DEVELOPMENT OF SOLID STATE FERMENTATION SYSTEM FOR ENZYME PRODUCTION AND ITS USAGE IN A PILOT SCALE DEINKING OF PRINTED WASTE PAPER

by

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TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

A. niger	*Aspergillus niger*
APHA	American Public Health Association
A_w	Water activity
BC	Before Century
Bhd	Berhad
BOD	Biological Oxygen Demand
BP	Before Present
C/N	Carbon per nitrogen
cm	Centimeter
CMC	Carboxymethyl cellulose
CMCase	Carboxymethyl cellulase
CMFAB	Continuously mixed force aerated bioreactor
Co	Corporation
CO_2	Carbon dioxide
$CoCl_2 \cdot 6H_2O$	Cobaltous Chloride Hexahydrate
COD	Chemical Oxygen Dissolve
COOH	Carboxyl group
CSF	Canadian Standard Freeness
DNA	Deoxyribonucleic acid
DNS	Dinitrosalicylic acid
DO	Dissolved oxygen
E.C.	Enzyme Commision
e.g.	Example
etc	et cetera

FAS	Ferrous ammonium sulfate
$FeSO_4 \cdot 7H_2O$	Ferrous sulfate heptahydrate
Fig.	Figure
FPU	Filter Paper Activity Units
g	Gram
G. sepiarium	*Gloeophyllum sepiarium*
g/cm^3	Gram per centimeter cubic
g/L	Gram per liter
g/m^2	Gram per meter square
h	Hour
HC	High consistency
HCI	Hydrochloric Acid
HW	Heavy weight
IBRL	Industrial Biotechnology Research Laboratory
IMFAB	Intermittently mixed, forcefully aerated bioreactors
Inc	Incorporated
$K_2Cr_2O_7$	Potassium dichromate
KCI	Potassium chloride
kDa	Kilo Dalton
Kg	Kilogram
KH_2PO_4	Potassium dihydrogen phosphate
K_La	Volumetric oxygen transfer coefficient
K_m	Michaelis-Menten constant
kPa	Kilo pascal
kPa m^2/g	Kilo pascal meter square per gram

K_s	Affinity constant biomass/substrate
kWh	Kilo watt hour
L	Liter
L/h•g	Liter per hour per gram
L/Kg•m	Liter Per kilogram per meter
L/min	Liter per minute
LC	Low consistency
LiP	Lignin peroxidase
LTD	Limited
LW	Light weight
M	Molar
m^2	Meter square
m^2/cm^3	Meter square per centimeter cubic
m^2/g	Meter square per gram
m^3	Meter cubic
MC	Medium consistency
Met	Methionine
mg/ml	Milligram per milliliter
$MgSO_4•7H_2O$	Magnesium sulfate heptahydrate
min	Minute
ml	Milliliter
mm	Millimeter
mM	Millimolar
mm^2	Millimeter square
mmol	Millimole
mN m^2/g	Milli Newton meter square per gram

MNI	Malaysian Newsprint Industries
MnP	Manganese-dependent peroxidase
$MnSO_4 \cdot 4H_2O$	Mangan (II) Sulfate Tetrahydrate
MOW	Mixed Office Wastepaper
N	Normality
NaCI	Natrium chloride
NaOH	Sodium hydroxide
NH_4NO_3	Ammonium nitrate
nm	Nanometer
Nm/g	Newton meter per gram
O_2	Oxygen
OD	Optical density
ONP	Old newspaper
PKC	Palm kernel cake
PM	Paper machine
ppm	Part per million
psi	Pounds per square inch
RD	Rotating drum
RH	Relative humidity
RM	Ringgit Malaysia
rpm	Rotation per minute
RT	Room Temperature
s	Second
SD	Stirred drum
Sdn	Sendirian

SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SEM	Scanning Electron Microscope
SmF	Submerged Fermentation
sp.	Species
SSF	Solid State Fermentation
T. reesei	*Trichoderma reesei*
T80	Tween 80
TAPPI	Technical Association of the Pulp and Paper Industry
TM	Trademark
Tris-HCI	Trimethylpyridine hydrochloric
µm	Micrometer
µmol/mg•min	Micromole per milligram per minute
U/mg_G	Unit Activity per milligram
U/g	Unit Activity per gram
UK	United Kingdom
USA	United State of America
USD	US Dollar
USM	Universiti Sains Malaysia
UV	Ultraviolet
v/v	Volume per volume
v/w	Volume per weight
V_{max}	Maximum velocity
w/v	Weight per volume
w/w	Weight per weight
X	Multiply
$ZnSO_4•7H_2O$	Zinc Sulfate heptahydrate

LIST OF SYMBOLS

β	Beta
%	Percentage
&	And
p	Para
^{o}C	Degree Celsius
α	Alpha
$\pm$	Plus minus
=	Equal
+	Plus
-	Minus
$	Dollar
μ	Specific growth rate
μ_{max}	Maximum Specific Growth Rate
>	More than
<	Less than
t_d	Doubling time
P<	Probability less than
P>	Probability more than
®	Register

PEMBANGUNAN SISTEM FERMENTASI KEADAAN PEPEJAL UNTUK PENGHASILAN ENZIM DAN KEGUNAANYA DALAM SKALA PERINTIS PENYAHDAKWATAN KERTAS BUANGAN BERCETAK

ABSTRAK

Kajian ini memberi tumpuan kepada pembangunan fermenter keadaan pepejal untuk penghasilan enzim dan pembangunan sistem penyahdakwatan secara enzimatik berskala rintis. Fermenter yang baru dibangunkan itu dikenali sebagai FERMSOSTAT®. Fermentor keadaan pepejal yang baru dibangunkan itu digunakan dalam kajian penghasilan enzim selulase dan xilanase dengan menggunakan pencilan tempatan, *Aspergillus niger* USM AI 1. Pengoptimuman untuk penghasilan enzim telah dijalankan dan didapati keadaan optimum penghasilan enzim adalah 0.5 kg substrat (gabungan PKC dan hampas tebu, nisbah 1:1); 70% (b/b) kandungan kelembapan; pengeraman pada suhu 30°C; pengudaraan pada 4.0 L/j•g substrat pemfermentasian selama 5 minit dan pengadukan pada 0.5 psm selama 5 minit. Di bawah keadaan optimum pemfermentasian keadaan pepejal, aktiviti CMCase, xilanase dan FPase yang diperolehi masing-masing adalah sebanyak 62.6 U/g, 390.8 U/g dan 3.4 U/g. Ini mewakili kira-kira 9.6, 7.8 dan 3.4 kali ganda peningkatan dalam aktiviti masing-masing selepas proses pengoptimuman berbanding dengan sebelum proses pengoptimunan. Apabila kajian perbandingan penghasilan enzim dijalankan dengan menggunakan *Trichoderma reesei*, aktiviti CMCase, xilanase dan FPase yang diperolehi masing-masing adalah 57.3 U/g, 563.0 U/g dan 2.2 U/g. Enzim yang dihasilkan digunakan dalam kajian penyahdakwatan campuran kertas terpakai pejabat (CKTP) dan surat khabar lama (SKL) dengan menggunakan sistem

