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MALAYSIA
KELANTAN

# Environmental-Friendly Bio-technologies for Sustainable Agrowaste Management

Ibrahim Che Omar, DEng

**Agricultural wastes – global problem**

3R concept is a must

**Environmental impact**

**Recycling of wastes**

Concept of sustainable development

**Disadvantages**

(cost intensive, unsafe, requires treatment, high energy requirement, environmental unfriendly)

Physical, Chemical, Biological methods
Value added for Economic development

**Why biological methods?**

- **Cheap, safe and effective**
- **Utilization of agrowastes for the production of added value and commercial products**
- **Viable large scale operation of enzymatic hydrolysis with low production cost**
- **Environmental friendly system/process**
- **Reproducible, efficient, low operational cost, non-detrimental approach & good quality products**
- **Strategic management of agricultural solid wastes**

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Malaysian golden crop

Dried palm kernel cake

Empty fruit bunches

Oil palm shells

Oil palm fresh fruit fibers

Sugarcane bagasse

Paddy straws

**Some of the lignocellulosic materials from Malaysian agrowastes**

Sustainable development of natural resources – generation of agrowastes with a volume of >100 million tons a year

SCB


**Environmental impact**

Commercializable and high value products/processes in recycling of waste materials *via* biotechnological approach

Solid State Fermentation (SSF)

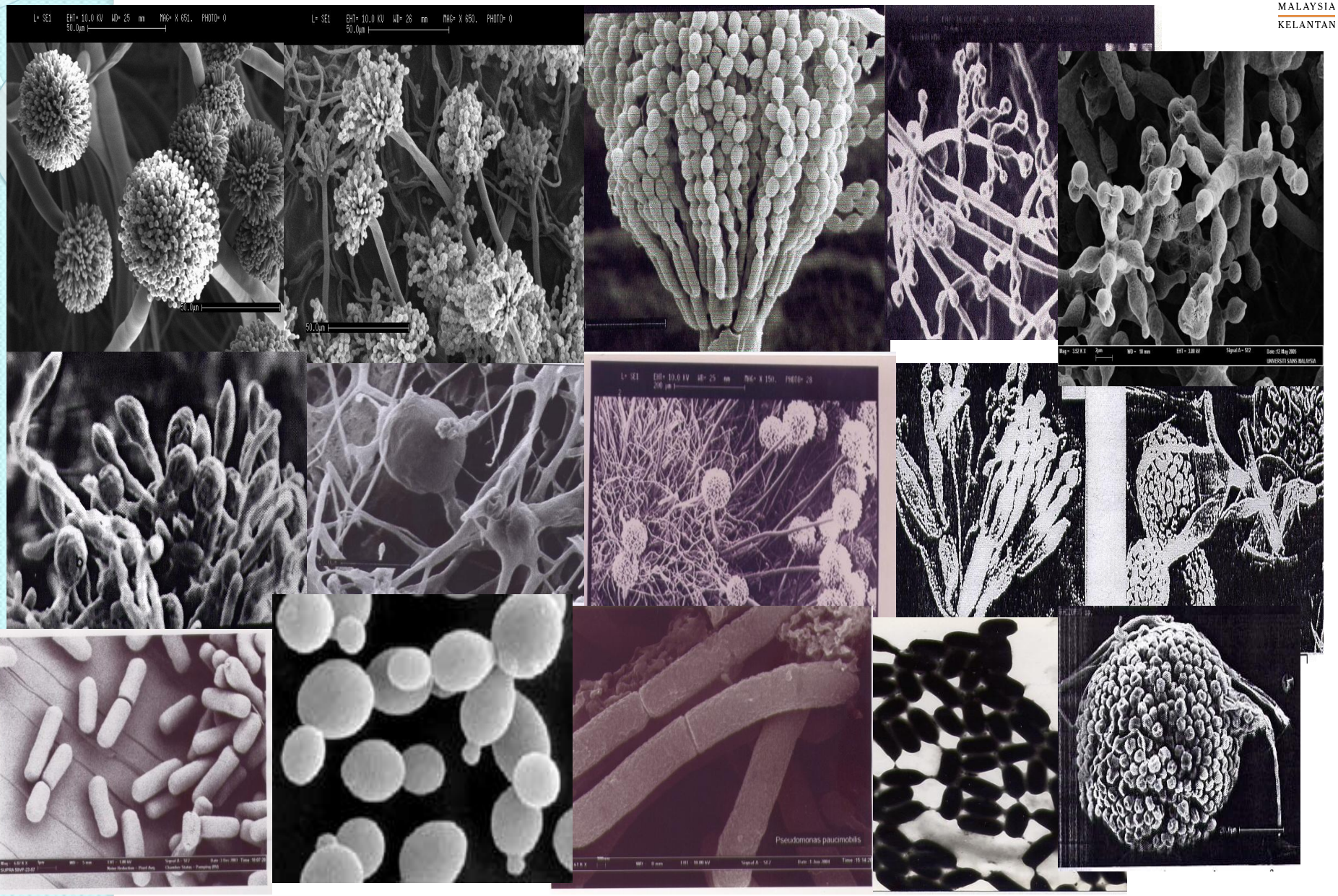
*Potential fungal isolates*

**Enzymes**, organic acid, biopesticides, compost, mushroom, animal feed, fertilizers

**Why SSF?**

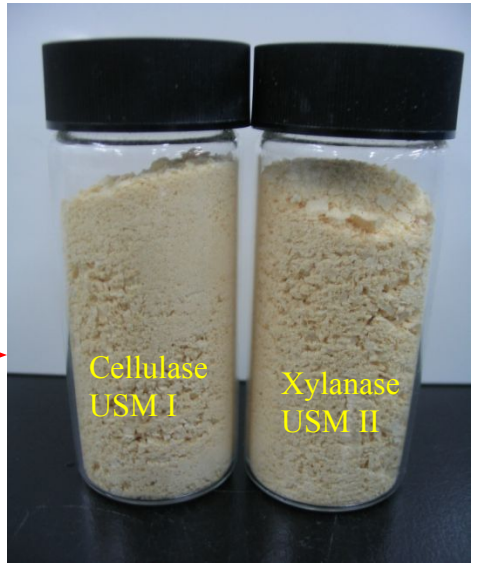
- Simple
- Cost effective
- **Environmental friendly**
- High product concentration
- Ease in product purification
- Less contamination risk
- Applicable for production of various microbial products

