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Enzyme Engineering

Review Article

Characterization and Advantages of Lignolytic Enzymes Produced by Solid State Fermentation

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ABSTRACT

Solid state fermentation has immense importance in pharmaceutical, textile and food industry for the manufacture of organic compounds, enzymes and flavors etc. Due to amendment and improvements in the design of reactors this technique is a well-known alternative to submerged fermentation (SmF). The present paper aimed to review the synthesis of ligninolytic enzymes by SSF technique and discuss the ability of various ligninolytic enzymes (Laccases, manganese peroxidase and venitile (peroxidase) in food, textile, and Bioremediation and ethanol production. This shows auspicious uses of SSF in the production of several laccases enzymes, favorably used in the detoxification of risky and dangerous compounds.

Keywords: SSF; Ligninolytic Enzymes; Bioremediation; Ethanol Production

INTRODUCTION

Due to unique nature of ligninolytic enzymes they are very useful in the breakdown of recalcitrant and complex polymers[1-3]. Production of ligninolytic enzymes is done by various enzymes but the most productive way is white rot fungi that produce several enzymes including lignin peroxidase (LiP), and manganese peroxidase (MnP).

Industrial processes like denim washing in textile industries, fruit juices in food industries, stabilization of wine, cosmetics and industry for biosensor production[4].

The word "Lignin" came from lignum" meaning wood. All types of vascular plants have lignin in them and it is considered as the second carbon source most abundantly present on earth after cellulose. the structure of lignin is complex. It contains three primary hydroxyl-cinnamyl alcohol, coniferyl, p-coumaryl and sinapyl alcohols which are linked together by the monolignols oxidative coupling [3]. The process of synthesis of lignin is called “lignification”.

Lignification occur either through radicals that are produced by oxidase enzymes by polymer-polymer coupling or by the monomeric units cross linking with growing polymer. due to coupling of radicals at different sites, the polymer formed as a result of cross linkage would have structural units array formed by C-C and ether linkages. High structural concentration of acylated units are present in "lignin" and

“Kenaf” white grains have esterified hydroxycinnamicacids at γ-position of lignin's propyl side chain [5].

Lignin degradation is also done by several other enzymes like aryl alcohol, glyoxal oxidase, quinone oxidoreductase, catalase aromatic aldehyde oxidase, dioxygenase veratryl alcohol and vanillate hyoxylase other than MnP, LiP and laccase Properties and characteristics of these enzymes are dependent upon the source from where they are originated mainly on the microbial source that’s why they are less significant. Degradation of lignin can be catalyzed by the production of H_2O_2 that results in the induction of peroxidase activity [6].

Sources of ligninolytic enzymes

Plants, insects, bacteria are different sources for obtaining unique groups of ligninolytic enzymes. In plants extracellular glycoprotein called laccase are the most studied group of enzymes that are obtained from different varieties of plants including mango, the Japanese lacquer tree, tobacco, peach, mung bean, zea mays etc.

In plants different laccases show different functions like lignin synthesis, regeneration in injured tissues and oxidation of Fe (II)

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Received: January 27, 2020; **Accepted:** February 10, 2020; **Published:** February 17, 2020

Citation: Tabassum S, Ali S (2020) Characterization and Advantages of Lignolytic Enzymes Produced by Solid State Fermentation. Enz Eng 9:166. Doi: DOI:10.4172/2329-6674.20.9.166

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to Fe (III). However, the most significant laccase function is the synthesis of lignin in plants [7].

In bacteria, three groups are involved in the breakdown of lignin i.e. α-proteobacteria, actinomycetes and γ-proteobacteria.

Insects on degradation of wood microflora described that these bacteria show delignification in both *in vivo* and *in vitro* analysis [8]. LiP activities are exhibited by thermobifidafusca and actinomycetes species. Laccases found in bacteria are intracellular with multimeric, homotrimeric or monomeric structure without presence of carbohydrate moiety [7].

Laccases are used by bacteria in pigmentation and spore formation and exhibited high resistance to temperature and p^H [7].

Ligninolytic enzymes are also found useful in the delignification process in insects that are produced by either insect's micro flora or by insects themselves. Ligninolytic enzymes are found in different parts of insects including Nephotettixcincticeps (salivary glands) [8], Manduca sexta (midgut, Malpighian tubules, epidermis and fat body [9].Triboliumcastaneum (cuticles) and Reticulitermes ravines (gut) [10,11]. Ligninolytic enzymes are significant in toxic compound oxidation, cuticle sclerotization, polymerization reaction and pigmentation [7,8].