penyahdakwatan berenzim pada skala rintis yang telah dibangunkan. Kajian pengoptimuman proses penyahdakwatan berenzim mendapati bahawa keadaan optimum CKTP adalah pempulpaan pada konsistensi 2% selama 60 min; hidrolisis berenzim pada suhu 50°C, pH 5.5, 0.8 U CMCase aktiviti dan 4.0 U xilanase aktiviti bagi 1 gram pulpa kering dan hidrolisis selama 60 minit dan proses pengapungan pada pH 8.0, 0.20% (b/b) Tween 80 dan masa pengapungan selama 5 minit. Manakala, keadaan optimum penyahdakwatan SKL yang perolehi adalah pempulpaan pada konsistensi 3% selama 45 minit; hidrolisis berenzim pada suhu 50°C, pH 5.5, 0.4 U CMCase aktiviti dan 2.0 U xilanase aktiviti bagi 1 gram pulpa kering dan hidrolisis selama 45 minit dan proses pengapungan pada pH 8.0, 0.55% (b/b) Tween 80 and masa pengapungan selama 20 min. Tahap kecekapan penyahdakwatan yang diperolehi oleh CKTP dan SKL di bawah keadaan optimum masing-masing adalah kira-kira 6.0% and 6.3%. Manakala, tahap kecekapan penyahdakwatan secara kimia bagi CKTP dan SKL masing-masing adalah kira-kira 2.9% dan 3.5%. Kedua-dua proses penyahdakwatan berenzim dan kimia bukan sahaja mempengaruhi dengan ketara ciri optikal dan mekanikal kertas terpakai yang dikaji tetapi juga ciri pulpa dan air buangan yang dihasilkan. Penyahdakwatan berenzim CKTP menunjukkan peningkatan dalam kecerahan (4.7 unit), indeks tensil (14.1%), indeks pecah (3.4%), kebebasan (2.0%) dan penyingkiran sisa dakwat (44.5%) tetapi penurunan dalam kelegapan (2.6%) dan indeks koyak (9.6%). Manakala, penyahdakwatan berenzim SKL menunjukkan peningkatan dalam kecerahan (2.5 unit), indeks tensil (10.2%), indeks pecah (3.8%), kebebasan (2.9%) dan penyingkiran sisa dakwat (51.15%) tetapi penurunan dalam kelegapan (0.4%) dan indeks koyak (3.9%). Berbeza dengan penyahdakwatan berenzim, penyahdakwatan CKTP secara kimia menunjukkan peningkatan dalam kecerahan

(2.3 units), indeks tensil (1.1%), indeks pecah (1.2%), kebebasan (1.9%) dan penyingkiran sisa dakwat (31.1%) tetapi penurunan dalam kelegapan (1.4%) dan indeks koyak (1.9%). Manakala, penyahdakwatan SKL secara kimia menunjukkan berlakunya peningkatan kecerahan (1.4 unit), indeks tensil (6.8%), indeks pecah (3.0%), kebebasan (2.3%) dan penyingkiran sisa dakwat (49.8%) tetapi penurunan dalam kelegapan (0.1%) dan indeks koyak (1.1%). Nilai keperluan oksigen kimia (COD) yang diperolehi daripada process penyahdakwatan berenzim ke atas CKTP dan SKL masing-masing adalah 33.9% and 33.8% lebih rendah berbanding dengan proses penyahdakwatan secara kimia. Manakala, nilai keperluan oksigen biokimia (BOD_5) yang diperolehi daripada process penyahdakwatan berenzim ke atas CKTP dan SKL masing-masing adalah 47.1% and 39.3% lebih rendah berbanding dengan proses penyahdakwatan secara kimia. Ini secara langsung akan mengurangkan kos rawatan air buangan. Tambahan lagi, berdasarkan Akta Persekitaran Malaysia 1974, rawatan tidak perlu dijalankan ke atas air buangan daripada penyahdakwatan CKTP kerana air buangan tersebut berada di bawah paras yang dibenarkan untuk dibuang ke persekitaran. Hasil yang diperolehi daripada kajian ini mencadangkan bahawa penyahdakwatan berenzim boleh menjadi satu alternatif yang berpotensi tinggi kepada kaedah kimia, yang menunjukkan beberapa masalah dan keburukan berbanding dengan penyahdakwatan berenzim dalam pengitaran semula kertas buangan.

DEVELOPMENT OF SOLID STATE FERMENTATION SYSTEM FOR ENZYME PRODUCTION AND ITS USAGE IN A PILOT SCALE DEINKING OF PRINTED WASTE PAPER

ABSTRACT

The present work deals with the development of solid state fermenter for enzymes production and the development of a pilot scale of enzymatic deinking system. The newly developed solid state fermenter, was named FERMSOSTAT®, which was used for the production of cellulases and xylanase using a local isolate; *Aspergillus niger* USM AI 1. Optimization of enzymes production was carried out and the optimum conditions obtained were 0.5 kg substrate (a combination of PKC and sugarcane baggase at 1:1 ratio); 70% (w/w) moisture content; incubated at 30°C; aeration at 4.0 L/h•g fermented substrate for 5 min and mixing at 0.5 rpm for 5 min. Under the optimum SSF conditions, the CMCase, xylanase and FPase activities obtained were 62.6 U/g, 390.8 U/g and 3.4 U/g, respectively. This represents about 9.6, 7.8 and 3.4 folds increased in their respective enzymes activities after the optimization process. When comparative study on the enzymes production was performed using *Trichoderma reesei*, the CMCase, xylanase and FPase activities obtained were about 57.3 U/g, 563.0 U/g and 2.2 U/g, respectively. The enzymes produced were used in the deinking studies of mixed office waste paper (MOW) and old newspaper (ONP) using newly developed pilot scale enzymatic deinking system. Optimization of enzymatic deinking process was carried out and the optimum conditions obtained for MOW were pulping at 2% consistency for 60 min; enzymatic hydrolysis at 50°C, pH 5.5, 0.8 U of CMCase and 4.0 U of xylanase per gram of air