# Potential microorganisms with good growth on agrowastes



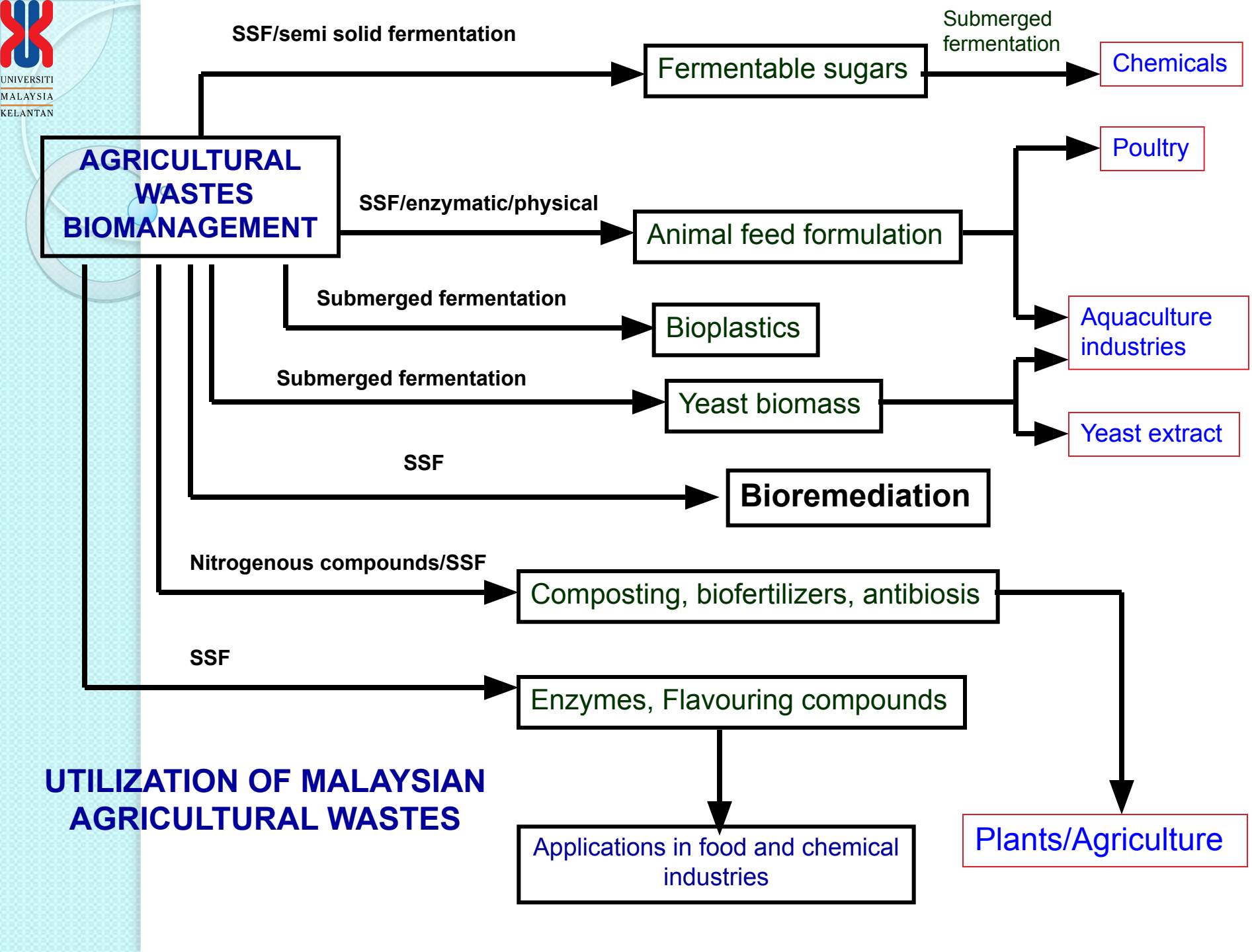
# Solid state fermentation (SSF) for the production of microbial metabolites

The utilization of agrowastes as substrates for SSF




Medium formulation and fermentation conditions

Enzyme preparations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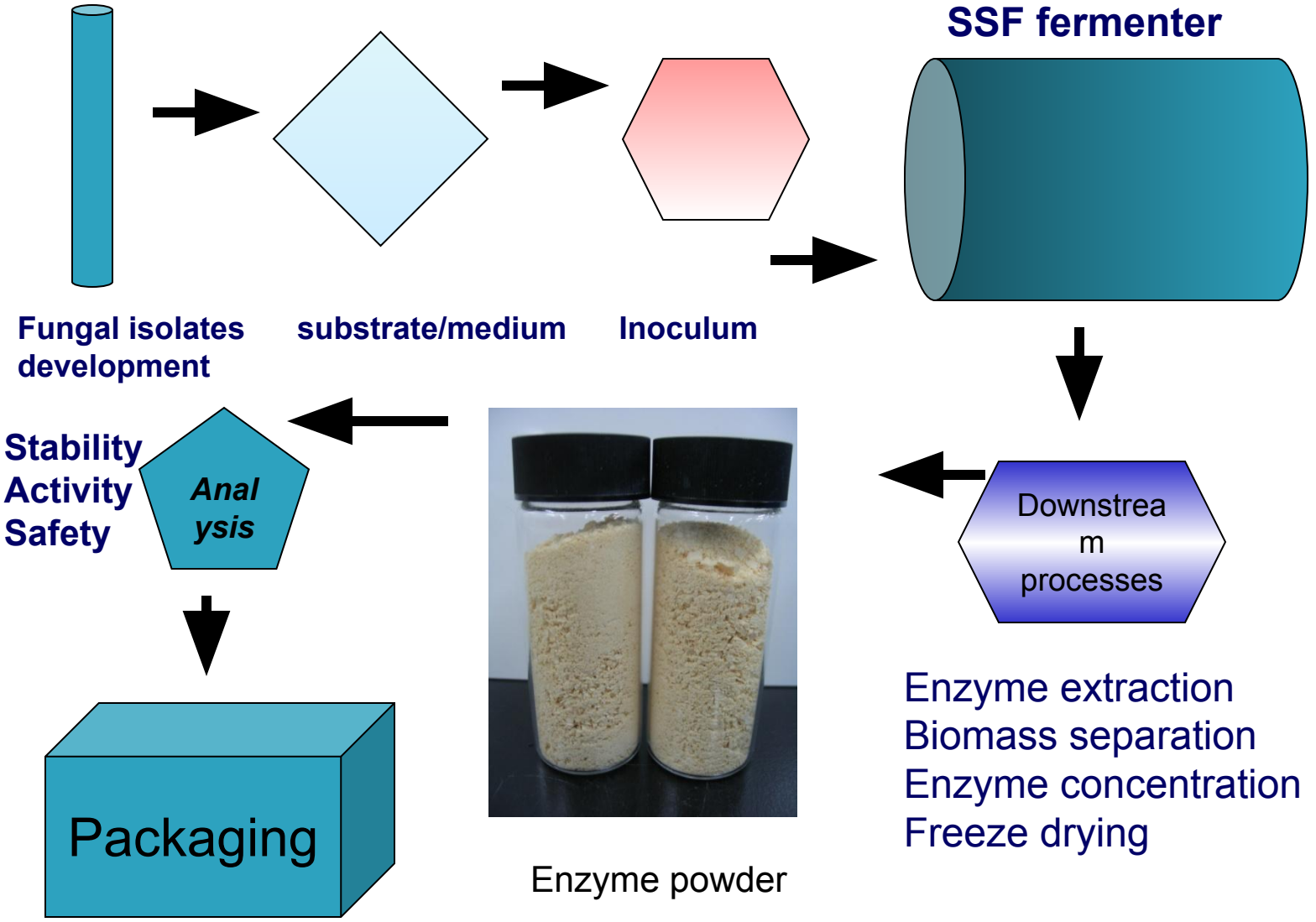
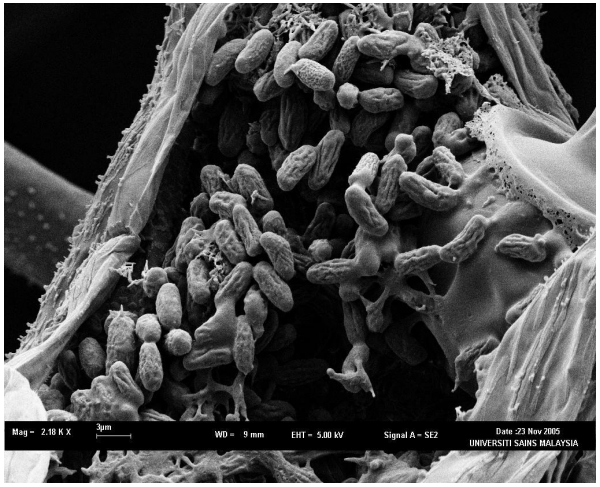
AGRICULTURAL WASTES BIOMANAGEMENT
SSF/semi solid fermentation
Fermentable sugars
Submerged fermentation
Chemicals
SSF/enzymatic/physical
Animal feed formulation
Poultry
Aquaculture industries
Submerged fermentation
Bioplastics
Submerged fermentation
Yeast biomass
Yeast extract
SSF
Bioremediation
Nitrogenous compounds/SSF
Composting, biofertilizers, antibiosis
SSF
Enzymes, Flavouring compounds
Applications in food and chemical industries
Plants/Agriculture
UTILIZATION OF MALAYSIAN AGRICULTURAL WASTES

