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CHAPTER TWO

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LITERATURE REVIEW

2.0 AGRO-RESIDUES AS ABUNDANT SOURCE OF LIGNOCELLULOSE

Vast quantities of agricultural and agro-industrial residues that are generated as a result of diverse agricultural and industrial practices represent one of the most important energy-rich resources. Accumulation of this biomass in large quantities every year results not only in deterioration of the environment but in a loss of potentially to recover or produce value-added products such as food, fuel, feed and a variety of chemicals. Cellulose, hemicellulose and lignin are the major constituents of these lignocellulosic residues while minor quantities of protein, pectin, soluble sugars, vitamins and minerals are also present (Bisaria, 1999). Proper strategies for the treatment of these wastes are now required prior to safe and environmentally sound disposal. However, the complete utilization of lignocellulosic residues remains an intractable problem.

2.1 AGRO-RESIDUES AS SUBSTRATES FOR SOLID SUBSTRATE FERMENTATION

The selection of a substrate for SSF process depends upon several factors mainly related with cost and availability and thus may involve screening of several agro-industrial residues. Research on the selection of a suitable substrate has mainly centred around tropical agro-industrial crops and residues. These include crops such as cassava, soybean, sugar beet, potato, crop residues such as bran and straw of wheat and rice, hull of soy, bagasse of sugarcane and cassava, residues of the coffee processing industry, fruit-processing industry, oil-processing mills such as palm oil mill waste and others

such as sawdust (Pandey *et al.*, 2000). Table 2.1 describes some of the most common agro-residues found in tropical countries.

Table 2.1 : Potential biodegradable agro-industrial residues

Source	Residues
Agro-industrial wastes and by-products:	
1. Rice milling industry	Rice husk, rice bran
2. Sugar industry	Baggase, pith
3. Cotton processing industry	Cotton linters, cotton seed hulls
4. Jute industry	Jute sticks, jute mill wastes
5. Sawmill industry	Sawdust, woodchips, bark, shavings
6. Coconut industry	Coconut husk, shell and pith
7. Palm oil processing industry	Empty fruit bunches, fronds, palm kernel shell,
8. Sago starch processing industry	Fibrous pith residue (hampas)

Source : Bisaria (1991); Khozirah and Khoo (1991); Abu Hassan and Azizan (1992); Kumaran *et al.* (1997).

2.2 OIL PALM FROND PARENCHYMA TISSUE (OPFPt), SAGO 'HAMPAS' AND RUBBERWOOD SAWDUST AS SUBSTRATES FOR SSF.

One of the by-products generated from the oil-palm plantations is oil palm fronds (OPF) which can be obtained during pruning and replanting activities. The estimated total availability of OPF for the year 2000 is 19.19 million tonnes. Currently,

OPF are widely used for mulching the inter-rows of oil palm plantations (Mohamad Husin *et al.*, 1986). Besides this, OPF have also been used for extraction of vitamin E (Ab Gapor and Kato, 1987), production of pulp and well-bonded paper (Ashari *et al.*, 1991) and in animal feed (Abu Hassan and Azizan, 1992). In processing OPF using the shredder-hammermill rotating sieve, the OPFPt was obtained in a mash-like form which only requires drying and sieving before being suitable for SSF (Figure 2.1). Physico-chemical characteristics of OPFPt (Table 2.2) such as crude protein of 5.4%, cellulose 15.2% and lignin 19.2% proves that this agro-residue is rich in lignocellulosic material and stands as a potential substrate for solid substrate fermentation. (Dinesh, 1994).