Solid state fermentation

SSF is used most commonly in pharmaceutical, textile and food etc. industries for the production of useful metabolites by using solid support instead of liquid support medium. The solid support includes oil seeds cake that are de-oiled, grain brans and substances like these. Solid state fermentation produces minimum amount of waste and effluents that are beneficial for environment [12]. Enzymes production, flavors, organic acid production and colors are some of the products of food industry produced by SSF [13]. SSF has direct application in food processing industry such as traditional orient soy sauce preparation then goes to bread preparation and cheese ripening in west [14].

Fungi was considered to be active in very low activity of water therefore they were used in such fermentation but later on yeast and bacterial species were being used in these types of fermentation [15]. Cost of raw material should be affordable so that acceptable amount of product could be obtained. However, in food processing industry, for obtaining right flavors and essence of processed food, high regulation of processes like curing, ageing and ripening should be attained. Addition of flavors occur externally.

However, in some cases like in ripening of cheese it is not required to be purified to great extent and can be produced during the process of fermentation [16]. Fermentation is also used in a process like pickling of meat, eggs, fish and vegetables. Inhibition of spoilage bacteria occur by lactic acid bacteria manufactured in company with various bacteriocins that helps in the preservation of foods. So, it is also notice that submerged liquid fermentation SLF is involved in the manufacture of lactic acid instead of SSF in which carbohydrates are released from compounds either by pressing or by brining [17].

GENERAL ASPECTS OF SSF

SSF is a type of fermentation process that occur in the dearth of freely flowing water, can give an inert or natural support as a solid material [18]. However, this technique is not been used after 1940 because of the production of penicillin by submerged fermentation (SmF).

Environmental and economic problems could be resolved by using these kinds of support generated by their disposal. However, by the use of organic wastes many important substances serve as inducers for enzymes, are produced like "lignin peroxidase". SSF techniques produced ligninolytic enzymes which contain large amount of lignin [19]. Whereas laccases production can be stimulated by employing wastes rich in cellulose and rice. This will lead to the production of certain enzymes like amylase containing high starch content [20]. Table 1 shows SSF technique helps in the production of ligninolytic enzymes by showing the organic waste composition.

Table 1: Chemical composition of various organic wastes that are used in the production of ligninolytic enzymes [21].

Waste	**Lignin Cellulose Hemicellulose**	(%)	
	(%)	(%)	
Barley bran 21.4	23	32.7	
Cane	14		
bagasse	33	22	
Corn cobs	20.3	31.7	34.7
Corn leaves 12.6	37.6	34.5	
Hardwood 20.3	45.8	30.7	
Grape seeds 43.54	7.1	31.13	
Grape stalks 22.94	29.95	35.33	
Oat straw	18	49.3	25
Rice straw 17	35	25	
Softwood	29.5	43.8	24.5

Production of surfactants, antibiotics, enzymes and biocides has been increased by the application of SSF. However, enzymes produced by SSF also includes ligninolytic enzymes by the support of agro-wastes (Table 1). Product obtained by natural support is more complex as compared to inert support because extracellular product can be extracted easily and with minimum impurities [21]. Product purification and extraction by natural support is an expensive process. However, their maximum use results in higher activities and much lower product cost [22].

APPLICATION OF SSF

Living standards have been significantly improved by the potential advantages of low technology requirement of inputs by SSF. Different authors have studied the potential application of SSF which is mostly involved in the synthesis of traditional foods by fermentation mainly 'Koji" referred as "Tempah" in Indonesia or "Ragi" in India. SSF has also vital role in the manufacture of potential compounds like organic acids, flavors, enzymes, bio-fuel and bio-pesticides. New studies reveal the use of SSF in the environmental control strategies like biodegradation of hazardous compounds, bioremediation and agro-industrial residue detoxification.

SSF BIOREACTOR FOR LIGNINOLYTIC ENZYMES

In comparison, less water is present in the solid media of SSF but there exists important gas present between the space of particles of solid media. As air thermal conductivity is relatively poor as compared to water but this feature gained immense importance. However, there are variation among water holding capacity mechanical resistance, porosity and composition in SSF.

These factors affect the reactor design and overall efficiency of SSF. On the other hand, in SmF there is one difficulty that is the microorganism oxygen transfer whereas in SSF problems and difficulties become more complex and it effect two important control parameters i.e. water content and temperature of solid-state medium. Fungal morphology and resistance agitation mechanism and not having a sterile process necessity also key factors that affect the design of bioreactor. Generally, there are several bioreactors that require small amount of medium to run in laboratory scale but on the other hand scale up process is complex due to the generation of heat and heterogeneity in the system [23].