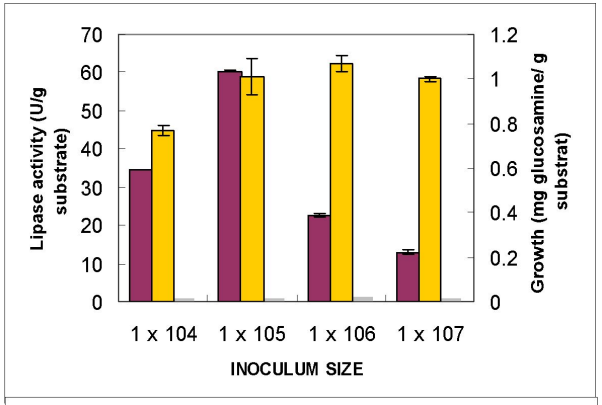
# Enzyme production *via* SSF

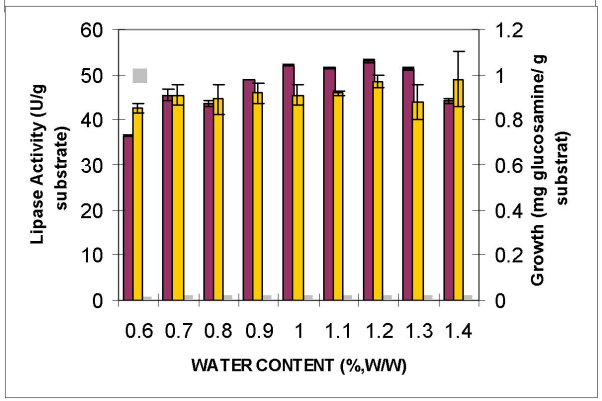


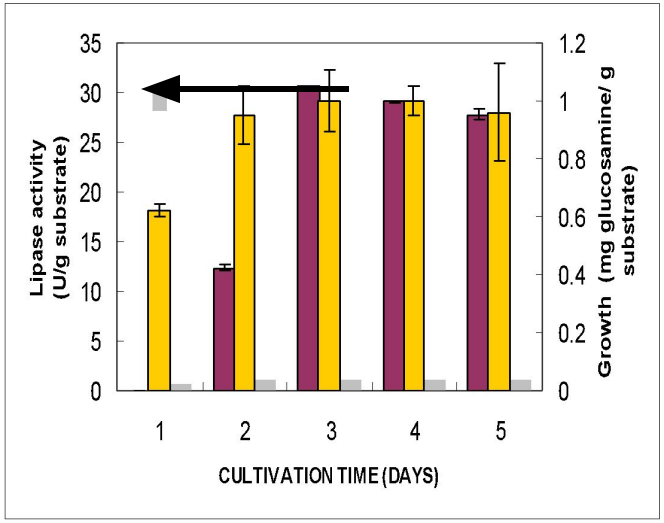
# Lipase production by *Mucor miehei* by solid state fermentation



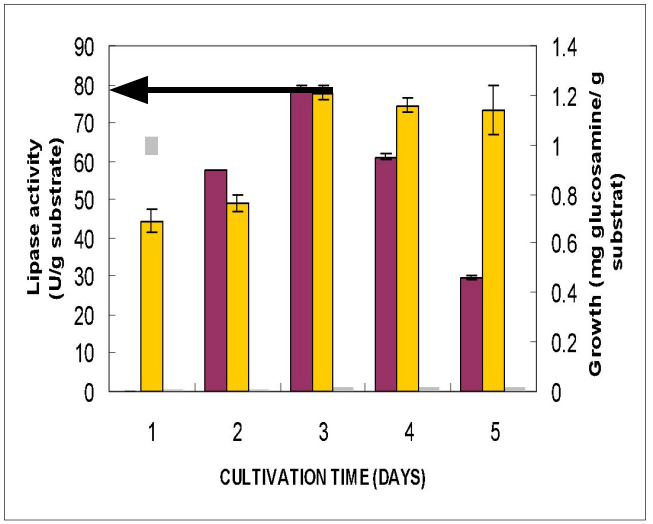
Growth of *M. meihei* in flask system



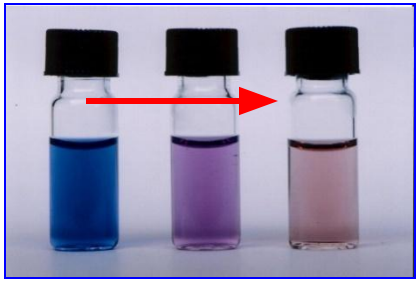

Before optimization : 10 U/g/day




After optimization : 27 U/g/day

# PRODUCTION OF ENZYMES AND ITS INDUSTRIAL APPLICATIONS


- Lipase (Fine chemical synthesis and detergency)
- Protease (Allergenic protein degradation in latex and feeds and detergency)
- Xylanase/hemicellulase (Enzymatic deinking in paper recyling, production of fermentable sugars)
- Cellulase (Similar to xylanase)
- Lignin peroxidase (Lignin degradation, dye decolorisation)
- Manganese peroxidase (Similar to LP)
- Laccase (Similar to LP dan MnP)
- Manannase (Degradation of mannan in palm kernel cake)
- Phytase (Feed formulation)
- β-glucosidase (Feed formulation and fermentable sugar production)