Sarawak, Malaysia is a major producer of sago starch and Sibu division alone accounts for about 95% of the starch from Sarawak (Chew and Shim, 1993). Debarking is the first stage of sago processing to produce starch where a conventional factory may generate about 8-15 tonnes of bark per day, for every 750-1200 log sections processed (Chew and Shim, 1991). The debarked pith is soft and pale pink in color. The pith contains almost all the starch in the sago palm. The debarked pith is then fed into a rasper or hammer mill where water is added and renders the pith into a fine pulp which goes through several rotating drums with sieves for starch extraction. Large amounts of treated river water is mixed with the pulp to separate and wash the starch granules out of the 'repos' in the rotary sieves. At this stage 'hampas', the leftover fibrous pith residue is produced. The 'hampas' is usually washed off into the waterways together with the wastewater (Cecil *et al.*, 1982). It was estimated that Sarawak would have generated about 39,240 tonnes of 'hampas' in 1992 (Kumaran *et al.*, 1997) which contributes to high Biological Oxygen Demand (BOD) of between 1,824 and 6,750 mg/L and Chemical Oxygen Demand (COD) of between 5,682 and 16,974 mg/L and particularly

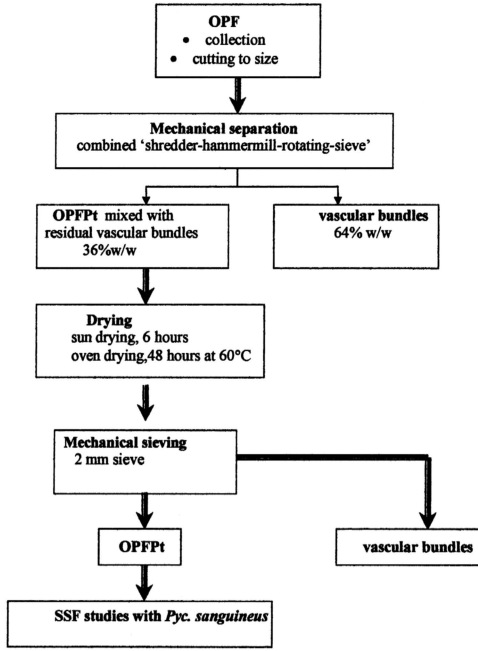

Figure 2.1: Flow diagram of processing of OPFPt before being used as a substrate in SSF using *Pyc. sanguineus.*

Source: Dinesh, 1994.

high suspended solids in the combined effluent (Chew and Shim, 1990; Anton, 1992). It was estimated from several studies that sago 'hampas' contains around 65% to 93% starch on dry weight basis, 6 to 16% crude fiber and insignificant amounts of protein and fat (Table 2.2) (Kumaran *et al.*, 1997). Besides being used as dietary fiber supplement for ruminants, the large amount of 'hampas' produced is otherwise poses an environmental and disposal problems. Some practical utilization of sago 'hampas' have been attempted such as in ruminant feed (Pongsapan *et al.*, 1984; Yahya *et al.*, 1992), biogas production (Bisaria *et al.*, 1990) and production of bulk enzyme through SSF (Kumaran *et al.*, 1997).

Table 2.2 : Physico-chemical properties of OPFPt and sago 'hampas'

	OPFPt *	**Sago 'hampas' ****
Dry matter (%)	96.70 ± 0.02	89.90 ± 0.10
Crude fat	1.86 ± 0.04	Nd
Crude protein	5.46 ± 0.30	1.15 ± 0.12
Crude fibre	30.89 ± 0.31	14.45 ± 0.25
Cellulose	15.17 ± 0.22	71.18 ±1.32
Lignin	19.23 ± 0.24	24.79 ± 0.82

Nd= not detectable

All units are in % dry matter

Source: * Dinesh (1994); ** Kumaran (1996)

Another cheap and easily available lignocellulosic material is rubberwood sawdust which is a by-product from rubberwood processing industries. In Malaysia, this agro-residue is the main substrate used in cultivation of several mushroom species which are of commercial importance such as *Lentinus edodes, Pleurotus sajor-caju* and *Ganoderma* spp. (Chang, 1993; Kuan, 1999). Rubberwood sawdust would be a good substrate for SSF as it can be easily degraded by white-rot fungi.