dry weight of pulp and 60 min of hydrolysis time and flotation process at pH 8.0, 0.20% (w/w) of Tween 80 and 5 min of flotation time. Meanwhile, the optimum conditions obtained for the deinking of ONP were pulping at 3% consistency for 45 min; enzymatic hydrolysis at 50°C, pH 5.5, 0.4 U of CMCase and 2.0 U of xylanase per gram of air dry weight of pulp and 45 min of hydrolysis time and flotation process at pH 8.0, 0.55% (w/w) of Tween 80 and 20 min of flotation time. The deinking efficiency obtained for MOW and ONP under their respective optimum conditions were about 6.0% and 6.3%, respectively. The deinking efficiency detected by chemical deinking of MOW and ONP were about 2.9% and 3.5%, respectively. Both enzymatic and chemical deinking processes not only significantly influence optical and mechanical properties of the deinked paper but also the pulp properties and the effluent generated. Enzymatic deinking of MOW showed improvement in brightness (4.7 units), tensile index (14.1%), burst index (3.4%), freeness (2.0%) and residual ink removal (44.5%) but decreased in opacity (2.6%) and tear index (9.6%). Meanwhile, enzymatic deinking of ONP showed improvement in brightness (2.5 units), tensile index (10.2%), burst index (3.8%), freeness (2.9%) and residual ink removal (51.15%) but decreased in opacity (0.4%) and tear index (3.9%). Unlike enzymatic deinking, chemical deinking of MOW demonstrated increased in the brightness (2.3 units), tensile index (1.1%), burst index (1.2%), freeness (1.9%) and residual ink removal (31.1%) but loss in opacity (1.4%) and tear index (0.1%). Meanwhile, chemical deinking of ONP showed enhanced in brightness (1.4 units), tensile index (6.8%), burst index (3.0%), freeness (2.3%) and residual ink removal (49.8%) but decreased in opacity (0.1%) and tear index (1.1%). Biological oxygen demand (BOD_5) and chemical oxygen demand (COD) analysis indicated that, effluent produced from the enzymatic deinking process was lower compared to

chemical deinking process. About 33.9% and 33.8% lower in COD values were obtained after enzymatic deinking of ONP and MOW compared to chemical deinking process, respectively. Meanwhile, BOD_5 obtained from enzymatic deinking of MOW and ONP were 47.1% and 39.3% lower compared to chemical deinking process, respectively. This directly will reduce the wastewater treatment cost. In addition, based on the Malaysia Environmental Act 1974; no wastewater treatment was required on the effluent obtained from deinking of MOW, where the effluents obtained were within the permitted value for safety discharged into the environment. The results obtained from this work suggested that the enzymatic deinking can be a highly potential alternative to the chemical method, which showed several problems and disadvantages compared to enzymatic deinking in the recycling of waste paper.

CHAPTER ONE: INTRODUCTION

1.1 Paper Industries in Malaysia and the Need for Paper Recycling

The paper and paper products industries are essentially part of the larger wood-based products sector. Meanwhile, the paper making industry has a close correlation to the packaging and writing industry. The paper industry continues to grow even though the current drive in many nations is to go "paperless" including Malaysia. Indeed, according to Food and Agriculture Organization, the global demand for paper would increase by about 3.1% annually and it is predicted to reach about 440 million metric tons by the year 2010 (Daily Express newspaper, 2005). Therefore, the Malaysian government has identified this industry as one of the priority areas for investment in the second Industrial Master Plan (Center for International Forest Research, 2006). The strategy is to achieve self sufficiency, to reduce import and encourage foreign capital. With that, the government had given duty-free for importation of industrial paper machines and also the pulp and waste papers recovered.

Malaysia has extensive forest resources with forest covering close to 60% of its land area, but remained to be a major importer of paper. The country's pulp and paper industry is not as well developed as those in other countries. It is crowded and highly fragmented, which made up more than 300 companies with more than 5000 workers. Meanwhile, the capital investment in pulp and paper industry is more than RM 250 millions (Ministry of International Trade and Industry, 2005). Basically the industry included pulp/paper mills, paper converter and producers of paper containers/corrugated carton boxes. Out of 300 companies, less than 30% are pulp and paper mills. The paper mills in Malaysia are small by the standard of the industry in the developed countries. Only 6 out of 20 mills producing more than 100, 000

metric tones and up to 300, 000 metric tones per annum of papers. Unfortunately, none of these companies reach the critical level of 500, 000 metric tons per year, which is often seen as the standard for a minimum competitive level in terms of world competition (Center for International Forest Research, 2006). Local production has a total capacity of more than 1.3 million metric tones per annum in the year 2005. However, it was not able to meet the total consumption of 115 kg per capital or more than 2.2 million metric tones per annum in year 2005 (World Resources Institute, 2005).

Malaysia needs to import pulp, newsprint, printing/writing papers and industrial papers. According to Department of Statistics (2003), Malaysia spent more than RM 3.0 billion importing 1.40 million metric tones of all types of paper. Out of RM 3.0 billion, more than RM 1 billion was spent on importing printing/writing paper. The import is inevitable, since local consumption is more than 380, 000 metric tons per year (The Star Newspaper, 2009). Meanwhile there is only one mill in Malaysia (Sabah Forest) produces printing/writing paper with the capacity of 165,000 metric tones per year. On the other hand, Malaysia produces 280,000 metric tons of newsprint per annum with almost 99% of it are produced by Malaysian Newsprint Industries Sdn Bhd. However, that did not meet the local demand of about 320,000 metric tons of newsprint per annum (Malaysian Newsprint Industries Sdn Bhd, 2007).

The pulp and paper industry in Malaysia is heavily dependant on imported fiber particularly virgin pulp due to the lack of domestically sourced of fresh fibers. In addition, the industry is also facing the need to find new sources of fiber to

strengthen and retain the quality of secondary fibers as the use of recycled paper is growing in Malaysia (Center for International Forest Research, 2006). This was due to the fact that waste paper constitutes 95% of raw materials in Malaysian paper mills. Moreover, for the year 2002, import of pulp and paper was 7 times greater than its export (Department of Statistics, 2003). Therefore, there is a need to increase the waste paper recovery rates in Malaysia, which only hovers around 60% (Malaysian Newsprint Industries Sdn Bhd, 2007). However, these present a challenge for pulp and paper mill with current chemical deinking process and technologies, which is less effective in deinking of mixed office waste paper (MOW). MOW is a fast growing source of materials for recycling and it is the most difficult raw material to be de-inked (Prasad, 1993; Gubitz *et al.*, 1998a). Nevertheless, it has to be borne in mind that MOW is a large, virtually untapped source of high quality fiber that could be used for high quality grade papers and many other products only if the current deinking process could be improved.

