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An Overview on Paddy Crop Residue Decomposition: A Biochemical Analysis of the Process

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Abstract
The most common crop in India is rice, which is grown on 43 million planted hectares and produces 746 million tones of grain annually. Due to the fact that straw accounts for 50% of the dry weight of the rice plant, a tremendous amount of straw is produced as a byproduct of rice farming each year. We produce 65% of our biomass on land, according to estimates. Lignin is the most prevalent natural polymer of that biomass after cellulose and a significant renewable supply of aromatic carbon on earth. Since lignin, cellulose, and hemicelluloses make up the structural elements of higher land plant vascular tissues, the biodegradation of these elements is a crucial step in the recycling of terrestrial biosynthetic carbon. This study focus on biochemical analysis of decomposition of paddy straw and find out different fungi present in soil after decomposition.

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INTRODUCTION

Paddy straw is one of the most abundant lignocellulosic waste on earth reaching 731 million tons per year. The amount of rice straw can potentially produce 205 billion liters bioethanol per year, which would be the largest amount from a single biomass feedstock. By selecting high- biomass yielding species, combined with high nutrient and water use efficiency, economically efficient production of biofuel feedstock may be realised on less optimal land without pressuring prime grain crop territories (Pandey, et al., 2009, Wahdan, et al., 2023). Rice straw or the whole crop that had been milled to small size was mixed with water and alpha- amylase, and the mixture was held for 1 hour at 90°C to liquefy the starch. The temperature was then reduced to 30°C to start the fermentation process with simultaneous addition of glucoamylase and yeast. The results show that 135 L of ethanol and 580kg of distillation residues can be obtained from ton of rice straw, whereas 242 L of ethanol and 550 kg

of distillation residues can obtain from one ton of the whole crop (Gadde, et al., 2009). These distillation residues are more valuable as a nutrient than the original raw materials. The organic mulch such as paddy straw and rice husk are rich in carbon content in which microbial population is very high (Singh et al., 2022). The microbial population from the soil are mostly bacteria and fungi. The organic mulching treatments used for study are paddy straw (6 cm thickness) and rice husk (6 cm thickness) as compared to control. The microbial analysis of soil was evaluated after harvesting of fruits. The analysis of soil has revealed that the microbial population of the soil was found to be highly significant. The higher microbial population count for bacteria was observed in paddy straw mulch (83.45 × 10^5cfu / g) (Satlewal *et al.,* 2018). Paddy straw has high cellulose content but the lignin complex and silica incrustation shields the microbial action for biogas production. Therefore, paddy straw needs to be pretreated to enable cellulose to be more accessible to the microbial / enzymatic attack (Rathour et al, 2023). Many physical (mechanical and non-mechanical), chemical (alkaline, hydrolysis, oxidative delignjfication and solvent extraction), physico-chemical (Ammonia fibre explosion, CO2 and steam explosion) and biological pretreatments (lignocellulolytic micro-organisms and the enzymes) have been proposed in the recent years (Saratale et al., 2008; Hendriks and Zeeman, 2009). However the physical and chemical pretreatments require high energy and corrosion resistant, high – pressure reactors, which increase the need of equipment and cost of pretreatment. Furthermore, the chemical pretreatments can be detrimental to the methanogens apart from generating acidic or alkaline water, which needs pre- disposal treatment to ensure environment safety (Keller et al., 2003, Chaturvedi, 2022). Thus, alternative approach is microbial pretreatment especially fungi to increase digestibility of paddy straw. Advantages of biological pretreatment include inexpensive, low energy requirement and mild environmental conditions (Saratale *et al.,* 2008). Most of the white- rot fungi degrade lignin and cellulose simultaneously. A selective white-rot fungus, *Ceriporiopsis subvermispora* is known to selectively degrade lignin in softwood and hardwood (Okano *et al.,* 2005).

Lignocelluloses are mainly composed of cellulose, hemicellulose and lignin which have been naturally developed to block their deconstruction from microbes and enzymes, defined as “biomass recalcitrance” (Hammel and Picataggio, 2008, Periyasamy et al., 2023).Lignin is a complex , hydrophobic , cross linked , aromatic polymer composed phenylpropanoid units. It works as an adhering agent which tangles with cellulose and hemicellulose and protect them against degradation. Therefore, delignification of biomass prior to the bio conversion process is compulsory. Consequently, several chemical (acid, alkaline and Organo solvents pretreatment, wet oxidation), physical (mechanical pretreatment, pyrolysis, microwave), physicochemical (ammonia fiber explosion , steam explosion, carbon dioxide explosion, liquid hot water) and biological lignin removal methods have been suggested (Sarkar *et al.,*2012 Abdallah, *et al.,*2006; Sun and Cheng, 2002 . With the exception of bio- treatments, the majority of these methods suffer disadvantages like difficult operating conditions (such as high pressure, temperature and pH), production of inhibitors or hazardous wastes and having harmful side effects (Iranmahboob *et al.,* 2002). On the other hand, the most significant drawback of the biological method is their low efficiency and slow rate of lignin removal which hindered their application in the large scale (Chandra *et al*., 2007). Evidently, improving the efficiency and low rate of delignification besides the other inherent merits of the biological pretreatment makes it a potentially proper option. Fungi which are capable of producing ligninolytic enzymes play the key role in biological pretreatments.