The selection of an ideal substrate for microbial processes for the production of value-added products is essential for economical utilization of agro-residues. Application of agro-industrial residues in bioprocesses such as bioconversion or biotransformation into value-added products such as enzyme is of commercial interest and on the other hand, helps solve the pollution and disposal problems. Extensive research has been done on the production of enzymes through solid substrate fermentation using agro-industrial residues (Table 2.3). In this study, OPFPt, sago 'hampas' and rubberwood sawdust was chosen as substrates for solid substrate fermentation using a white-rot fungi, *Pyc. sanguineus* for the production of bulk enzyme.

Table 2.3: Production of enzymes through SSF using various agro-residues

Enzyme produced	Micro-organisms used	Substrates
Cellulases, laccase and xylanase	Strains of *Aspergillus* sp., *Trichoderma* sp., *Lentinula* sp., *Penicillium* sp., *Pleurotus* sp., *Neurospora* sp., *Sporotrichum* sp., *Gliocladium* sp., *Botrytis* sp., *Phanerochaete* sp.	Baggase, coconut coir pith, rice husk, rice straw, wheat bran, wheat straw, tea waste, sweet sorghum, silage, sugar beet pulp, sawdust, grape-wine cutting waste, palm oil mill waste, sago hampas, cassava waste, soy-hull, paddy straw, etc.
Xylanases	Strains of *Aspergillus* sp., *Trichoderma* sp., *Penicillium* sp., *Phlebia radiata, Melanocarpus albomyces, Pyc. sanguineus, Thermomyces lanuginosa, Humicola lanuginosa, Thermascus aurantiacus, Talaromyces emersonii, Thermomonospora* sp.	Rice straw, corn hull, corncobs, wheat bran, wheat straw, bagasse, rice straw, cotton stalks, soy-hull, kraft pulp, sugar beet pulp, rice husk, apple pomace, corn cobs, coffee processing waste, barley straw, oat straw.
Laccase, lignin peroxidase, phenol oxidase and manganese peroxidase	Strains of *Penicillium* sp., *Pleurotus* sp., *Phlebia radiata, Trametes versicolor, Flammulina velutipes, Panus tigrinus, Trichoderma versicolor.*	Bagasse, wheat bran, wheat straw, sawdust, cotton stalk, kraft lignin, wood chips.

Source : Modified from Pandey *et al.*(2000).

2.3 CHARACTERISTICS OF LIGNOCELLULOSIC MATERIALS

Cellulose is one of the three major components of lignocellulosic residues. Cellulose is linear polymer of D-glucose residues (building blocks), held together by β1-4 glucosidic linkages. This linkage acts as a functional group along with the hydroxyl groups mainly determines the chemical properties of cellulose. Lignocellulosic residues also contain hemicellulose and lignin. It has been proposed (Fengel and Wegner, 1984) that the microfibrils of cellulose, which are about 12 nm in cross–section and about 30 nm in length, are surrounded by hemicelluloses and lignin in wood. Pretreatment of cellulosic materials, aimed at degrading the protective layers of hemicelluloses and lignin around elementary fibrils and microfibrils, is essential to rapid enzymatic hydrolysis. The molecular structural features of cellulose, the elementary fibril and the microfibril are important for enzymatic degradation of cellulose. The ability of the cellulolytic micoorganisms or that of enzymes to hydrolyse cellulose varies greatly with the nature of cellulosic materials (Bisaria,1999).

The second major constituent of lignocellulosic biomass is hemicelluloses, the amorphous polysaccharides made up of xylose, galactose, mannose, arabinose, glucose and their uronic acids (Visser *et al.*, 1992). The proportions of these pentoses and hexoses vary widely in the hemicelluloses of different biomass origin.