A major difficulty in dealing with secondary fiber is the removal of ink through the process of deinking. The degree of difficulty in ink removal depends primarily on the ink types, printing process and fiber type. Currently, Malaysian paper mills used chemical approach to de-ink the waste paper, which generally is considered to be more efficient with respect to the ink detachment. However, it requires the use of large amount of chemical agent and not environmental friendly (Prasad *et al.*, 1993; Woodword *et al.*, 1994). In addition, deinking in an alkaline environment induces a smeared pulp or low brightness. Furthermore, this chemical method resulted in a significant increase in the level and concentration of COD in the effluent water caused by the dissolution of carbohydrates and organic additives present in the

fibrous materials. Besides that, the effluent will also contain high impurities of chemicals used in the deinking process. Ultimately, this results in a highly environmentally damaging and costly wastewater treatment to meet the environmental regulations (Prasad *et al.*, 1992).

Due to significant number of disadvantages resulted from the conventional chemical method; an alternative method for deinking process must be sought. Alternatively, the use of enzymes has been reported to be a potentially efficient solution to overcome the problem encountered by commonly employed deinking techniques (Prasad *et al.*, 1992; Putz *et al.*, 1994). The potential of enzymatic deinking has been assessed and proven successful using a number of different types of enzymes (Gubitz *et al.*, 1998a). For example, the removal of oil carrier-based inks can be facilitated by the addition of lipases and esterases. Meanwhile, hemicellulases and cellulases can be used to release ink from fiber surface by partially hydrolysis of carbohydrate molecules on the fiber surface.

To date, few studies on the effect of enzymes on deinking of MOW and ONP have been carried out using enzyme preparations containing mixed activities including both cellulases and hemicellulases (Prasad *et al.*, 1993; Jeffries *et al.*, 1994; Pala *et al.*, 2004). However, none of these studies has been performed in Malaysia. Therefore, the need to explore the possibility of using enzymes in the deinking of MOW and ONP with the aim to recycle the waste paper must be given priority as an environmental friendly approach in the Malaysia paper industries.

1.2 Economical and Industrial Advantages of Solid State Fermentation

Solid state Fermentation (SSF) has gained importance in recent years in biotechnology industries due to its applications in the production of biologically active secondary metabolites. There are several industrially and economically important advantages, in which SSF can offers over submerged fermentation (SmF). The fermenters used in SSF are simple in design, constrict and small reactor and do not have much automation in the control system. In SSF, slow mixing is performed with no foam generation, in which normally found in SmF by using antifoam sensor. Additional benefit includes substrate volume used is higher due to low moisture content and less water is used during the fermentation. Therefore, smaller volume of bioreactor is required and smaller working space is needed, which led to lower capital investment (Pandey *et al.,* 2008b). On the other hand, the substrates use in SSF are generally agro-industrial residues with minimal or no pretreatment and thus is relatively simple, uses less energy than SmF (Wang and Yang, 2007). Besides that, the substrate itself contains most of the necessary nutrient required by the microbial to grow and this makes the fermentation media simpler (Cannel and Moo-Young, 1980a; Steinkraus, 1984; Kumar and Lonsane, 1990; Raimbault, 1998; Perez-Guerra *et al.,* 2003).

The products produced by SSF process achieved higher yields, relatively high concentration and higher product titers than those obtained in SmF. This is because small volumes of solvent or water are required to extract the desired product if necessary. Less water is needed in up-stream processing resulting in lesser wastewater generation in the down stream processing and lower recovery cost, which form some of the advantages point of SSF processes. Additional benefits include

easy and simple disposal or treatment of the fermented residue due to the low water content of the fermented residue. Furthermore, the fermented residue can be dried and stored at room temperature without any significant loss in the activity. On the other hand, the fermented substrates can be used directly as fertilizer or animal feed (Kumar and Lonsane, 1990; Perez-Guerra *et al.,* 2003) without the need to isolate the product. Another example is the production of glucoamylse using wheat bran mixed with corn flour. After fermentation the fermented substrates can be used directly for the hydrolysis of cassava flour to produce fermentable sugars (Wang and Yang, 2007).

Cost is another factor that makes SSF favorable over SmF process. About 40% of the industrial enzymes are produced using SmF. However, compared to SmF, SSF shows lower cost and high productivity. It has been reported that the production cost of crude cellulases in SmF is approximately USD20/kg, which was about 100 fold more expensive compared to SSF (Tengerdy, 1996; Pandey *et al.,* 2000a, 2008a). The low production cost by SSF method may due to low cost of the agro-industrial residues, which is used as substrates, minimum sterility and low equipment requirement. On the other hand, SSF can be an economical alternative for phytase enzyme production due to high enzyme concentration and activities. Furthermore, the fermented substrates containing phytase can mixed with animal feed as a value added supplement. Thus, reduces the animal feed cost (Bogar *et al.,* 2003)

SSF possesses a unique characteristics which normally is not done in SmF is the employment of mixed microbial cultures. It has been reported that, mixed culture can be used in production of broad range of enzymes (Koroleva et *al.,* 2002; Stepanova *et*

al., 2003; Yang *et al.,* 2004) with different productivity (Gutierrez-Correa *et al.,* 1999; Massadeh *et al.,* 2001). Meanwhile, mixed cultures used in food industry are important for food flavour production. For example, the production of various aroma-active components was reported by Nout and Aidoo (2002). Whereas, 70 volatile compounds can be produced when bamboo sprouts was processed by undefined mixed cultures (Fu *et al.,* 2002).

Global demand for citric acid is large and still growing all over the world. It is one of the world's largest fermentation products with an estimated annual production of 1 million tons (Soccol *et al.,* 2004). At present, it is mainly produced using the SmF. However, the production by SSF using low cost agro-industrial residues has attracted attention. The substrates that can be used include bagasse (Prado *et al.,* 2004), apple pomace (Shojaosadati and Babaeipour, 2002) coffee husk, fruits waste (Kumar *et al.,* 2003a), sugar cane bagasse (Kumar *et al.,* 2003b) and cassava bagasse (Vandenberghe *et al.,* 2000). Higher productivity of lactic acid from SSF was obtained compared to SmF (Soccol *et al.,* 1994a, 1994b). In addition, the production of lactic acid using SSF was able to overcome some problems faced in SmF such as difficulty in mixing and aeration using the fungal fermentation. This also indicates that SSF is highly potential for economic production of lactic acid.

Biological control agent such as fungal spores can be produced by both SSF and SmF. However, spores production is the only process where SSF dominates over SmF in all aspects such as better yield, abundant, robust, morphology, healthy and high stability (Holker *et al.,* 2004; Ramachandran *et al.,* 2007; Wang and Yang, 2007). This may due to the SSF conditions used are close to natural habitat of the

fungi. Spore of *Coniothyrium minitans* can be used as biopesticides against the plant pathogen *Sclerotinia sclerotiorum*. Meanwhile, *C. minitans* spores produced by SSF are of better quality, with greater resistance to UV-irradiation and desiccation during recovery procedures and survive longer after storage (McQuilken and Whipps, 1995; McQuilken *et al.,* 1997; Oostra *et al.,* 2000; Vrije *et al.,* 2001; Wang and Yang, 2007).