There should be adequate utilization of production system for the utilization of ligninolytic enzymes in industrial process like decolonization and bioleaching of industrial wastes. SSF, despite its potentially valuable products still at underutilized state [24]. Due to limited knowledge of the operation and design of large-scale bioreactor, it become difficult to utilize the application of SSF [25].

However, there is no such information that indicates ideal bioreactor for SSF process. Some mathematical bioreactor being divided into two categories i.e. micro scale models and macro scale models, proposed in the area of SSF. In order to optimize SSF bioreactor operation, a perfect framework that describes heat and mass transfer mechanism and biological phenomena is required [26]. However, construction of given bioreactor is quite difficult that determines the scarcity and complexity of the bioreactor behavior [27].

Review on bioreactor design is also published and described by Durand [28]. Many designs that have been emerging for over 10 years were highlighted by him in order to scale up the potential of each group of reactors. Some engineering features including design, scale up, control, mass and heat transfer and monitoring have also been observed recently [29-31]. However, Bhattacharya and Banerjee explained evolutionary operation (EVOP) to be of immense importance for complex system optimization like SSF. There was need for the modification and configuration of bioreactors having advantages as well as difficulties in performance of solid-state process by the bioreactor designs.

These bioreactors should be operating continuously with excessive enzyme production for elongated time periods without problems related to performance as well as permit the activity scale up. Several research groups worked on bioreactor development in order to produce ligninolytic enzymes working in solid state condition. Such type of bioreactors is used that can be distinguished by types of mixed system or aeration employed are shown in Figure 1a.

Immersion bioreactor

It consists of round bottom wrapping glass cylinder vessel. Several colonies of fungus were filled in wire mesh basket providing support to it. They move downward and upward by the help of pneumatic system, having more time outside then inside of medium (Figure 1a).

Packed bed bioreactor

It consists of bio-particle system filled in jacketed glass column. Humidified air was continuously supplied (Figure 1b).

Rotating drum bioreactor

It consists of cylindrical glass container having culture medium in its lower part with wire in the cylinder which has the ability to rotate as slowly as lower than 3 rpm wire network cylinder support along with the fungus. Both the fungus and carrier continue to impregnate with culture medium when rotation of wire mesh cylinder occurs. At the same time, they permit adequate oxygen transfer by keeping air constant in the upper part of vessel (Figure 1c).

Tray bioreactor

In this bioreactor bio particles are placed on a flat tray forming a layer of almost 1.5-2 cm in thickness. It was kept at constant temperature in a chamber with submissive aeration (Figure 1d).

Such noteworthy work results in the formation of new bioreactor design called immersion bioreactor, that are working with inert nylon sponge acting as Rivela [32]. However, it is also able to run continuously without any operational difficulty, obtaining Lip activities about 132 U/L and MnP activities of almost 60 U/L [33]. When the configuration of bioreactor was confirmed to be adequate for solid state processing with inert support then ligno-cellulosic support was taken to operate the bioreactor. Difficulties were found in operating bioreactor with these types of support system than the inert support such as support acceleration and degradation also occur together with the process of fermentation carrying oxygen and mass restriction as obstacles. All these factors will reduce the accurate performance of bioreactor.

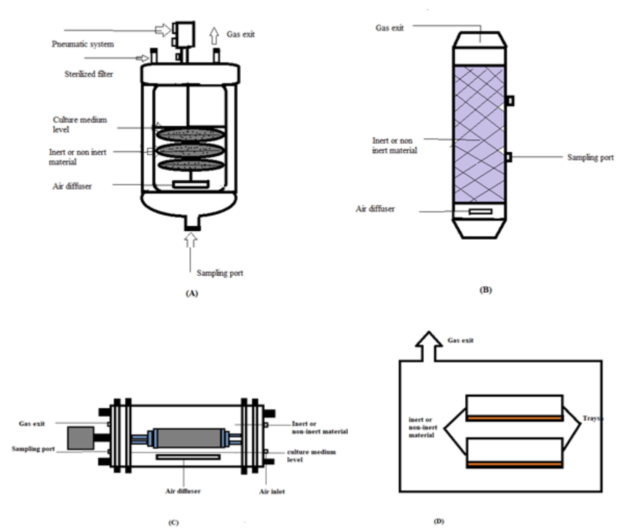

Figure 1: Shows different types of bioreactors (a) Immersion bioreactor (b) Packed bed bioreactor (c) Rotating drum bioreactor (d) tray bioreactor [34]

However, there were several advantages of the materials mentioned above as use of wood shavings was employed as ligninolytic support. LiP and MnP activities of 509 and 803 U/L were obtained.