Lignin, the third major constituent of lignocellulosic residues, is an amorphous three-dimensional polymer composed of three phenylpropanoid units coniferyl alcohol, sinapyl alcohol and *p*-coumaryl alcohol (Crawford, 1981). Lignin is a highly recalcitrant compound, forming the second most abundant biopolymer in nature after cellulose

(Worral *et al.*, 1997) and until degraded, prevents access of degradative enzymes to the cellulose and hemicelluloses in woody plant tissues (Millet *et al.*, 1975). Lignin is decomposed predominantly by higher basidiomycetous fungi that cause the white-rot type of wood decay (Ander and Eriksson, 1978). Since the accessibility of cellulose by hydrolytic enzymes is inhibited by the presence of lignin, the digestibility of carbohydrates from the waste can be increased considerably by the decrease of its lignin content. As the chemical delignification of wastes is too expensive for the fodder industry, the alternative way may be the bioconversion of lignocellulose by lignolytic microorganisms including several mushroom species. During the growth of mushrooms on lignocellulosic wastes, a significant portion of the lignin content of the residue is decomposed. Further, a simultaneous increase in the protein content as a result of the mycelial growth is observed (Zetelaki-Horvath, 1984). There are effective chemical procedures which can fractionate the lignocellulose into its major constituent components and further hydrolyse them into essential building blocks, i.e. glucose from cellulose, pentoses and hexoses from hemicellulose, and phenylpropanoid units from lignin. However, these chemical processes besides being energy intensive, require expensive corrosion-resistant equipment, extensive washing and disposal of chemical wastes. Severe conditions of chemical treatment may also result in partial degradation of desired end-products to toxic byproducts such as furfural in acid hydrolysis. Because of the drawbacks associated with hydrolysis of lignocellulosics, extensive research efforts have been directed towards their enzymatic hydrolysis (Bisaria, 1999).

2.4 CELLULOSE DEGRADING ENZYMES

Cellulose forms the basic structural material of the cell walls of all higher plants. The main characteristics of cellulose are its great strength, fibrous nature, insolubility and inertness. Cellulose is basically composed of long, linear chains of β-1,4 linked glucose units. Cellulases are enzyme complexes, which stepwise, breakdown native cellulose or derivatives of cellulose to glucose. The three enzymes involved in cellulose breakdown are exocellulase or exobiohydrolase (EC 3.2.1.91), endocellulase or endoglucanase (EC 3.2.1.4) and β-glucosidase or cellobiase (EC 3.2.1.21) (Wood, 1989).

Extensive research on cellulases have revealed their biotechnological potential in various industries. Cellulases have been found to improve the nutritional quality of animal feed and thus the performance of ruminants and monogastrics (Galante *et al.*, 1998b). Cellulases have achieved their worldwide success in textile and laundry industries because of their ability to modify cellulosic fibres in a controlled and desired manner, so as to improve the quality of fabrics (Uhlig, 1998). Latest best-known applications of cellulases in the textile industry are in bio-stoning and bio-polishing which is currently an environmentally friendly processes in the textile industry for producing high quality garments (Galante *et al.*, 1998a). Paper and pulp industry also makes use of cellulases in the area of bio-characterization of pulp fibres and bio-mechanical pulping where partial or complete hydrolysis of pulp fibres are done (Buchert *et al.*, 1998).

2.5 XYLAN DEGRADING ENZYMES

Xylanases degrade xylan which is the major hemicellulose component, ranking second to cellulose in nature (Bastawde, 1992). Unlike cellulose, xylan is a complex polysaccharide comprising of a backbone of xylose residues linked by β-1,4-glycosidic bonds (Gilbert and Hazlewood, 1993).

Complete breakdown of a plant heteroxylan requires the concerted action of a number of hydrolytic enzymes. The key enzyme for xylan degradation is endo-β D-xylanase, which attacks the main chain, generating non-substituted or branched xylo-oligosaccharides (Buswell, 1998). The presence of xylan degrading enzymes has been found to expose more surface area of cellulose for the action of cellulase enzymes (Shamala and Sreekantiah, 1986).