They can biodegrade lignin and increase the accessibility of cellulose in the biomass structure. As a result, the modified biomass is more susceptible to enzymatic digestion (Jang, *et al.,* 2007). It has been shown that lignin can be degraded by a ligninolytic system mainly consists of lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase (lac) (Lechner and Papinutti, 2006; Zeng, 2006; Elisashvili *et al.,*

2008; Rodrigues *et al.,* 2008) and involved mechanism in the biodegradation of lignin through this system has been well understood (Sanchez, 2009).

Presumably, the low efficiency problem of bio based delignification methods could be alleviated by a few modifications in the process. Firstly, by increasing the activity of the ligninolytic enzymes a high efficiency of delignification could be obtained (Winquist *et al.,* 2008). This depends on the cultivation conditions of the micro-organisms and possible improvements of their genetic system.

Secondly, physical constraints such as those cause mass transfer limitations should be removed. For example, it is well known that diffusion of enzyme molecules into the fibrous structure of lignocellulosic biomass is a slow phenomenon which could drastically decrease the rate of the process. Therefore, increasing the contact surface area of biomass through size reduction has been suggested (Silva *et al.,* 2012). Finally, a good contact between the enzyme active site and the lignin molecules should be facilitated to have an effective and fast delignification process. Thus, it has been suggested that disruption of the lignin-hemicellulose association (e.g. by adding xylanase to the system) can enhance the process efficiency (Kroon, *et al.,* 1999). In addition, application of surfactants to modify the hydrophobicity of lignin has been recommended (Qing *et al.,* 2010).

However, a highly efficient bio- delignification process with an acceptable rate has not been achieved yet. The goal of the present study was to improve bio- delignification pretreatment of rice straw, using fungal enzymes, optimization the operating parameters i.e. temperature, carbon source concentration and biomass to liquid culture ratio (w/v) of the process. Additionally, the effect of milling and existence of Surfactant in the system was also investigated.

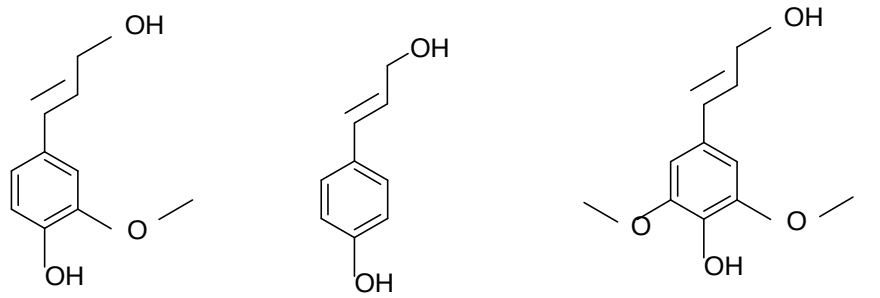
The increase in world population and its effect on food demands, in addition to reducing environmental impact derived from the food production, has raised a challenge for the agricultural industry in order to improve resource utilization and diminish food industry impact on non- renewable resources (Godfray *et al.,* 2010). Within this context, crop residue plays an important role in agricultural sustainability. Thus, it is necessary not to consider them as waste, but to acknowledge them as a renewable resource (Misselbrook *et al.,* 2012). Rice straw is one of the most produced agricultural residue worldwide. For each kg of cropped grain 1 to 1.5 kg rice straw is produced. Therefore, a worldwide estimate of byproduct is about 650-975 million tonnes / year (Binod *et al.,* 2010).