# SCALING UP OF SOLID STATE FERMENTATION - FERMSOSTAT®

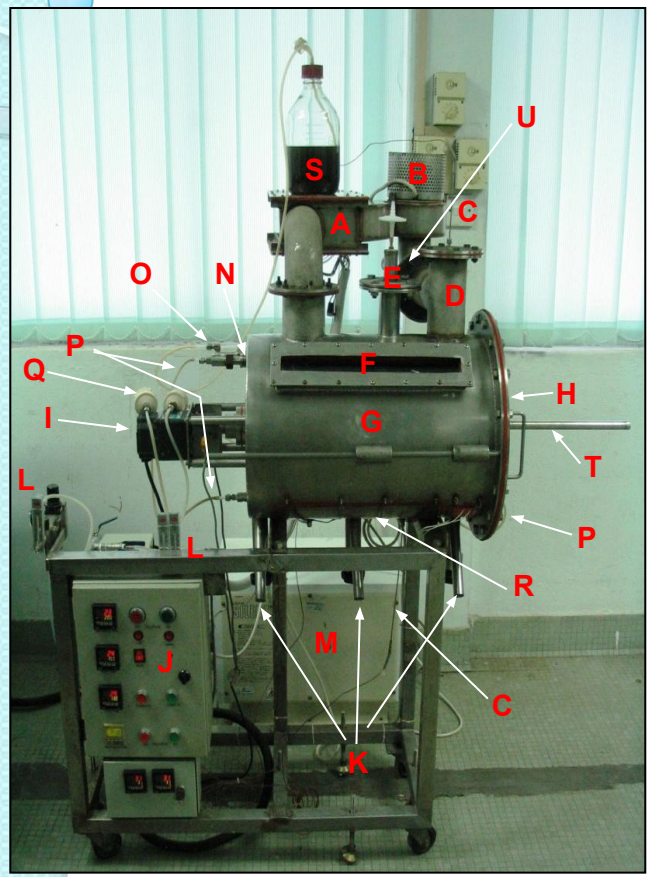



**Plate 1:** FERMSOSTAT with complete fermentation system.

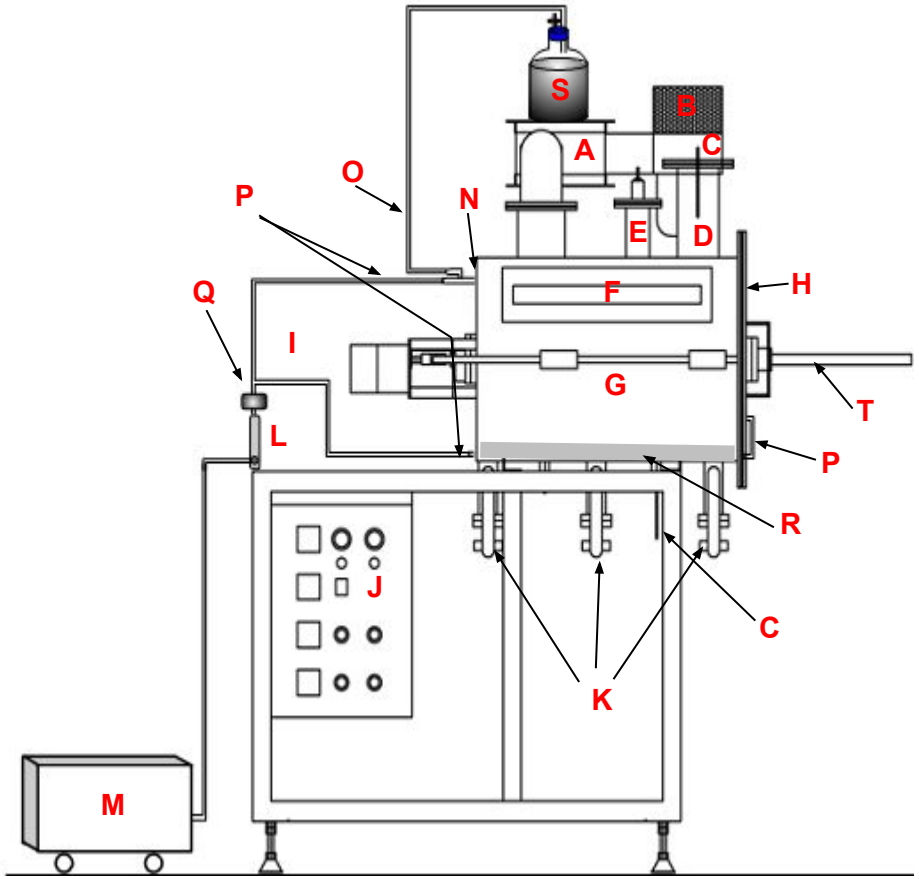
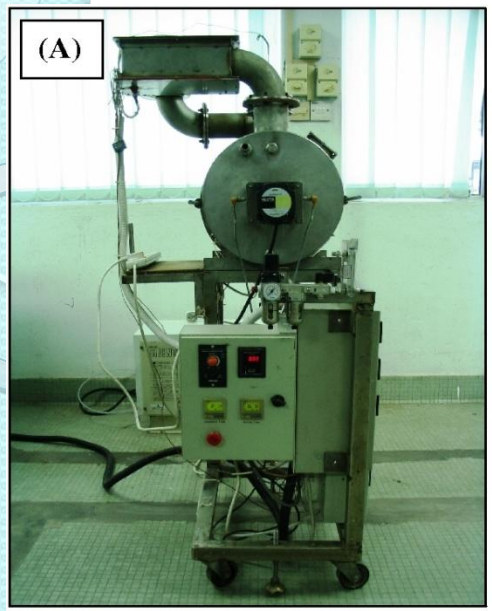
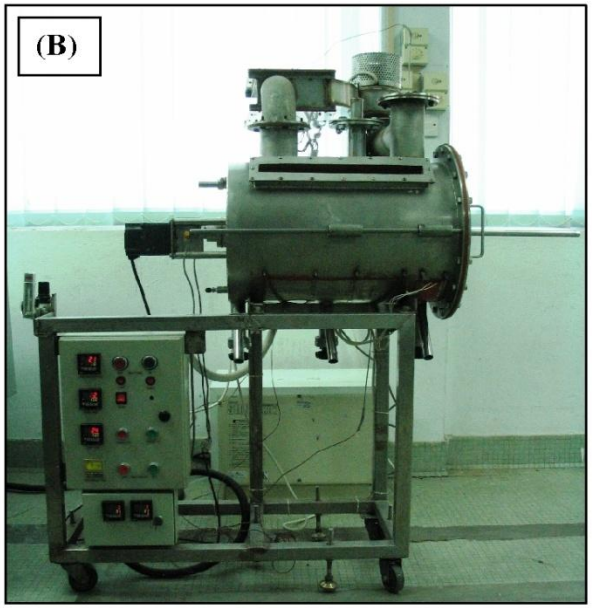
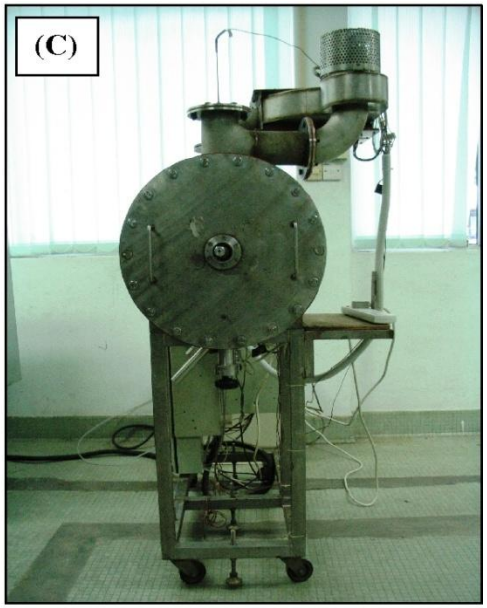
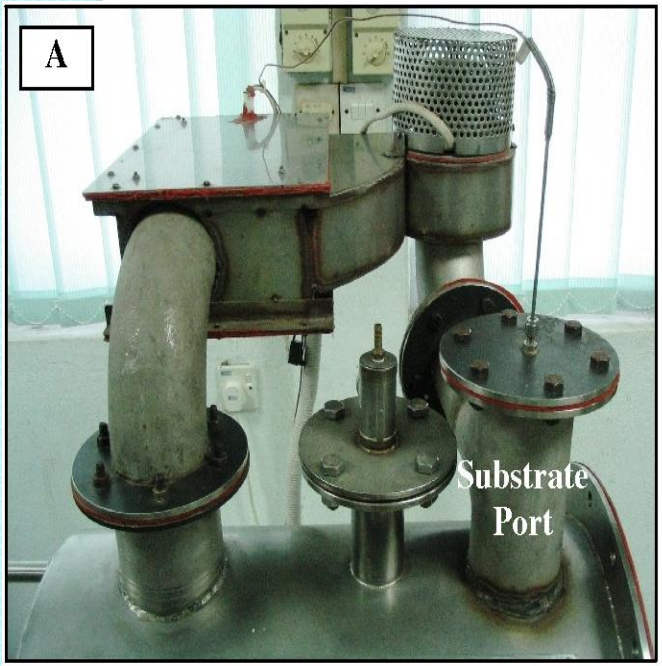