Xylanases have potential applications mainly in the pulp and paper industries for bio-bleaching of kraft pulp which is also an environmental friendly process (Buchert *et al.*1998). In recent years, endo-xylanases have also been used to improve the quality of bakery products (Pountanen, 1997). Xylanases have been successfully used in the area of animal feed biotechnology where improvement in the feed digestion and absorption of feed components as well as weight gain by broiler chickens and hens have been proven (Galante *et al.*, 1998b).

2.6 LIGNIN DEGRADING ENZYMES

Glenn *et al.* (1983) and Tien and Kirk (1983) independently and almost simultaneously, reported the discovery of a lignin degrading enzyme. Ligninases are a family of isoenzymes, oxidases and peroxidases responsible for the oxidative depolymerization of lignin. The enzymes implicated are lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase (phenol oxidase) (Tien, 1987).

Several different mechanism have been postulated for different types of oxidizing enzymes involved in lignin degradation (Kirk and Farrell, 1987; Hatakka, 1994; Tour *et al.*, 1995; Robles *et al.*, 2000) :

1. Lignin peroxidases catalyze the cleavage of arylpropane side chains, ether bond cleavage, aromatic ring opening, and hydroxylation in the presence of H_2O_2.
2. Manganese peroxidases oxidize the phenolic components of lignin in a process that requires Mn(II) and H_2O_2.
3. Laccases oxidize a variety of phenolic compounds in an O_2-dependent process that does not require hydrogen peroxide or manganese ions
4. Other enzymes, such as oxidases, supply H_2O_2.

Ligninolytic enzymes or ligninases are produced by fungi, actinomycetes and bacteria, especially wood-rotting fungi. *Phanerochaete chrysosporium* is the most studied wood-rotting fungus for production of ligninases. Carbon, nitrogen and manganese are the critical nutritional variables for production of ligninases by *Pha. chrysosporium* (Bonnarme *et al.*, 1991) and also for lignin degradation. Carbon limitation causes the rapid onset of lignin mineralization but it is short lived as the cells

undergo autocatabolism accompanied by a rapid loss of dry weight (Jeffries *et al.*, 1981). On the other hand, nitrogen limitation also limits the ability of the organism to produce extracellular proteins (enzymes). Therefore, the supply of carbon and nitrogen is most critical in the production of ligninases in *Pha. chrysosporium*.

The application of ligninolytic enzymes for the delignification of lignocellulosic materials as a pretreatment for their conversion into biofuels and single cell proteins has been suggested by Chahal (1991). The use of ligninases would also reduce the energy and chemical requirement for the pulping process (Tien, 1987). The inherent ability of the ligninolytic enzyme system of white-rot fungi to cleave varieties of carbon-carbon and ether bonds in lignin suggests that such organisms may be useful for the biotransformation or biodegradation of recalcitrant environmental pollutants. Studies have been done to evaluate the ability of white-rot fungus, *Pha. chrysosporium*, to degrade a variety of toxic recalcitrant chemicals : TCB (3,4,3',4',-tetrachlobiphenyl), TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin), DDT [1,1-bis (4-chlorophenyl) –2,2,2-trichloroethane] and benzo(a)pyrene (Fernando and Aust, 1994). Pentachlorophenols (PCBs), polyaromatic hydrocarbons (PAHs), polychlorinated hydrocarbons (mainly insecticides), dioxins and benzoprene are some of the highly toxic environmental pollutants which have been reported to be degraded by the ligninolytic enzyme system of *Pha. chrysosporium* and *Tra. versicolor* (Field *et al.*, 1993; Collins *et al.*, 1996; Johannes *et al.*, 1996).