In conclusions, for the past few years, development in SSF technology has been significant and it is on the way for economical commercialization. It has also been found economically viable for various processes such as secondary metabolites, enzymes, biotechnology and pharmaceutical products.

1.3 Benefits of Enzyme Application in Paper Recycling Industries

The use of enzyme in deinking of various type of waste paper has been assessed and reported successful using various type of enzymes and mixture of enzymes preparations (Gubitz *et al.*, 1998a). Enzymatic deinking has been described to be able to deink high quality waste paper such as MOW, in which its reuse is usually limited by the high content of toners (Jeffries *et al.,* 1994). MOW is the most difficult raw materials to be deinked by conventional deinking process. This is because the toners contain thermoplastic binders, which polymerize and fuse onto the paper fibers during the high temperature printing process. They will remain as flat, large and rigid particles and when treated with chemical are poorly separated from the fiber during flotation process (Quick and Hodgson, 1986; Shrinath *et al.*, 1991; Jeffries *et al.,* 1994, 1995; Viesturs *et al.,* 1999). Longer mechanical action will degrade the

fiber but not efficient to break down the toner particles to size which is favorable to be removed by flotation process (Shrinath *et al.*, 1991).

Conventional chemical deinking process is carried out under alkaline environment, which is generally considered more efficiency. However, it requires the use of large amounts of chemical agents such as sodium hydroxide, sodium carbonate, sodium silicate, hydrogen peroxide and surfactants (Prasad *et al.*, 1993; Woodword *et al.*, 1994). This resulted in significant increase in the level and concentration of COD in the effluent water. Alternatively, the use of enzymes has been reported to be a potentially less polluting solution to overcome the problem encountered by chemical deinking techniques (Prasad *et al.*, 1992; Putz *et al.*, 1994). Furthermore, enzymatic deinking avoids the alkaline environment commonly requires in the traditional deinking which cause yellowing of the recycled pulp. In addition enzymatic deinking reduces chemical usage means lower waste treatment costs and less impact to the environment (Putz *et al.*, 1994). Besides the ink removal, during enzymatic deinking cellulases acts directly on fines and microfibrils protruding out from the surfaces. This action would remove fines content and improved the interfibrillar bonding, which may result in better paper strength properties (Jeffries *et al.*, 1994; Gubitz *et al.*, 1998b; Lee and Eom, 1999). In addition, enzymatic deinking enhances the brightness and cleanliness of the pulp. Additional benefits include the improved operation of thickeners due to better drainage. Improved drainage may results in faster machine speed which yields significant savings in energy and thus the overall cost (Heise *et al.*, 1996).

The use of bleach chemicals is usually lower for enzymatic deinking than for conventional chemical deinking. The reduction of chemical use would result in lower waste treatment costs while minimizes the impact on the environment. Lower bleaching costs and less pollution can also be achieved, since enzymatic deinked pulps have been proved to be easier to bleach which consequently require less bleaching chemicals than the pulps deinked by chemical methods (Pratima and Pramod, 1998).

In addition, successful development of enzymatic deinking may directly increase the recycling of waste paper, which indirectly may increase the economic of paper recycling industry. This is because cheaper operational cost of enzymatic deinking compared to current chemical deinking process and less pollution. Thus, this will encourage more capital investment in the industry and more working opportunity for the local residents. In addition, it may also help in the enzymes industry by increasing the enzyme production at a cheaper cost *via* solid state fermentation process using agro-industrial residual as valuable substrates. Furthermore, the use of recycled fiber reduces the need for virgin pulp. This may result in great savings on the water and energy required for pulping, bleaching and refining, which would also eventually reduce pollution problems (Pratima and Pramod, 1998). Additional benefits include reduction in the amount of waste paper into landfill which will reduce associated disposal cost.

1.4 Research Scope and Objectives

In this study, solid state fermenter, which named FERMSOSTAT® was designed and developed for laboratory use. It was fabricated by a local fabricator based on the

findings obtained by Pang *et al.,* (2005) using tray SSF system. The developed solid state fermenter was able to monitor and control the parameters that may affect the solid state fermentation process. FERMSOSTAT® was used for the production of cellulases and xylanase enzymes using *Aspergillus niger* USM AI 1 (Pang *et al.,* 2005) grown on palm kernel cake and sugarcane baggase as substrates. Parameters that affect the enzymes production were optimized in order to increase the enzymes productivities. The parameters that were optimized included; amount of substrates, moisture content, incubation temperature, aeration rate and aeration time, mixing rate and mixing intensity. The production of cellulases and xylanase by *A. niger* USM AI 1 was compared using *T. reesei* grown on the same substrates under optimized conditions. The enzymes produced by *A. niger* USM AI 1 were used in the deinking of mixed office waste paper and old newspaper.

A pilot scale of enzymatic deinking system was designed and developed. It was fabricated based on the data obtained by Lee *et al.,* (2007) using laboratory scale enzymatic deinking system. The strategy for the development of the deinking system was determined. The enzymatic deinking system developed was used throughout this study for deinking of MOW and ONP. Parameters that affected the deinking process include pulping process, enzymatic hydrolysis and flotation process. Optimization of pulping process was carried out involving the pulping consistency and pulping time. The optimization of enzymatic hydrolysis of MOW and ONP were performed in order to determine the most effective conditions for ink removal. The conditions that were optimized include the temperature, pH, enzymes concentration and hydrolysis time. The efficiency of the deinking process was greatly influenced by flotation process. Therefore, the parameters that were involved in flotation process were

optimized in order to improve the deinking efficiency and brightness. The parameters that were optimized in the flotation process include the flotation pH, surfactant concentration and flotation time. Conventional chemical deinking process was carried out in order to compare the effectiveness of enzymatic deinking of MOW and ONP.

After optimization of enzymatic deinking process, the quality of the de-inked paper was determined and compared with chemical deinking process using the standard TAPPI tests methods. The properties examined include not only the optical properties of the paper such as brightness and opacity but also mechanical properties of the paper like burst strength, tensile strength and tear resistance of paper. Furthermore, pulp property such as pulp freeness was also determined. In addition, the residual ink remaining on the deinked paper after deinking process was examined using Spec Scan 2000 software. The wastewater effluent generated from both enzymatic and chemical deinking processes were examined by determining the BOD_5 and COD.