**Figure 1:** Schematic diagram of FERMSOSTAT with complete fermentation system.



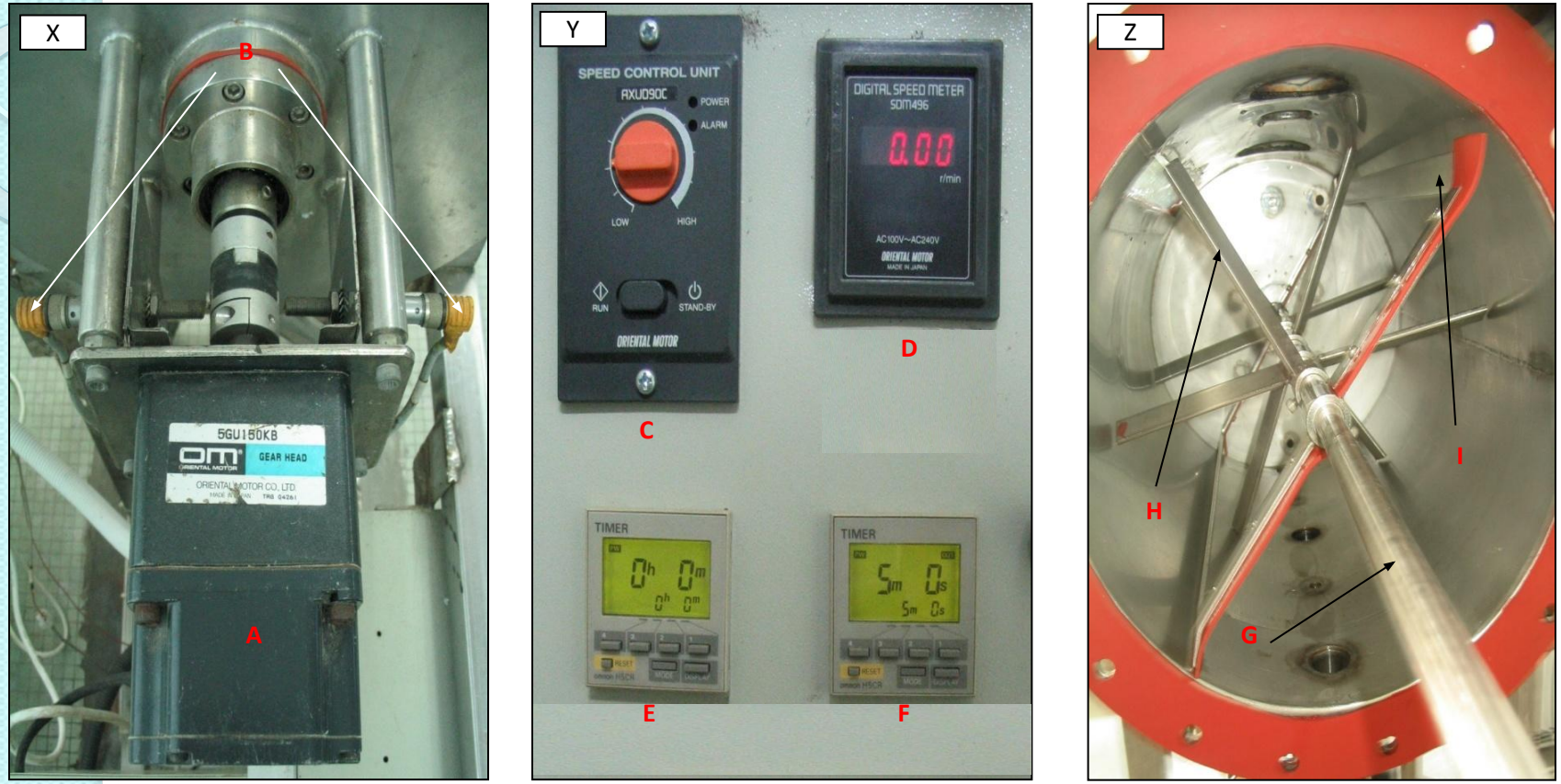



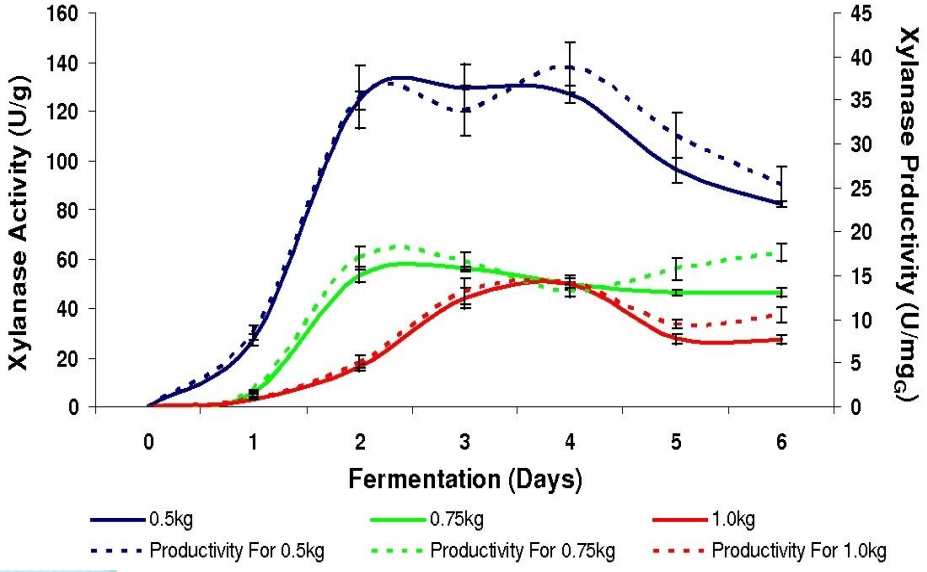


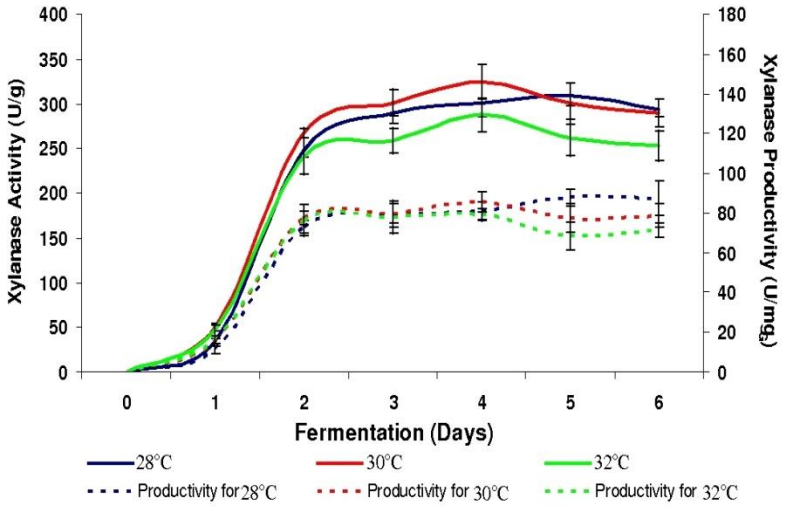
**Substrate port (A) and Sampling port (B) provided in FERMSOSTAT®.**