Laccase is a type of copper-containing polyphenol oxidase discovered in 1883 (Yoshida, 1883) in the exudates of the Japanese lacquer tree *Rhus succedanea* and *Rhus vernicifera*. This blue copper oxidase (laccase) catalyzes the one-electron oxidation of

phenols to phenoxy radicals, eventually transferring four electrons to O_2 (Reinhammar, 1984). Laccase partially depolymerizes methylated lignin and also causes cleavage between the α and β carbons of aromatic side chains (Tien and Kirk, 1983). Laccase activity of fungi is therefore equally important for selective delignification and lignin transformations as the generalized ligninase-type reactions. Two or more of the three families of enzymes are quite widely distributed in the basidiomycetes that cause white-rot decay of wood (Kantelinen *et al.*, 1989; Maltseva *et al.*, 1991; Coll *et al.*, 1993). It was also reported that laccase and other lignocellulose modifying enzymes in marine fungi are often found on decaying branches, leaves and driftwood (Pointing *et al.*, 1998).

Laccases are able to depolymerize synthetic lignin (Kawai *et al.*, 1999) and delignify wood pulps (Bourbonnais *et al.*, 1997) when they are combined with various low molecular weight electron transfer agents. These so-called laccase/mediator systems are potentially applicable for pulp bleaching (Srebotnik and Hammel, 2000). Laccases are also useful in the detoxification of phenolic pollutants (Collins *et al.*, 1996; Johannes *et al.*, 1996; Hublik and Schinner, 2000).

While the potential of fungal laccase are being studied extensively for various functions, there are already some commercialised applications of laccase .One of it is called 'DeniLite" which was launched by Novo Nordisk in 1997 for the bleaching of jeans (Novozymes). Another latest laccase application to be developed is for hair dyeing. Another commercial laccase called "TienZyme" is a preparation from *Pleurotus ostreatus* which shows optimum acitvity at pH 4.0-7.00 between 25°C to 60°C and is shipped on ice in 50mM phosphate buffer,pH 7.0. One unit of this enzyme will produce

an absorbance change of 0.001 per minute at pH 5.5, 25°C using syringaldazine as substrate. 100,000 units of this laccase is priced at US$ 275.00 (Tienzyme).

Some researchers have also focused on studies dealing with species which produces only laccase as the sole lignolytic enzyme. *Pyc. cinnabarinus* and *Pyc. sanguineus* have been reported to produce laccase without any lignin peroxidase or manganese peroxidase (Eggert *et al.*, 1996; Sigoillot *et al.*, 1999; Pointing *et al.*, 2000). This is of beneficial for industrial applications, where purification steps are both costly and time consuming.

2.7 ENZYMES IN BIOREMEDIATION

Paper and pulp industry is one of the biggest water polluters and hence is potentially harmful to aquatic ecosystems. Increasing demands for improvement in pulp quality and environmental safety standards forces this industry to make changes continuously. At present, the pulp and paper industry more than ever needs new technologies in order to minimise the production of hazardous substances (El Haji *et al.*, 1999).

Wood processing industry also introduces certain amount of toxic pollutants into the waste water as wood contains minor portions of fatty and resin acids and other organic compounds. During conventional bleaching, complex reactions occur involving the chlorination, oxidation and demethylation of residual lignin. The major products of these reactions are adsorbable organic halides. Extracts of bleached, kraft-mill effluent (BKME) have been shown to contain mutagenic activity as well as to induce biochemical responses in fish, such as increased activity of the mixed-function

oxygenase (MFO) enzyme system (Rao *et al.*, 1995). Also, bleaching effluents contain toxic, chlorinated, phenolic compounds and recalcitrant, chlorinated, lignin fragments of higher molecular weight (Heizel *et al.*, 1992). It is shown that the chromophoric and aromatic lignin derivatives of the waste waters from the bleaching stage are toxic and are highly resistant to biodegradation by conventional treatment methods (Bergbauer *et al.*, 1992).

