CHAPTER SIX

GENERAL DISCUSSION AND CONCLUSION

Sago starch is considered by many plant scientists as the "starch crop of the 21st century". With the rapid increase in the amount of sago starch processed the amount of wastes generated is becoming alarmingly high. Alternative use of the wastes produced would not only minimize pollution of the receiving bodies, but also generate additional income for the sago industry. The sago *hampas* which contributes to a large proportion of solid residue produced at the sago factory represents a suitable material for fungal fermentation and mushroom cultivation (Vikineswary and Shim 1996). The degradation of the fibrous lignocellulosic portion of 'hampas' was of interest in the present study, as this would render *hampas* useful for other applications.

The sago 'hampas' used in this experiment was obtained from a factory in Johor Darul Takzim, Malaysia. Due to the high moisture content, drying of *hampas* within the shortest time possible was crucial to arrest natural fermentation. Other than drying, sieving of the dried 'hampas' through a 2mm sieve was necessary, as the dried substrate tended to clump together.

There were many reasons for choosing *Pycnoporus sanguineus* in this study. The main reason was the tropical distribution of this fungus making it a particularly good candidate for use in the biotransformation of lignocellulosic wastes such as sago-hampas, cocopeat and baggase, which are produced in large

quantities in tropical regions (Pointing *et al.*,2000a). Further, laccase was produced as the sole ligninolytic enzyme in liquid culture (Pointing *et al.*, 2000)

In this study, simple technologies and minimal processing methods were used to grow *Pycnoporus sanguineus* on sago 'hampas'. However, a major problem with technologies using fungi as bioconversion agent is the apparent necessity to operate under sterile conditions. In order to secure a successful colonization of the substrate by the fungus, potential competitors have to be suppressed by thermal pretreatment of the substrate by autoclaving at 15 psi and 121°C for 30 min. However, the autoclaving for 30 minutes did not eliminate all the contaminants as some culture flasks showed signs of contamination during the fermentation period. Nevertheless, it reduced the number of potential contaminants and the optimized culture conditions selectively favored the growth of *Pycnoporus sanguineus*.

The main constituents of 'hampas' being starch and fibers, its utilization by the ligninolytic microorganism, *Pycnoporus sanguineus* was found to be possible. As *P. sanguineus* is a slow-growing fungus, increasing the incubation time will increase the decomposition of the substrate. In this study, a 30- day incubation period was used to study the enzyme profile of this fungus. The drawback of long incubation time was apparent drying of the mycelium-substrate mixture due to lack of facilities to maintain the humidity content, which may affect the growth of the fungus. In any commercial application, the fermentation time has to be shorter for economical reasons.

The enzyme extraction was done best with a solvent volume just large enough to permit efficient extraction typically 1:2 to 1:4 culture to solvent (w/v)

(Leatham *et al.*, 1991). It is important to understand the limitations of extracting enzymes from lignocellulosic substrates. Leatham *et al.* (1991) assumed that only the enzymes present on the exposed hydrated surfaces of such solid substrates would be extracted. And although accessible, the enzymes will not necessarily be extracted at the same efficiencies. The notoriously tight-binding enzymes such as the exocellulases are likely to be extracted in low yield. This will be underestimating their original concentration in the cultures.

The results of this study on enzyme activity of *P. sanguineus* during SSF of 'hampas' on laboratory scale showed evidence. In the process of studying the utilization of sago 'hampas' for enzyme production, possibilities for further research were revealed. Lignolytic and cellulolytic enzymes were produced by *P. sanguineus* during biodegradation of 'hampas'. Enzymes, particularly laccase which is a good indicator of mycelial biomass, was detected in the highest amounts in the culture extracts. The result of the process showed that the activities of the enzymes quantitatively produced; were in the descending order of:

Laccase > xylanase > lignin peroxidase

Maximum activities of the various enzymes obtained during the fermentation of 'hampas' were as follows: laccase and xylanase with productivities of 18.35U/g and 6.36U/g of substrate respectively on day 10 of SSF. Lignin peroxidase activity was the lowest of 1.5U/g of substrate on day 5.The enzyme activities correlated well with the age of fermentation culture. The lignin peroxidase showed almost similar activities with slight variation throughout the fermentation period, inspite of significant increase in xylanase and

laccase productivity. The insignificant and low productivity of lignin peroxidase indicated that this enzyme was either not produced by *P. sanguineus* or the nutrients available were not favorable for the production of this particular enzyme *by P. sanguineus*. The soluble protein content, which was used to measure fungal biomass indirectly, showed a slight increase throughout the fermentation and stabilized towards the end of SSF. Overall, *P. sanguineus* produced laccase in higher quantities than the other enzymes during SSF of 'hampas'.

The optimization of the fungal inoculum parameters for efficient SSF of 'hampas' with the *P. sanguineus* were carried out and encouraging results were obtained. In this study the 2-week-old immature inoculum and 4-week too old inoculum were found not suitable for significant xylanase and laccase productivities. The 10% (w/w) 3-week-old inoculum density (w/w) with 0.76% urea (w/v) was found to be optimum for maximum enzyme production. In the follow up study, more urea concentrations with minimal difference for each parameter should be studied in order to determine the optimal conditions more precisely.

