laccase towards the non-phenolic subtrates. From the research conducted by Sun *et al.*, (2016), they have successfully immobilized both laccase and a mediator (acetylacetone) into a hydrogel to be used in conversion of malachite green. This finding helps to reduce the cost of the system since both laccase and mediator could be reused, a more effective biocatalyst is produced. For the future study, the finding should be implemented in the application of lignocellulosic biomass delignification as only less than 20% of lignin polymer is phenolic substrate (Fillat *et al.,* 2017). A suitable mediator and support system should be studied further for better result in the co immobilization of both laccase and mediator.

Finally, the search for a new support or matrix from nanosized material such as nanotube, nanoparticle, magnetic nanoparticle, nanofiber and so on had become common interest to the researchers recently due to the large surface area features. However, due to the complex preparation and high cost of the material for large scale synthesis, their applications in industries could be challenging.

6. CONCLUSION

There is a huge potential of immobilized laccase to be used in the biological delignification of lignocellulosic biomass. By using the immobilized laccase, the reaction time could be reduced compared to the fungal or microbial treatment and enzyme stability will be further enhanced. Deciding and selecting the suitable support system and immobilized method for the delignification should be the main concern in order to apply the system in this industry.

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