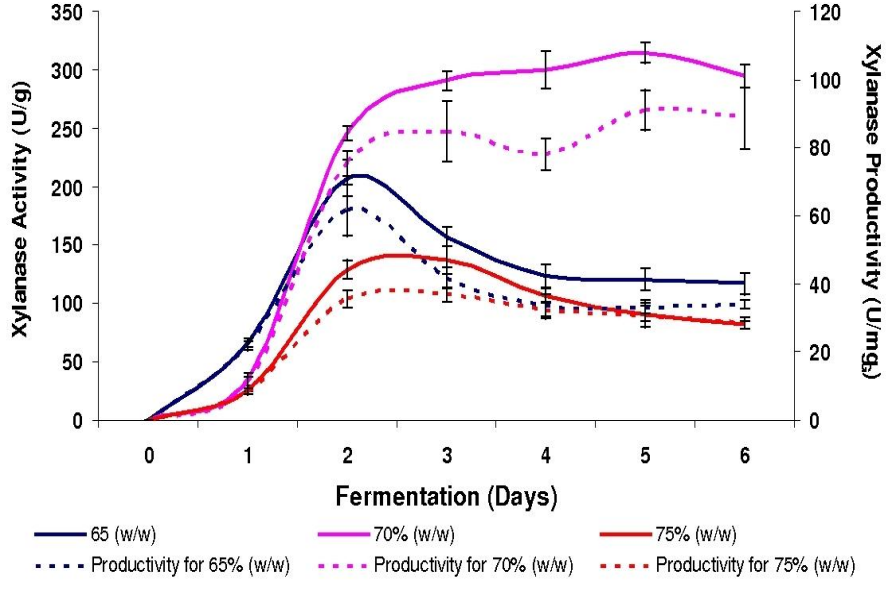


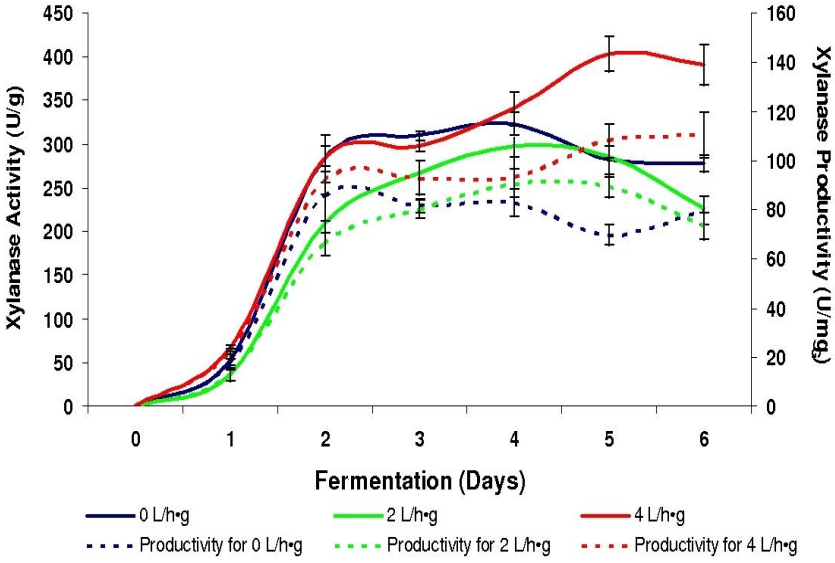

**Mixing system provided in FERMSOSTAT®. (X) Speed control motor, (Y) Various digital readouts and (Z) Impeller.**

# Xylanase production in the FERMSOSTAT



# Enzymes production in the FERMSOSTAT®

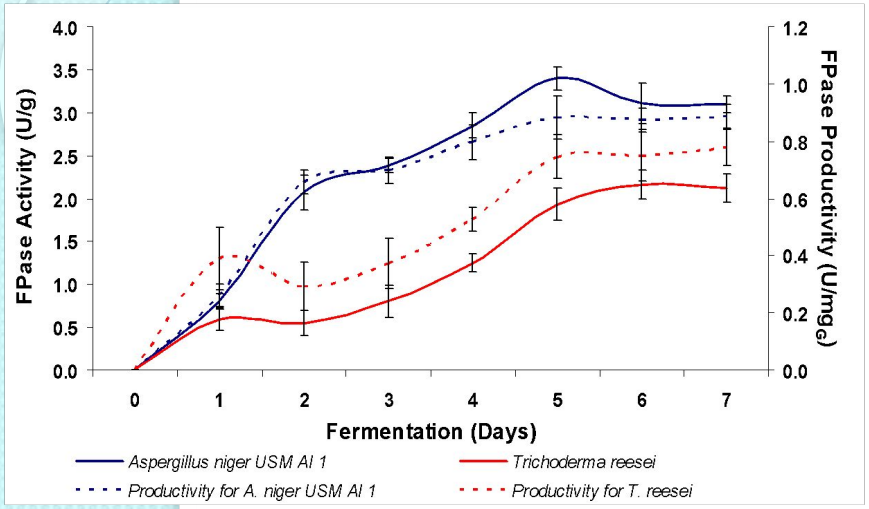



**Figure 3.25:** Production of FPase enzyme by *A. niger* USM AI 1 and *T. reesei* under optimized fermentation conditions.

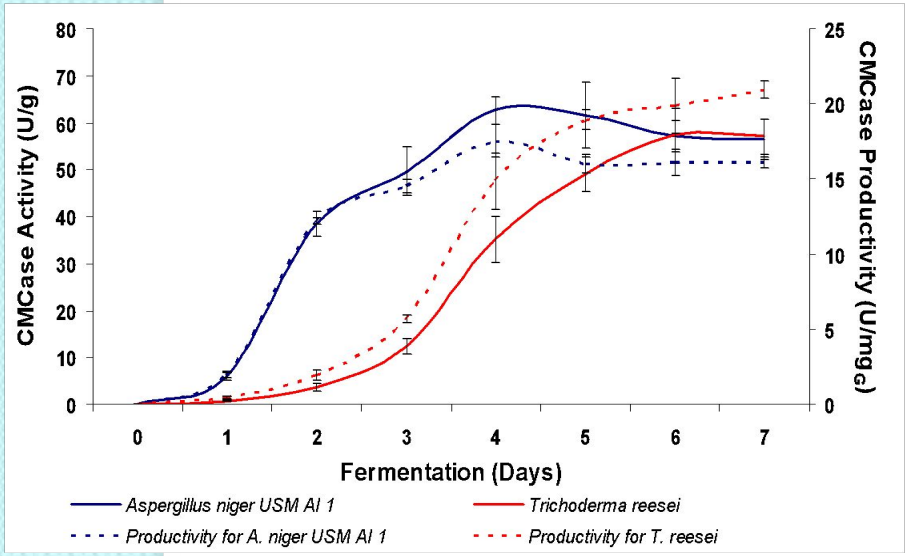



**Figure 3.23:** Production of CMCase enzyme by *A. niger* USM AI 1 and *T. reesei* under optimized fermentation conditions.