Bioremediation is a process that exploits the catalytic abilities of living organisms or enzymes to enhance the rate or extent of pollutant destruction and is an important tool in attempts to mitigate environmental contamination (Pandey *et al.*, 2000). Physical and chemical treatment techniques are also ineffective in the treatment of these toxic wastes. Techniques like ultrafiltration and ion exchange are expensive. Therefore alternative biotreatment process are needed (Pandey *et al.*, 2000).

The white-rot fungi, which degrade lignin biopolymers by a range of extracellular enzymes, have been used to degrade and detoxify polyaromatic hydrocarbons, polychlorinated biphenyls, and certain industrial dyes (Field *et al.*, 1993; Schliephake *et al.*, 2000). Some fungi have the capacity to produce enzymes such as laccases, which renders toxic compound less toxic via polymerization. Work has been done on the decolourisation of textile dyes by the white-rot fungus, *Pha. chrysosporium* and *Tra. versicolor*, to indicate ligninolytic activities of the enzymes present (Swamy and Ramsay, 1999). A number of trials using *Pyc. cinnabarinus* in a packed-bed bioreactors showed successful decolourisation of complex industrial effluents and the industrial-grade dyes (Schliephake *et al.*, 1993; Lonergan *et al.*, 1995; Schliephake *et al.*, 2000).

Although most studies have concentrated in using submerged liquid fermentation, but solid substrate fermentation appears to be useful tool for bioremediation and biodegradation of hazardous compounds (Pandey *et al.*, 2000). Masaphy *et al.*(1996) and Fan *et al.*(1999) have proposed a system for bioremediation using *Pleurotus* sp. Significant reduction in leachability and bioavailability of pesticide have been found through SSF (Berry *et al.* 1993). Biodegradation of polychlorinated biphenyls (PCBs) and volatile chlorinated ethenes (CIUs) in contaminated soils have also been reported by Kastanek *et al.*(1999). These studies also prove that solid substrate fermentation is a potential method for producing bulk enzymes which can be utilized for the bioremediation of some pollutants.

2.8 SOLID SUBSTRATE FERMENTATION FOR ENZYME PRODUCTION

Solid substrate fermentation (SSF) may be defined as fermentation in which microbial growth and product formation occur in solid substrates with low moisture contents (Moo-Young *et al.*, 1983; Mudgett, 1986). However, as pointed out by Pandey *et al.*(2000), the term 'solid substrate fermentaion' should be used to define only those processes in which the substrate itself acts as carbon or energy source, occuring in the absence or near-absence of free water where as solid state fermentation should define any fermentation processes occuring in the absence or near-absence of free water, employing a natural substrate as above, or an inert substrate used as solid support. Such processes are used on a commercial scale for the production of different types of fermented foods, particularly in the Orient and in some developing countries. In addition to their use in food production, SSF have been used in recent years for large scale production of fungal metabolites and for bioconversion of plant, animal, and

domestic wastes into useful products including biomass and enzymes (Aidoo *et al.*, 1982 and Moo-Young *et al.*, 1983). During the period 1940-80's, the neglect of SSF was not only caused by the popularity of the submerged culture process, but also because of the difficulties associated with the quantification of parameters such as microbial biomass, substrate consumption, concentration of end-products formed in SSF.

There are several important factors, which affect SSF processes. Among these are:

1. Selection of a suitable strain :

Research have been done using bacterial, yeast and filamentous fungal strains for enzyme production through SSF. Among the filamentous fungi, Zygomycetes (*Mucor* and *Rhizopus*), Ascomycetes (*Aspergillus* and *Penicillium*) and Basidiomycetes (white-rot fungi) continues to be the most preferred choice (Pandey *et al.*, 2000).