BIODEGRADATION OF PECTINS

Pectins have a very complex structure. Due to the complex structure, biodegradation of pectins requires several enzymes, collectively known as pectinases. These pectinases include pectin methyl, esterases, poly galacturonases, polymethyl galacturonases, poly galacturonatelyases, poly methyl galacturonate lyases, rhamnogalacturonase, arabinases and xylogalacturonases (G.B. Seymour; Valdespino et al., 2021). Most of the current commercial pectinolytic enzyme mixtures are produced by filamentous fungi. These organisms are very efficient in the degradation of plant cell wall polysaccharide and use broad set of enzymes to convert them into monomeric sugars that can be taken up as nutrients. However the composition of these enzymes sets differs significantly between fungal species and this is also observed for the subset of pectinolytic enzymes. For instance Rhizopus spp. mainly degrade the homogalacturonan part of pectin, while Aspergilli produce enzymes to hydrolyse all pecticstructural element (Xu, J et al., 2019). Pectin lyase, pectate lyase and rhamno galacturonan lyase also cleave the pectin backbone, using a β-elimination mechanism. lyases have different sensitivities to the acetylations or methyl esterifications (o-6)of the D-galacturonic acid backbone . In contrast to pectate lyases, pectin lyases prefer substrates with a high degree of methyl esterification. Rhamno galacturonan lyases favour non acetylated substrates. (Barnett 1972).

LIGNIN

Lignin is a complex aromatic polymer, which is deposite in the secondary cell wall of all vascular plant. It is tightily cross-linked with other cell wall component and can thus be considered the "cellular glue '' providing strength to plant tissue and fiber and stiffness to the cell wall. Phenylpropane aryl-C3 units make up the high-molecular heterogeneous biopolymer known as lignin (Tuomela et al., 2000, Sánchez, 2009, Muaaz-Us-Salam et al., 2020). P-hydroxyphenyl (H), syringyl (S), and guaiacyl (G) make up its fundamental aromatic structural unit (Asina et al., 2017). Due to its aromaticity and highly branched polymer network, lignin is resistant to both biological and non-biological reactions, which makes it more difficult to transform and degrade (Adler, 1977). The biotransformation and breakdown of lignin is one of the most significant ways that it can be used. Previous research has demonstrated that both fungus and bacteria are great at degrading lignin, do not create secondary pollutants, and are eco-friendly. Lignin can be broken down by fungi rather than bacteria. It is known that basidiomycetes, an aerobic white rot fungus, can totally degrade lignin (Knežević et al., 2013). Through the secretion of lignin enzymes, Bacillus may efficiently breakdown lignin (Zhu et al., 2020). The size of lignin substrates has an impact on the catalytic efficiency of lignin peroxidase (Lip), as smaller lignin substrates are easier to degrade. Laccase (Lac) is able to quickly oxidise the phenolic monomers of lignin and exhibits increased activity on aromatic compounds. The genome sequences of different fungi such as; *Phanerochaete chrysoporiumstrain* RP8, *Coprinopsis cinerea, Postia placenta, Pleurotus ostreos, Agaricus bisporus, Schizophyllum commune and Serpula lacrymans* have been revealed and its genomic information may greatly facilitate our understanding of the lignocelluloses biodegradation process (Niu, et al., 2021). In Fig 1 Coniferyl alcohol, sinapyl alcohol, and p-coumaryl alcohol are the three primary phenylpropane (monolignol) units that make up the phenolic macromolecule known as lignin, which has a high molecular weight (Figure 1). These monomers are known as p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) units, respectively, when they are integrated into the lignin structure. The lignin structure also includes more subunits besides these three basic ones (Silva et al., 2021).



Coniferyl akchol(G) p-Coumaryl alchol(H) Sinapyl akchol(S)

Figure 1: Chemical structure of the three monomeric precursors of the lignin mcromolecules

BIODEGRADATION OF LIGNOCELLULOSE RESIDUE

The organisms predominantly responsible for lignocellulose degradation are fungi, and the most rapid degrades in this group are basidiomycetes (Ten Have et al., 2001). The ability to degrading cellulose efficiently is through to be associated with the a mycelia growth habit that allow the fungus to transport scarce nutrents such as nitrogen and iron, to a

distance into the nutrient-poor lignocellulosic substrate that constitutes its carbon source. The fungal degradation occurs exocellularly, either in association with the outer cell envelop layer or extracellular, because of the insolubility of lignin, cellulose and hemicelluloses. Fungi have two types of extracellular enzymatic syatem: the hygrolatic system, which produce hydrolases that are responsible for polysaccharide degradation; and a unique oxidative and extracellular ligninolytic system, which degrades lignin and open phenyl ring. Several microoraganism system, mainly fungi, have been isolated and identified as lignocellulolytic organism. (Silva et al., 2021).The most widely studied white-rot organism is *Phanerochaete chrysosporium*, which is one of the holobasidiomycetes. *Trichoderma reesei* and its mutants are the most studied ascomycetes fungi, is used for the commercial production of hemicellulases and cellulases (Jorgensen *et al.,* 2003). Not even white-rot fungi are known to be capable of using liginin as a sole carbon and energy source, and it is generally belived that lignin break down is necessary to gain access to cellulose and hemicelluloses. Although white-rot basidiomycetes have been shown to efficiently mineralize lignin, species differ gross morphological patterns of decay they cause. *P. chrysosporium* strain simultaneously degrades cellulose, hemicelluloses and lignin, whereas other such as *Ceriporiopsis subvermispora* tend to remove lignin in advance of cellulose and hemicelloluse (Nieves *et al.,* 1998). Brown rot mechanism has likely involved independently multiple times from white rot decay fungi. Presumably, because lignin break down is energetically unfavorable, selection has favored a mechanism which can specifically unfavorable, selection has favored a mechanism which can specifically attack the cellulose and hemicellulose components.