The objectives of the current research are as follows:

- To design and develop a laboratory scale solid state fermenter.
- To optimize cellulases and xylanase production *via* solid state fermentation process using FERMSOSTAT®.
- To design and develop a pilot scale enzymatic deinking system and optimize the deinking process.
- To compare the efficiency of enzymatic deinking process and quality of the deinked paper with chemical deinking process.
- To analyze the wastewater effluent generated from the deinking process.

CHAPTER TWO: LITERATURE REVIEW

2.1 SOLID STATE FERMENTATION

2.1.1 General Considerations

2.1.1.1 Definition and History of Solid State Fermentation

Solid state fermentation (SSF) involves the growth of microorganisms (mainly fungi) on moist solid substrate in the absence or near absence of free flowing water (Stredansky and Conti, 1999; Adinarayana *et al.,* 2003; Kumar *et al.,* 2003b; Kashyap *et al.,* 2003; Pandey *et al.,* 2008b). In a wider definition, SSF can be seen as including processes during which microorganisms are cultivated in the presence of a liquid phase at maximum substrate concentrations (Mitchell *et al.,* 2000a) or on inert support (Ooijkaas *et al.,* 2000). On the other hand, SSF process can also be defined based on its properties as stated in Table 2.1. The basic principle of SSF is the "solid substrate bed". This bed contains moist substrates, which are polymeric in nature and insoluble in water act as a source of carbon, nitrogen, minerals, water and other nutrients to support the growth and metabolism of microorganism. SSF has been conventionally more applicable for filamentous fungi that grow on the surface of the solid substrates particle and penetrate through the inter particle spaces into the depth of the solid bed (Pandey, 2003). In addition, SSF simulates the fermentation reactions occurring close to the natural environment in which microorganisms are adapted and has been credited to be responsible for the beginning of the fermentation technique in ancient time (Pandey *et al.,* 2000a). Therefore, it is not surprising that almost all the fermentation processes used in ancient time were based on the principles of SSF.

The history and development of SSF have been reviewed by several authors from time to time. Enzymes production and food fermentation are the areas where SSF

originated. Typical examples of SSF processes in traditional fermentations are summarized in Table 2.2. SSF is the state of art technology that is used in many applications in the food industry in Asia (Udo and Jurgen, 2005).

Table 2.1: SSF process can be defined in terms of the following properties (Raimbault, 1998)

No	Condition
1	Solid porous matrix either can be biodegradable or not but with a large surface area per unit volume (10^3 to 10^6 m^2/cm^3) for ready microbial growth.
2	The matrix should possess water absorption capacity one or several times its dry weight with a relatively high water activity on the solid/gas interface in order to allow high rates of biochemical processes.
3	The support/carrier should be able to absorb or contain available nutrients/foodstuffs to support the growth of microbial such as water, carbohydrates, nitrogen sources and mineral salts.
4	The solid support should not be contaminated by inhibitors of microbial activities.
5	The solid matrix should be able to stand compression or gentle stirring as required for a given fermentation process. This requires small granular or fibrous particles that do not tend to break or stick to each other.
6	Air mixture of oxygen with other gases and aerosols should flow under a relatively low pressure and mix the fermenting mash.
7	The solid/gas interface should be a good environment for the fast development of specific cultures of fungus, moulds, yeasts or bacteria, either in single or mixed cultures.

Table 2.2: Typical examples of SSF process in traditional fermentations (Raimbault, 1998; Udo and Jurgen, 2005; Pandey, *et al.*, 2008b)

No	SSF Fermentation
1	"Blue cheese" making in French, which uses perforated fresh cheese as substrate and *Penicillium roquefortii* as inoculum.
2	Enzyme rich soy sauce "koji" which uses steamed rice as solid substrate and inoculated with *Aspergillus oryzae* as an enzymatic starter for different hydrolytic processes.
3	Indonesian "tempeh" or Indian "ragi" which use steamed and cracked legume seeds as solid substrate and a variety of non toxic moulds such as *Rhizopus* sp. as microbial seed.
4	Composting of lignocellulosic fibers, naturally contaminated by a large variety of organisms including cellulolytic bacteria, moulds and *Streptomyces* sp.
5	Saccharification of rice used for the production of alcoholic beverages such as "sake".
6	Production of "angkak", which is rice that is colored red by *Monascus purpurea* metabolites.

For example SSF had been used in cheese making by *Penicillium rouquefortii* before the birth of Christ. In 2000 BC, SSF process was used to make bread as were reported by Egyptians. SSF is used in the production of enzyme rich soy sauce Koji in China was reported in the years 1000 BP. In 17th century, the koji process was migrated from China to Japan by the Buddhist priests. During this period, several fermented food such as "tempeh" or Indian "ragi", miso were mentioned in many South East Asian countries. During the 18th century, SSF was used for the production of vinegar from the apple pomace. While in late 19th century, SSF processes were used in composting and solid waste treatment. For the first time the production of primary metabolites such as enzymes and organic acids using SSF were reported in the beginning of 20th century (Raimbault, 1998; Udo and Jurgen, 2005; Pandey *et al.,* 2008b).

SSF processes were nearly completely ignored in western countries in the period of 1940's due to dramatic discovery and development of penicillin using submerged fermentation (SmF) technology. Since the development of penicillin took place in SmF and due to enormous importance of penicillin during the world war, SmF became a role model technology for the production of any compound. Subsequently, researchers at that time put their entire attention and focus on development of SmF and somehow or other, knowingly or unknowingly, SSF got totally neglected. During the period of 1950-60, reports were published describing steroid transformation using fungal cultures. SSF attained another milestone achievement during 1960-70 when mycotoxins production by SSF was reported successful using fungal cultures that resulted a significant impact on cancer research. During this period, production of protein enriched cattle feed (single cell protein) was another important application

oriented finding of SSF research. Since then enormous work has been carried out and SSF regained a fresh attention from researchers and industries all over the world (Pandey *et al.,* 1999; Classen *et al.,* 2000; Medeiros *et al.,* 2000a; Haddadin *et al.,* 2001; Han *et al.,* 2001; Ogbonna *et al.,* 2001; Yang *et al.,* 2001; Pandey *et al.,* 2008b).

SSF offers numerous potential in processing of agro-industrial residues. This is mainly due to SSF processes required lower energy consumption, produce lesser wastewater, biomass energy conservation and are environmental friendly as they resolve the problem of solid wastes disposal. Substrates that have been traditionally used in SSF include wide variety of agricultural products or residues such as rice, cassava, wheat, millet, barley, grains, sugarcane, beans, corn, canola meal, soybeans and etc. However, non-traditional substrates such as abundant supply of agricultural, forest and food-processing wastes are also be of interest in industrial process development (Perez-Guerra *et al.,* 2003). A closer examination of SSF processes in several research centers throughout the world has led to the realization of numerous economical and practical advantages of SSF (Steinkraus, 1984; Lonsane *et al.,* 1985). Research on SSF has led to a wide range of applications at the laboratory scale (Gupte and Madamwar, 1997; Gutierrez-Correa and Tengerdy, 1998; Hang and Woodams, 1998).