2. Selection of a suitable substrate:

For SSF process, the selection of a natural substrate depends upon several factors mainly related with cost and availability and thus may involve screening of several agro-industrial residues. This substrate not only supplies the nutrients to the microbial culture growing in it, but also serves as an anchorage for the cells. It is difficult to obtain a natural substrate which has all the essential nutrients as some of the nutrients may be available in sub-optimal concentrations, or even not present. Thus, it would be necessary to supplement them externally. It also has been a practice to pre-treat (chemically or mechanically) some substrates before use in SSF processes (eg: lignocellulosics) which makes them more easily accessible for microbial growth (Pandey *et al.*, 2000)

3. Selection of process parameters (physical, chemical and biochemical):

Mudgett (1986) suggested a number of key variables including inoculum age, density, supplementation of substrate, pH, temperature, agitation and aeration which should be considered in process development of solid substrate fermentations.

For enzyme production, SSF may be attractive owing to its low-level technology, the high product concentration and reduced cost of dewatering (Pandey *et al.*,2000). Hatakka *et al.*(1994) postulated that protein enrichment of lignocellulosic wastes may be economically feasible if at the same time wood-rotting fungi are used for production of extracellular enzymes (e.g: cellulases, ligninases). Soccol *et al.* (1994) demonstrated the feasibility of production of α-amylase, glucoamylase and protein enrichment of cassava by *Rhizopus* strains in SSF. They have found that raw cassava could be an inexpensive source of starch and constitute a strategic biological matter for production of commercially valuable microbial metabolites and feed products as well.

Solid substrate fermentation is also a very valuable technology for the production of crude enzyme preparation for the food industry (Deschamps and Huet, 1985; Considine *et al.*, 1987). It has also been reported that toxic compounds can be drastically reduced through SSF processes (Bau *et al.*, 1994).

There are many advantages of SSF processes for bioconversions of wastes over the conventional submerged systems at both the laboratory and the industrial scale (Aidoo *et al.*, 1982). The most attractive advantages of SSF may be the high fermenter volumetric productivity, and the reduction in product recovery expenses, pollution problems and total cost of production.

Further, there are some disadvantages of SSF (Aidoo *et al.*,1982) such as :

1. the limited type of organisms which can grow at reduced water activities, namely fungi, some yeasts, and some bacteria and streptomycetes;
2. technical problems in controlling the heat generated during fermentation;
3. low product yields;
4. the slowness of fermentation;
5. the difficulty of using devices to measure/control some of the fermentation parameters (e.g: moisture, temperature, pH, oxygen, product concentration).

Disadvantages such as problems in controlling the heat build-up and the use of reliable monitoring devices are being reduced or eliminated as investigations on those factors are being developed (Gowthaman *et al.*, 1995; Rajagopalan and Modak, 1995).

2.9 *Pycnoporus sanguineus.*

Pycnoporus sanguineus is a basidiomycete which is widely distributed on lignocellulose in tropical forests and is associated with aggressive white-rot type decay (Pointing *et al.*, 2000). This pantropical species, easy to recognize and commonly found on oil palms and on a variety of trees. Cultures of these species are white at first, but soon take on the same orange red colour as the fruit bodies (Treu, 1998). It was reported that laccase was produced as a sole lignolytic enzyme under a variety of submerged culture conditions by this fungus including cultures supplemented with a native wood substrate (Pointing *et al.*, 2000). Therefore, this fungus was sugggested as a good candidate for use in the biotransformation of

lignocellulosic wastes such as sago hampas, cocopeat and bagasse which are produced in large quantities in tropical regions (Pointing *et al.*, 2000).

Fungal laccases have been proven to have a role in lignin degradation (Eggert *et al.*, 1996), dye decolorization (Schliephake *et al.*, 1993; Pointing *et al.*, 2000) and detoxification of organic pollutants (Collins *et al.*, 1996). Thus, with the increasing understanding of laccase physiology, fungi producing this enzyme such as *Pyc. sanguineus* are currently the focus of much attention. Studies have been done using this species in submerged liquid fermentation process (Pointing *et al.*, 2000).

The proposed study was to investigate the enzyme productivity profiles of *Pyc. sanguineus* in solid substrate fermentation of selected agro-residues.