NEW TRENDS IN SSF

Utilization of organic waste is being carried out and trending from the recent years such as the residues obtained from forestry, agricultural and alimentary industries can be used as raw material to produce valuable products by SSF technique [35]. Besides providing substrate these wastes also help to resolve environmental problems which could otherwise cause by their dismissal. These materials contain cellulose or lignin and hemicellulose that act as inducers for the activities of ligninolytic enzyme. Sugars are also a main source obtained from the waste water. SSF technique uses wheat ban for the production of ligninolytic enzymes [23].

However, there are also alternative wastes being employed by SSF technology such as laccases from Trametesspecies keeping fruit peelings as a source of support to get high value activities [34].

APPLICATIONS OF LIGNINOLYTIC ENZYMES

Potential of ligninolytic enzymes in the elimination of edc (endocrine disrupting compounds) in water

Endocrine disrupting compounds (EDC) has imposed high health and environmental risked factors when come in contact to humans and there is no such treatment for the complete EDC removal in wastewater. There are several challenges and difficulties on operation traditional methods for the removal of EDC in waste water; therefore, an alternative approach was made using ligninolytic enzymes which gained much attention as an alternative environmentally friendly method for removal [35].

PROPOSED SCHEME FOR WASTE WATER TREATMENT

Ligninolytic enzymes have advantage in the treatment of waste water produced by various industries. Several techniques were developed for its removal. Strategy for the removal of wastes from water is given in Figure 2.

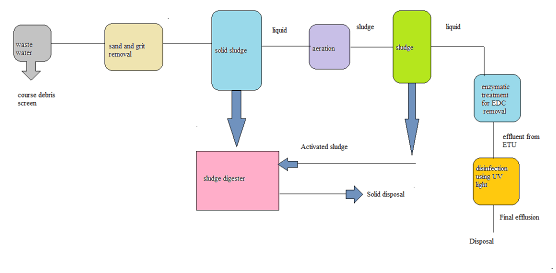

Figure 2(a): Schematic diagram for the removal of waste water. The process of waste water treatment for the removal of EDC by ligninolytic enzymes. Primary treatment invokes grit removal and debris screening and primary clarification. Secondary treatment involves: aeration and clarification. Tertiary treatment involves: disinfection and enzymatic treatment for EDC. ETU: Enzymatic Treatment Unit [36].

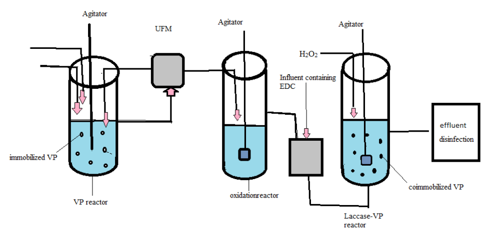

Figure 2(b): Continued stirred tank reactor is used for the elimination of EDC. It involves following stages: Stage I: Mn^{+3} is generated by MnP mechanism through immobilized VIP. Stage II: Mn^{+3} helps in the oxidation of EDC [37].

INDUSTRIAL APPLICATIONS OF LIGNINOLYTIC ENZYMES

Food industry

Baking products: Ligninolytic enzymes are used in the production of bread, improved flavor, texture and machinability [38]. It has examined that strength of gluten structure was improved by the addition of laccases into the dough. Depending upon the concentration of laccases which results in increase in the softness of the bread.

Paper and pulp industry: A large amount of waste is produced by paper and pulp industry which create severe issues in their disposal. Paper and pulp industry due to its high demand results in immense amount of pollutants consequently toxic gas are being released which ultimately results in the contamination of climate [39].

Bioremediation

Cyanide degradation: Photographic processing, extraction of metal ores, production of organic chemicals, synthetic fiber production and steel making processes, ligninolytic enzymes are used. Industrial effluents contain cyanide which can be removed either by using ozone or by H2O2. Biotechnological treatment is considered to be environmentally friendly or cost effective for the removal of cyanide [40].

Bioethanol production: White rot fungi has the ability to degrade lignin [41-43]. However, wood hydrolysates also resulted in the production of ethanol but it was a difficult process. Percentage of lignin in hardwoods and softwood is 20%-25% and 26%-32% respectively. Enzymes that helps in the decomposition process must present in large amount so that they can be studied easily e.g. Basidiomycetes Trametes versicolor, which induce secretion of peroxides and phenol oxidase laccases enzymes which take part in aromatic compound transformation [44-46].

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