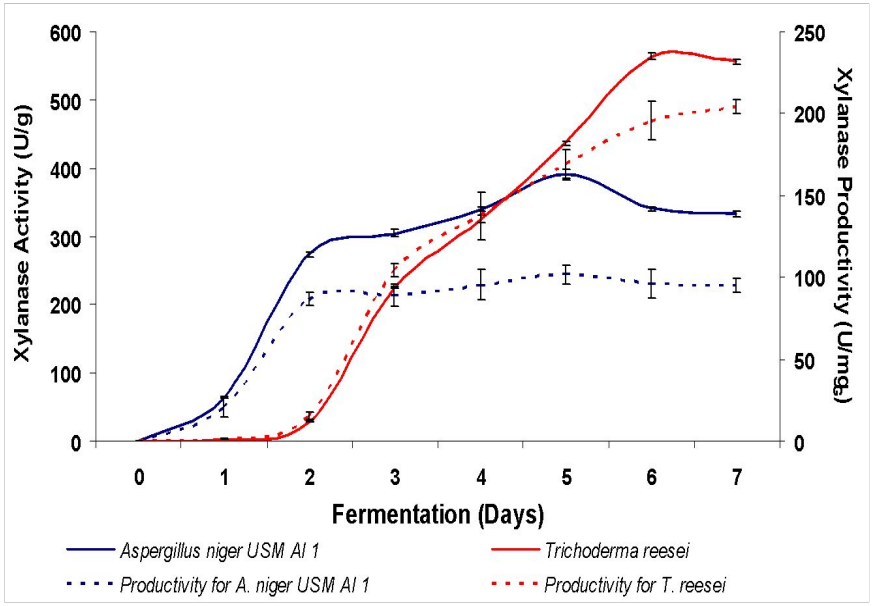
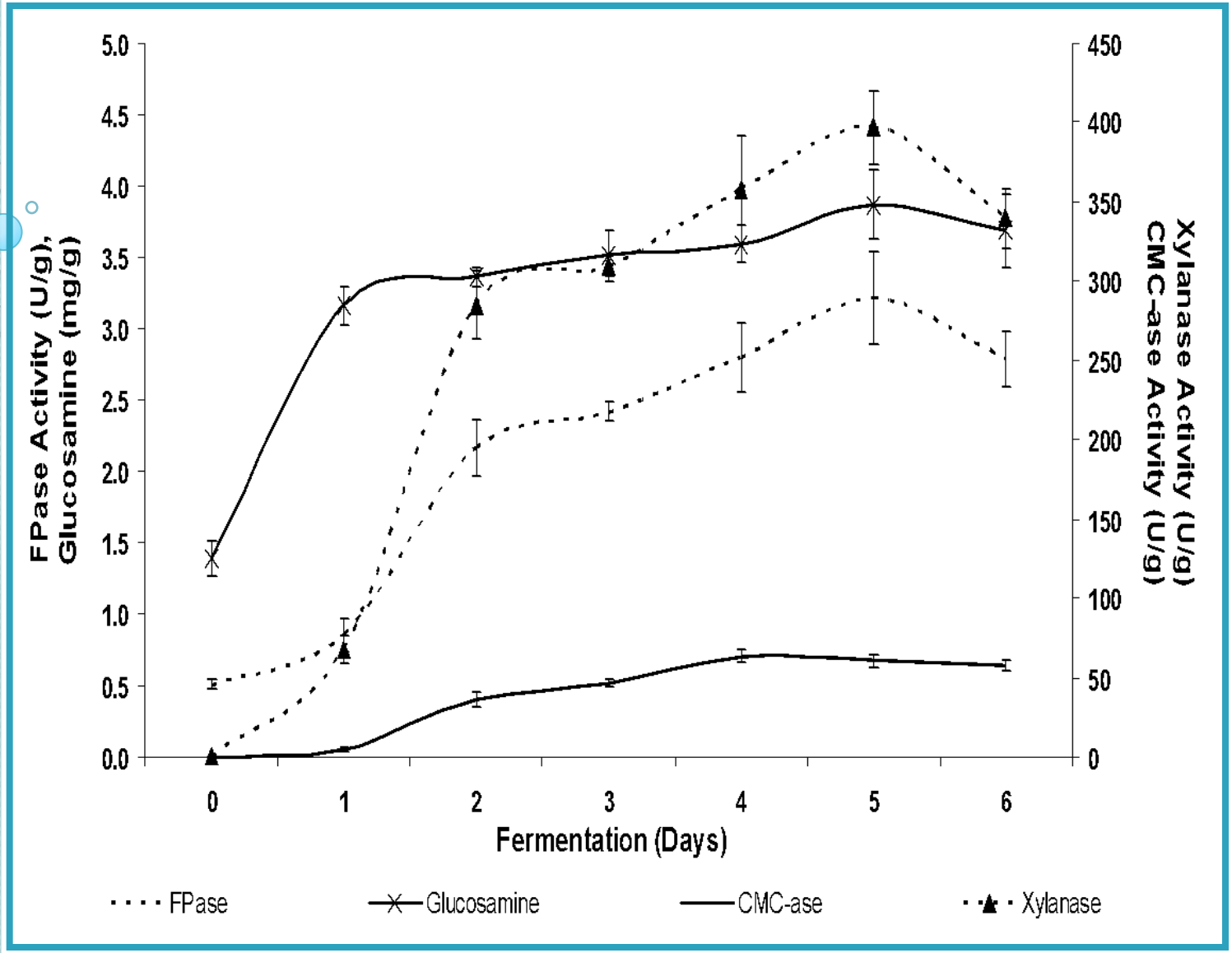



**Figure 3.24:** Production of xylanase enzyme by *A. niger* USM AI 1 and *T. reesei* under optimized fermentation conditions.

***Note:*** The SSF process was carried out under the indicated fungi; 0.5 kg substrate; 70% (w/w) moisture content; 30°C; aeration at 4 L/h.g fermented substrate for 5 min and mixing at 0.5 rpm for 5 min. Arrow bars indicate means with standard error of three replicates.

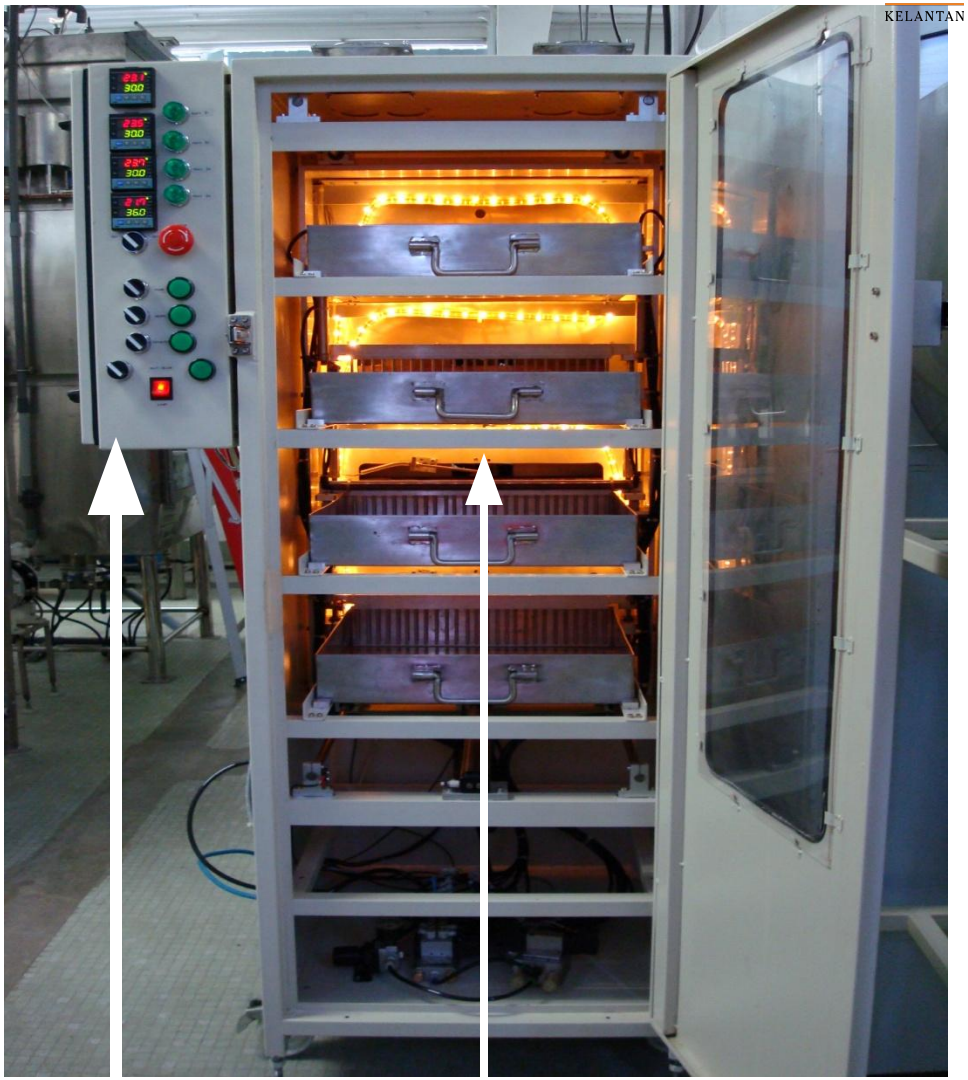
Summary of optimum conditions for production of cellulases and xylanase enzymes by *A. niger* USM AI 1.

| No | Parameters/Variables | Selected/optimum conditions | |
|---|---|---|---|
| 1 | Amount substrate | 0.5 kg | |
| 2 | Moisture Content | 70% (w/w) | |
| 3 | Incubation Temperature | 30ºC | |
| 4 | Aeration rate | 4 L/h•g fermented substrate | |
| 5 | Aeration time | 5 min | |
| 6 | Mixing rate | 0.5rpm | |
| 7 | Mixing intensity | 24 h interval | |
| | **Enzyme Activity** | **Before Optimization** | **After Optimization** |
| | CMCase (U/g) | 6.5 | 62.6 |
| | Xylanase (U/g) | 50.0 | 390.8 |
| | FPase (U/g) | 1.0 | 3.4 |