In addition, during 1991-2006, apart from special issue of journals and books more than 1400 publications have appeared in various journals, proceedings and books (Pandey *et al.,* 2008b). Current decade has witnessed much promise in the development of several bioprocesses and products (Smits *et al.,* 1996) such as

bioremediation and biodegradation of hazardous compounds, biotransformation of crops and crop-residues for nutritional enrichment, biopulping, and biological detoxification of agro-industrial residues and production of value added products such as antibiotics, enzymes and organic acids. In recent years, the increasing demand for the natural products in the food industry has encouraged the development of biotechnological processes for the production of flavor compounds. The use of SSF as a means to improve economical feasibility would be of potential benefit (Longo and Sanromffn, 2006; Pandey *et al.,* 2008b). These have led to the development of bioreactor, which was suitable for SSF process. However, SSF up-scaling, necessary for use on an industrial scale, raises severe engineering problems due to the build up of temperature, pH, 0_2, moisture and temperature gradients.

Mitchell *et al.,* (2003) reported that mathematical models have been considered as important tools for optimizing the design and operation of SSF bioreactors. The models must be able to describe the kinetics of microbial growth, how this may affect by the environmental conditions and *vice versa.* This can be done in two levels of sophistication. Moreover, simple empirical equations are used in many bioreactors to describe the models of kinetics (Mitchell *et al.,* 2004). Sensors development and measurement in fermentation processes are important developments in SSF. Bellon-Maurel *et al.,* (2003) discussed current online methods and applications of the methods with a potential to measure process variables in SSF that are not easily accessible and measurable.

2.1.1.2 Selection of Microorganisms

Fungi, yeast and bacteria can grow on solid substrates and find their application in SSF processes. Some examples of SSF processes for each category of microorganisms are summarized and presented in Table 2.3. However, based upon the type of microorganisms used, SSF processes can be generally classified into two main groups, indigenous or natural and individual culture of SSF. The former normally involved natural micro-flora and the latter mainly using pure culture or mixed-culture in the SSF process. Composting and ensiling are the two typical examples of SSF processes that involve natural micro-flora mainly bacteria (Pandey *et al.*, 2008b). Bacteria also have been used for some food processes such as sausages, Japanese natto, fermented soybean paste, Chinese vinegar (Ramesh and Lonsane, 1991; Prabhu and Chandrasekaran, 1995). On the other hand, yeasts can be used for ethanol, food and feed production (Saucedo-Castaneda *et al.*, 1992a, 1992b). However, filamentous fungi are the most important group of microorganisms used and dominate in SSF research works. Koji process is one of the most important applications of SSF with filamentous fungi and one such unique example of that involving pure culture of *Aspergillus oryzae*. Generally pure cultures are used in industrial SSF processes since it helps in increased productivity or optimum substrate utilization for the production of desired product. Bioconversion of agro-industrial residues such as wheat straw for protein enrichment by *Candida utilis* and *Chaetomium cellulolyticum* is one of the good examples of SSF processes involving mixed cultures (Pandey, 1992; Raimbault, 1998).

Selection of suitable microorganisms for a particular application is one of the most important criteria in SSF process. The wide varieties of wild type microorganisms are

Table 2.3: Major groups of microorganisms involved in SSF processes (Raimbault, 1998)

Microorganisms	SSF process
Fungi	
Aspergillus niger	➢ Feed, Amylase, Citric Acid, Protein
Aspergillus oryzae	➢ Koji, Food, Citric Acid
Penicillium notatum	➢ Penicillin, Cheese
Phanerochaete chrysosporium	➢ Composting, Lignin Degradation
Rhizopus oligosporus	➢ Tempeh, Soybean, Amylase, Lipase
Mucor sp.	➢ Composting, Food, Enzymes
Rhizopus sp.	➢ Composting, Food, Enzymes, Organic Acid
Yeast	
Endomicopsis burtonii	➢ Tape, Cassava, Rice
Saccharomyces cerevisiae	➢ Food, Ethanol
Schwanniomyces castelli	➢ Ethanol, Amylase
Bacteria	
Bacillus sp.	➢ Composting, Natto, Amylase
Pseudomonas sp.	➢ Composting

unable to meet commercial requirement in terms of productivity and yield of the end product. The importance of the microorganism can be seen from the fact that *Aspergillus niger* itself can produce at least 21 types of different enzymes such as cellulase, xylanases, lipase, protease and etc. On the other hand, one enzyme alone can be produced by several different microorganisms in different quantities. For example, α-amylase can be produced by as many as 64 different strains of microorganisms which include *Aspergillus* sp., *Bacillus* sp., anaerobic bacterium and etc (Pandey, 1992; Pandey *et al.*, 2008b).

Several groups of microorganisms are capable to grow on solid substrates; however theoretical classification based on water activity showed that only fungi and yeast are considered as suitable microorganisms for SSF process. Bacterial cultures might not be suitable for SSF owing to high water activity requirement. However, some authors reported that bacterial cultures can be well managed and manipulated for SSF processes (Pandey, 1992; Nampoothiri and Pandey, 1996; Selvakumar and Pandey,

1999; Pandey *et al.,* 2000a). Cultivation of the filamentous fungi in SSF processes have been carried out since long time ago for different applications either in laboratory or in pilot scale. SSF process can be used for koji and tempeh production (Raimbault, 1998), for mycotoxins production (Bhumiratna, 1980), for lignocellulose fermentation (Matteau and Bone, 1980) and for fungal spores production (Lotong and Suwarnarit, 1983). Three main classes of filamentous fungi that most commonly been used in SSF process include Ascomycetes (*Aspergillus* and *Penicillium*), Basidiomycetes (White rot fungi, *Polyporus*) and Phycomycetes (*Mucor* and *Rhizopus*). Several yeasts have been used for ethanol fermentation or protein enrichment. Protein production by *Sporotrichum pulverulentum* was reported by Smith *et al.,* (1986), while *Saccharomyces cerevisiae* has most commonly been used for ethanol production (Gibbons *et al.,* 1984; Kargi *et al.,* 1985).