LIGNIN BIODEGRADATION

The hard polymer lignin, which has a high molecular weight and complicated structure made up of phenolic subunits, is the second-largest contributor to the biomass of plants after cellulose. As a result, lignin can be exploited as a new, eco-friendly resource in the manufacturing of several polymers, dyes, and adhesives. Since it was discovered that laccase could break down lignin, the value of lignin has drawn more and more attention. The main areas of study have been the discovery of lignin-degrading enzymes, which are crucial to the biodegradation of lignin, and the possible uses of lignin degradation products (Cui et al., 2022). In this review, we detail the four types of lignin-degrading enzymes' origins, catalytic specificities, and enzyme reaction mechanisms. Fungi are recognized for their extreme facility in producing a large variety of extracellular enzymes. The principal organisms responsible for lignocelluloses degradation are Basidiomycetes (Dolphin, 1987). Wood-rotting Basidiomycetous fungi are usually divided into White-rot, brown-rot and litter-decomposing fungi. Basidiomycetous white-rot fungi and related litter- decomposing fungi are related litter-decomposing fungi (Preston *et al.,* 1990; Worrall *et al.,* 1997; Steffen, 2003) are the only organisms capable of mineralizing lignin efficiently. The white-rot fungi, belonging to the Basidiomycetes, produce various isoforms of extracellular ligninolytic enzymes: laccases and different peroxidases, including lignin peroxidase, manganese–dependent peroxidase hydrogen–peroxide oxidoreductase, and versatile peroxidase, the latter sharing LiP and MnP catalytic properties. (Tien and Kirk, 1994; Collins and Dobson, 1995; Collins *et al.,* 1997; Martinez, 2002).

CELLULOSE BIODEGRADATION

In cellulosic waste, cellulose bacteria can develop synergistic interactions with non-cellulolytic species; this interaction causes the cellulose to completely degrade. Cellulose-degrading bacteria produce a battery of enzymes with varying specificities that work together. The complex variety of enzyme proteins called cellulases, which are in charge of hydrolyzing cellulose, have diverse specificities for breaking down the 1,4-glycosidic linkage bonds. According to Goyal et al. (1991) and Rabinovich et al. (2002), there are three main categories of cellulases' activities. These include cellobiohydrolase, -glucosidase, and endoglucanases, also known as endo-1-4-glucanases. Endoglucanases, also known as carboxymethyl cellulases (due to the artificial

substrate used for their detection), are thought to attack amorphous regions of the cellulose fibre at random internal sites, creating openings for later attacks by cellobiohydrolases (Lynd et al., 1991). Cellubiohyhydrase, often called exoglucanase, is the major component of the fungal cellulase system accounting for 40-70% of the total cellulase proteins, and can hydrolyze highly crystalline cellulose (Esterbauer *et al.,* 1991; Rowell, 1992).

Monomers and dimers are removed from the end of the glucan chain by cellobiohydrolases. -glucosidase hydrolyzes cellulose oligosaccharides and glucose dimers to glucose. Endoglucanases and cellobiohydrolases typically cooperate to break down cellulose, although the precise mechanisms by which this happens are yet unknown (Rabinovich et al., 2002).

CONCLUSION

Crop residues are carbon-rich substances that are also rich in microelements, phosphorus, nitrogen, and potassium. A sustainable method of raising soil quality without upsetting its biological balance is to add crop residues. Crop residue decomposition can raise soil organic carbon content and availability of phosphate and potassium, which can offer nutrients for microorganisms and crops. Additionally, soil porosity, aggregate stability, and moisture can all be enhanced. Rice straw will continue to be a plentiful and easily available agricultural waste as rice production rises to meet the needs of the world's population, which now stands at more than half. Gathering and handling of Anaerobic digestion of rice straw is not only a practical method for generating clean, renewable energy, but it will also eliminate a significant source of greenhouse gas emissions from conventional methods of open burning or tilling the straw back into the fields. In nature, distinct enzymatic systems—free enzymes, multifunctional enzymes, and self-assembled, multi-enzyme complexes (cellulosomes)—are used to break down cellulose polymers.

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