About 50ml of moisture was added to every 10g of 'hampas' this gave a sufficient moisture content for growth of *Pyc. sanguineus*. Enough water was necessary to ensure good fungal development but too much water decreased porosity and oxygen diffusion in the mass and could favor bacterial contamination (Raimbault and Alzard, 1980).

Assays of the activities in the crude culture extracts were useful to indicate the enzymes available for recovery. Extracts from *P. sanguineus* culture

typically exhibited a range of enzyme productivities present in quantities apparently sufficient for isolation and characterization (Chapter 5). However, the values obtained with crude culture extracts were only qualitative. Often, they did not accurately estimate the quantities of the individual enzymes present.

The properties and roles of enzymes involved in the bioconversion of lignocellulosics have received considerable attention. Enzymes hold great potential for the industrial-scale processing of a wide variety of biologically-derived materials. Leatham and Himmel (1991) mentioned the advantages of enzymes over chemicals in biomass processing. These include higher specificity in the targeted conversions, resulting in the decreased generation of side products; the ability to carry out novel conversions; and the potential avoidance of producing toxic, difficult to degradable, or environmentally damaging by-products.

Currently, the effective utilization of enzymes in many new industrial applications is a possibility. Successful application will require increased knowledge about the range of enzymes available, their characteristics, and the need for improvements in the production of enzymes and how to utilize the enzymes. The abundance of sago 'hampas' suggests strongly that there is a good potential to profitably and commercially exploit sago 'hampas' for large scale processes such as mushroom, animal feed or enzyme production (Fig. 6.1).

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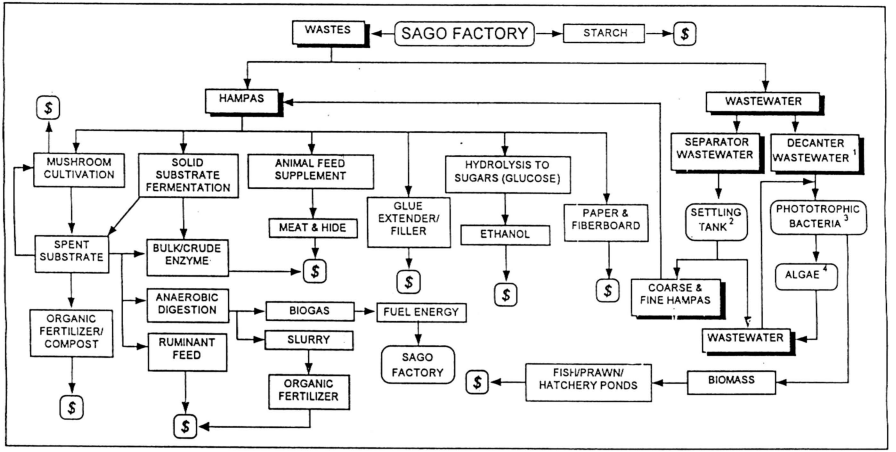

Fig 6.1: Proposed integrated system for the utilization and treatment of sago starch processing wastes

= Product = By-product ($) = Revenue

[1]Getha (1995), [2]Simple settling technique, [3]Phototrophic bacteria treatment system, [4]Algal treatment ponds

RECOMMENDATIONS FOR FURTHER WORK

The SSF of 'hampas' using *Pycnoporus sanguineus* showed excellent potential for further studies as follows:

1. Natural selection of *Pycnoporus sanguineus* for finding the variant, which will provide more laccase.
2. The SSF of sago *hampas* with *Pycnoporus sanguineus* can be optimized to produce maximum laccase extract. The feasibility of detoxification of polyphenols using the extract can be studied.
3. Studies on more cost effective laccase purification methods.
4. The organism and the substrate, in particular the degradative enzymes associated with *P. sanguineus* require further investigation in order to understand more fully their modes of action.
5. In assessing the potential of laccase in delignification, decolorization and detoxification processes, information on cell-free laccase with regard to their action on phenolics in natural substrates should be collated at the laboratory scale.
6. Economic analysis must be undertaken to evaluate the viability of the various utilization processes proposed. It is necessary to strike a balance between optimism and pessimism in the extrapolation of the laboratory observations to industrial scales. The ability to introduce novel biotechnological processes will in most case be dependent on the price of the products versus equivalent products obtained by the existing practices.

CONCLUSION

More effective treatments for enhancing lignocellulosic byproducts using ligninolytic fungi are dependent on coordinated research aimed at optimization of the solid substrate processes involved.

The future prospects for the development of the utilization of sago by-products for recovering value-added by-products need to be studied with a multi dimensional approach. The complete bioconversion of sago 'hampas' with the main aim of minimizing pollution must fit into an integrated treatment system together with sago waste utilization

The integrated system may incorporate, among others enzyme extraction, ruminant feed, mushroom cultivation, phototropic bacteria, algae and fish cultivation.