SSF research works have received increased attention and focus due to vast variety of interesting compounds that can be efficiently produced, which includes gibberellic acid (Kumar and Lonsane, 1987), penicillin (Barrios-Gonzalez *et al.,* 1993), enzymes (Solis-Pereira *et al.,* 1993) and citric acid (Hang *et al.,* 1987). About 50% of the microorganisms used in applications of SSF belong to the class of filamentous fungi, 30% to yeast, 15% to actinomycetes and 5% to bacteria (Gutierrez-Rojas *et al.,* 1995). The remarkable preference of filamentous fungi for the SSF process is due to the low water activity requirements, their efficient way to adherence, colonize on suitable substrate surfaces, penetrate into the substrate and their ability to assimilate mixtures of different polysaccharides used (Mitchell *et al.,* 1991).

In addition, filamentous fungi are the best adapted to grow due to their physiological, enzymological and biochemical properties. The hyphal mode of fungal growth gives a major advantage to filamentous fungi over unicellular microorganisms in the colonization of solid substrates and the utilization of available nutrients. Moreover, their ability to grow at low water activity (A_w) and high osmotic pressure conditions (high nutrient concentration) makes fungi efficient (Mitchell and Lonsane, 1992). In addition, the hyphal mode of growth gives the filamentous fungi the power to penetrate into the substrates particles. Hyphal extension provides an alternative to motility, enabling hyphae to extend toward new sources of food (nutrient), branching provides a means to optimize substrate utilization and colonization which ensures a firm and solid structure. The hydrolytic enzymes are excreted in high quantity at the hyphal tip with minimum dilution makes the action of the enzymes very efficient in hydrolysis and allows further penetration into most substrates particles. Penetration will increase the accessibility of all available nutrients within the substrate particles. The resulting contact catalysis is very efficient and the converted simple products are in close contact, enter through the mycelium across the cell membrane to promote biosynthesis and fungal metabolic activities (Raimbault, 1998).

2.1.1.3 Substrates and Particles Size

Selection of suitable substrate is another important key aspect in SSF process. It seems that two terms, solid state fermentation and solid substrate fermentation have often been equivocally used. It would be logical to distinguish these two terms. Solid substrate fermentation should only be used to define those processes in which the substrate itself acts as a carbon or energy source, occurring in the absence or near-absence of free water. But solid state fermentation should be defined as any

fermentation process occur employing a natural substrate as above or an inert substrate used as solid support in the absence or near absence of free water (Pandey *et al.,* 2008b). The substrate not only must possess enough moisture, all needed nutrients to support growth and metabolism of microorganism, but also serves as an anchorage for the cells. However, it must keep in mind that some nutrient may not be enough or even present in the particular substrates. In such case, it is necessary to supplement them additionally.

The selection of a substrate for SSF process depends upon several factors, mainly related with the cost, availability and consistency of the raw materials. Research on the selection of a suitable substrate for SSF process has mainly centered around tropical origin due to the agro-industrial crops and residues offer potential advantages for their application as substrates (Pandey *et al.,* 2000c; Pandey *et al.,* 2008b). The agro-industrial crops and residues that have potential have been used as substrates in SSF process are presented in Table 2.4. Among all the potential substrate, wheat bran has been the good choice. A lot of SSF research work has been developed using this substrate for the production of bulk chemicals and value added fine products.

Table 2.4: Agro-industrial crops and residues that have potential to be used as substrates (Pandey *et al.,* 2000c, 2008a, 2008b; Hoogschagen *et al.,* 2001; Peralta-Perez *et al.,* 2001)

Crops or Residues	Example
Crop	➢ Cassava, soybean, sugar beet, sweet potato, potato, sweet sorghum
Residues	
Bran, straw	➢ Wheat, rice
Hull	➢ Soy, corn, rice
Bagasse	➢ Sugarcane, cassava
Pulp, husk, spent ground	➢ Coffee processing
pomace	➢ Fruit processing (Apple, grape)
Wastes	➢ Pineapple and carrot processing
Coconut cake, soybean cake, peanut cake, canola meal	➢ Oil processing mill

Among the several other factors, substrate particle size and moisture level or water activity are the most important critical for the microbial growth and activity. Generally, reduction in substrate particle size through milling provides smaller substrate particles that would provide larger surface area for the microbial attack and growth. However, at the same time, very tiny substrate particles may result in substrate agglomeration, matrix compaction in most of the cases, which may interfere with microbial respiration and aeration, increasing mass and heat transfer problems. Thus, these may result in poor microbial growth and low productivity of the desired end products. On the other hand, larger substrate particles size provides better respiration and aeration efficiency owing to increase substrate inter-particle space, but provides limited surface area for the microbial attack. Thus, it must be remarked that particle size could show an optimum range and necessary to come through at a compromised particle size for a particular process (Pandey *et al.*, 2000a; Pandey *et al.*, 2008b).

Generally when dealing with agro-industrial natural materials, it has been a common practice to prepare and pre-treat some substrates to convert them to a more suitable and easily accessible for microbial attack (Pandey *et al.*, 2008b). Table 2.5 shows the common substrates pre-treatment steps before its being used in SSF process. Size reduction of substrate by chopping, grinding or grating is one of the most common substrates pre-treatment steps. It must be noted that the substrate particle size in SSF processes does not remain constant and tends to diminish through out the fermentation process. Mycelium formation is the main cause for void fraction variation. This is particularly true in fermentation which involves fungi.

Table 2.5: Common substrates pre-treatment steps (Mitchell *et al.*, 1992; Raimbault, 1998)

No	Steps
1	➢ Size reduction by chopping, grinding, sifting or grating
2	➢ Damage to outer substrate layers by grinding, pearling or cracking.
3	➢ Chemical or enzymatic hydrolysis of polymers to improve substrate accessible by the fungus.
4	➢ Supplementation with nutrients, determine the moisture content through a mineral solution.
5	➢ Setting the pH growth medium.
6	➢ Cooking or vapor treatment of substrate (macromolecular structure) as pre-degradation and elimination of major contaminants.

Solid substrate material, which are polymeric in nature and insoluble in water, acts as both physical support (sugarcane bagasse) and source of nutrients. The solid substrate can be a naturally occurring agricultural crops, residues, inert support or synthetic materials (Pandey, 1992; Pandey *et al.*, 2000b; Pandey *et al.*, 2000c; Pandey *et al.*, 2001a). In term of selection of suitable substrate, two major considerations must take into account; first is the specific substrate that requires suitable value-addition and/or disposal. The second could be related with the goal of producing a desired product from a suitable substrate.

Based on the nature of the solid substrate used/involved, two types of SSF process can be distinguished. First is the solid substrate serves as a support and a nutrient source, while the second is an inert support. The former utilized substrates that are heterogeneous and water insoluble materials from agro-industrial or by-products from food industry which have lignocellulosic nature (Pandey, 1992). The latter is saturated with liquid medium, which contains all the needed nutrients. However, this process is less used and presents economical disadvantages (Ooijkaas *et al.*, 2000).