****

CHAPTER ONE

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1.0 INTRODUCTION

Lignocellulosic material is one of the most abundant biopolymer in nature produced by forest and agricultural industries as residual wastes. In Malaysia, vast amount of lignocellulosic agricultural waste materials are readily available. Among the agro-industries which produce wastes in large scale are sago starch processing industry, palm oil processing industries as well as industries which produces rubberwood products (Agamuthu, 1997).

In Sarawak, East Malaysia, the sago industry faces solid and liquid waste disposal problems as it has been estimated that 300-1000m^3 of wastewater, 80-150 tonnes of bark and 50-110 tonnes of 'hampas' are produced daily within the industry in the Sibu division itself (Chew and Shim, 1993). Sago 'hampas', the fibrous pith residue obtained after starch extraction from the rasped sago pith, contains about 66% starch, 1% crude protein and 15 % fibre on a dry weight basis, of which about 25% is made up of lignin (Vikineswary and Shim, 1996). With these properties, sago 'hampas' is a potential substrate for microbial bioconversion into value-added products such as human food (mushroom), organic fertilisers and bulk hydrolytic enzymes such as cellulases, xylanase and laccase. The use of 'hampas' however, has been only as a diet supplement in ruminant feed (Pongsapan *et al.*, 1984).

The oil palm frond parenchyma tissue (OPFPt) is obtained after fresh pruned oil palm fronds are processed and the fibrous vascular bundles are separated. The dried OPFPt contains complex carbohydrates, proteins and small amounts of fibres which may be used as an organic fertilizer or as an animal feed supplement (Dinesh, 1994).

Another cheap source of agro-residue in Malaysia is rubberwood sawdust which is obtained during sawmilling operations and it is largely used by local farmers as a substrate to cultivate edible mushrooms (Kuan, 1999).

The most effective lignin biodegraders are the white rot fungi, belonging to the basidiomycetes. The direct cultivation of white rot fungi on lignocellulosic material has been used for feed production (Zadrazil *et al.*, 1996). Bioconversion of sago 'hampas' into value-added products have been attempted by several workers (Horigome *et al.*, 1991; Paridah, 1992; Tuen, 1994). Studies have been done on the utilisation of sago 'hampas' by amylolytic fungi and ligninolytic fungi (Vikineswary and Shim, 1996; Kumaran *et al.*, 1997). Shim (1992) has reported high activities of cellulases and α-amylase during growth of *Myceliopthora thermophila* on sago 'hampas', whereas Kumaran *et al.* (1997) had found high laccase activity together with variable cellulase and xylanase in the cultivation of *Pleurotus sajor-caju* (Fr.) Singer on sago 'hampas'. Recently, *Pycnoporus sanguineus* (Linn.: Fr.) Murrill, a fungus associated with aggressive white rot decay in tropical regions have been reported to produce laccase as the sole ligninolytic enzyme in a defined liquid growth medium (Pointing *et al.*, 2000).

Solid substrate fermentation (SSF) of lignocellulosic materials by white rot fungi have received much attention recently, primarily because of the possibility of converting these materials to more digestible feedstuffs for ruminants (Dinesh, 1994 and Zadrazil *et al.*, 1996). Solid substrate fermentation are often simpler and require less processing energy than the corresponding liquid submerged fermentations (Moo-Young *et al.*, 1983). Now, SSF is gaining importance in enzyme production because of its favourable energetics, lower capital and operating expenses as compared with liquid

culture (Pandey *et al.*, 2000). Favourable results have been reported in enzyme production using SSF (Kumaran *et al.*, 1997; Benjamin and Pandey, 1998).

Enzymes from fungi have gained much attention lately, mainly due to its industrial importance. The three major groups of enzymes produced by fungi during the breakdown of agro-residues are cellulases, hemicellulases and lignin modifying enzymes (LME). Cellulases are produced by many fungi for degradation of cellulose in wood and leaf litter. Industrial application of cellulases are in the textile technology and are used in denim bleaching, as an alternative to stone washing (Viikari *et al.*, 1991). Fungal catalases is used to remove surplus hydrogen peroxide during bleaching of cotton, a treatment which reduces the number of washing steps, thus reducing the amount of wastewater produced (Viikari *et al.*, 1991). Xylanases have importance in pulp and paper industry. Due to the increased environmental concern, xylanases are applied as a bleach booster to decrease consumption of chlorine for bleaching of kraft paper pulp. Lignin, which is the darkening constituent of pulp, is covalently bound to xylan, and xylanase action assists in removing lignin from the pulp (Viikari *et al.*, 1991). Although bacterial enzymes are currently used in bleaching processes, which functions at pH 10 and at 70°C, bleaching processes are being developed to better suit fungal enzymes.

Three classes of extracellular lignin modifying enzymes (LME) involved in the degradation of lignin are lignin peroxidase, manganese-dependent peroxidase and laccase (Hatakka, 1994). Laccase is an extracellular phenol oxidase produced by basidiomycetes that cause some oxidative degradation of lignin and to catalyze the polymerization of small phenolic molecules arising from this degradation (Higuchi,

1990; Betts and King, 1991). The role of laccase in the degradation of phenolic lignin (Thurston, 1994), oxidation of nonphenolic lignin (Bourbonnais and Paice, 1990) and also partial mineralisation of some nonphenolic xenobiotics (Collins *et al.*, 1996) makes fungi capable of producing laccase as the dominant LME to be significant ecologically. Besides this, laccase is also generally produced in larger titres than lignin peroxidase and manganese peroxidase by white rot fungi, thus stimulating interest in laccase-producing fungi for degradative biotechnological applications in biopulping, biobleaching and bioremediation (Schliephake *et al.*, 1993; Collins *et al.*, 1996). It was reported that laccase was produced as the sole extracellular phenoloxidase by a white-rot fungi, *Pyc. sanguineus*. The enzyme caused partial decolorization of two azo dyes namely Orange G and Amaranth, and complete decolorization of two triphenyl methane dyes Bromophenol blue and Malachite green was achieved (Pointing *et al.*, 2000).

Solid substrate fermentation holds tremendous potential for the production of enzymes. The selection of a particular strain, however, remains a tedious task, especially when commercially significant enzyme yields are to be achieved. Agro-industrial residues are generally considered the best substrates for the SSF processes, and enzyme production in SSF is not an exception to that (Pandey *et al.*, 2000). For both economic and ecological reasons, biological degradation of easily available agro-residues using white-rot fungi has become an increasingly popular alternative for the production of bulk enzymes and also for the remediation of hazardous wastes.

Objectives of study

The aim of this study was to investigate and compare the enzyme production of a white-rot fungi, *Pyc. sanguineus*, using various agro-residues such as sago '*hampas*', oil palm frond parenchyma tissue (OPFPt) and rubberwood sawdust as substrates through solid-substrate fermentation. Enzymes such as cellulase, xylanase and laccase were assayed and characterised. The specific objectives were :

a) to study the enzyme productivity profiles during fungal growth on sago 'hampas', rubberwood sawdust and oil palm frond parenchyma tissue (OPFPt).
b) to optimise extraction and recovery of enzymes from the fermented substrate.
c) to optimise selected parameters such as inoculum age and density as well as the nitrogen supplementation of the substrate for enzyme production.