**Profiles of batch production of enzymes using the FERMSOSTAT®**

# SSF INTELLIGENT FERMENTER



Water/inoculum storage

Control panel

Tray system

# Optimization of enzymes production via solid state fermentation using PKC

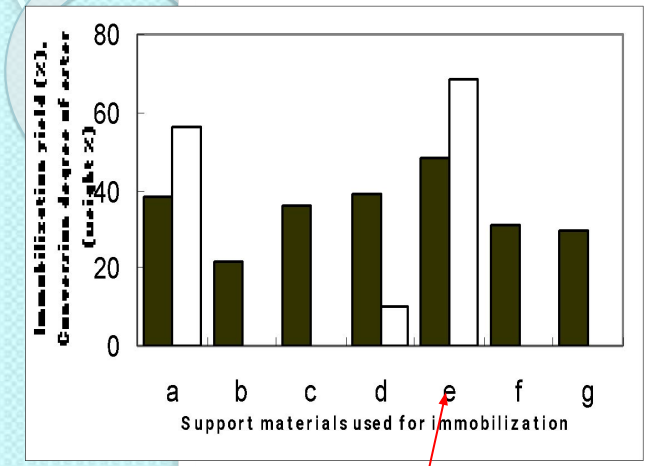
| **Xylanase production by *Aspergillus niger* USM AI 1** | **Cellulase production by *Aspergillus niger* var. *awamori* USM B1** | **Lipase production by *Mucor hiemalis* NRRL 13009** |
|---|---|---|
| Amount of PKC 10 g/tray<br>Ambient temperature<br>Water content : 1 : 0.75<br>Inoculum size : 1 X $10^4$ spores/ml<br>Moistening agent : tap water<br>Xylose as inducer at 0.75%<br>Cultivation time : 7 days<br>Production : 33 U/g PKC | Amount of PKC 10 g/tray<br>Ambient temperature<br>Water content : 1 : 0.75<br>Inoculum size : 1 X $10^5$ spores/ml<br>Moistening agent : tap water<br>Cellulase as inducer at 0.75%<br>Cultivation time : 7 days<br>Production : 10 U/g PKC | Amount of PKC 10 g/tray<br>Ambient temperature<br>Water content : 120%<br>pH : 9.0<br>Inducer : olive oil 2%<br>Sucrose 0.3%<br>Ammonium sulphate 0.4%<br>Cultivation time : 3 days<br>Production: 81 U/g PKC |

# Application of lipase for esterification reaction of acetone glycerol acyl esters

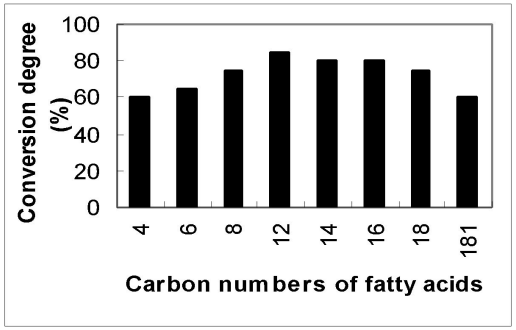
1,2-*O*-isopropylidene glycerol + fatty acids → (Lipase of ***Pseudomonas*** sp.) → 1,2-*O*-isopropylidene acyl glycerol + water

↓ *Mild hydrolysis*

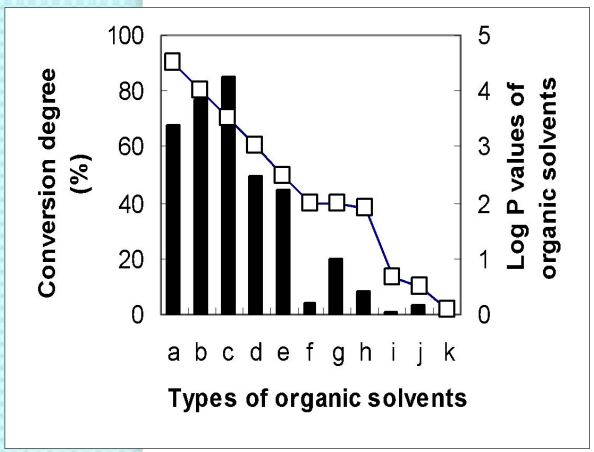
monoacylglycerol + acetone

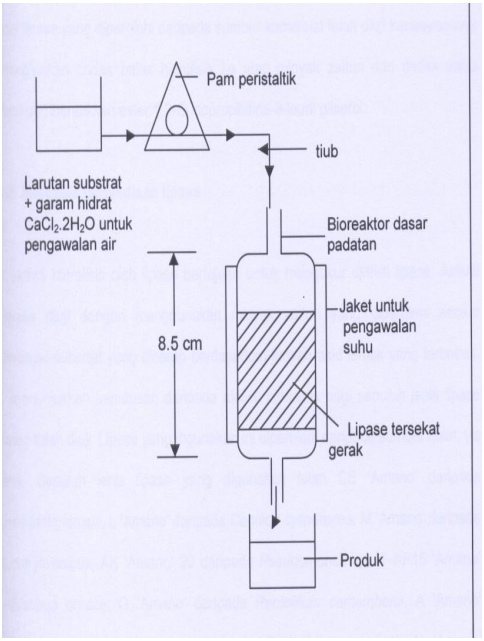


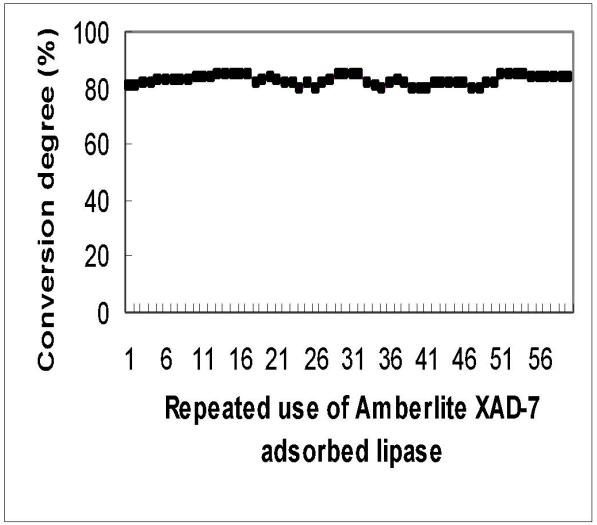

a. Celite 545, b. Florisil, c. Kieselguhr, d. Amberlite XAD-4, e. Amberlite XAD-7, f. Eupergite C, and g. Eupergite C250L.




**Fatty acid specificity**



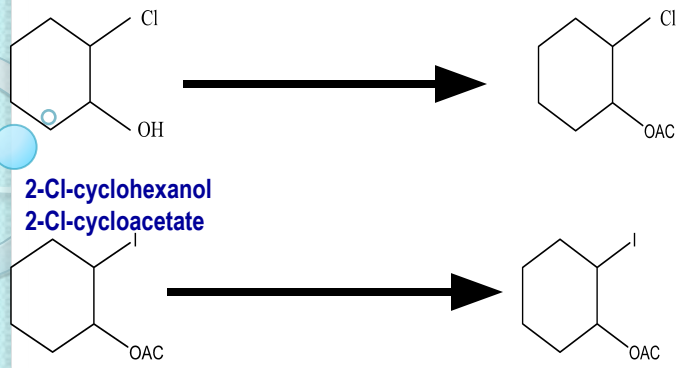



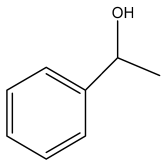




**CONTINUOUS PACKED BED BIOREACTOR SYSTEM**

(Each cycle was for the reaction time of 24 hr )

# The use of SSF materials as enzyme source in organic synthesis

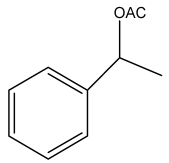


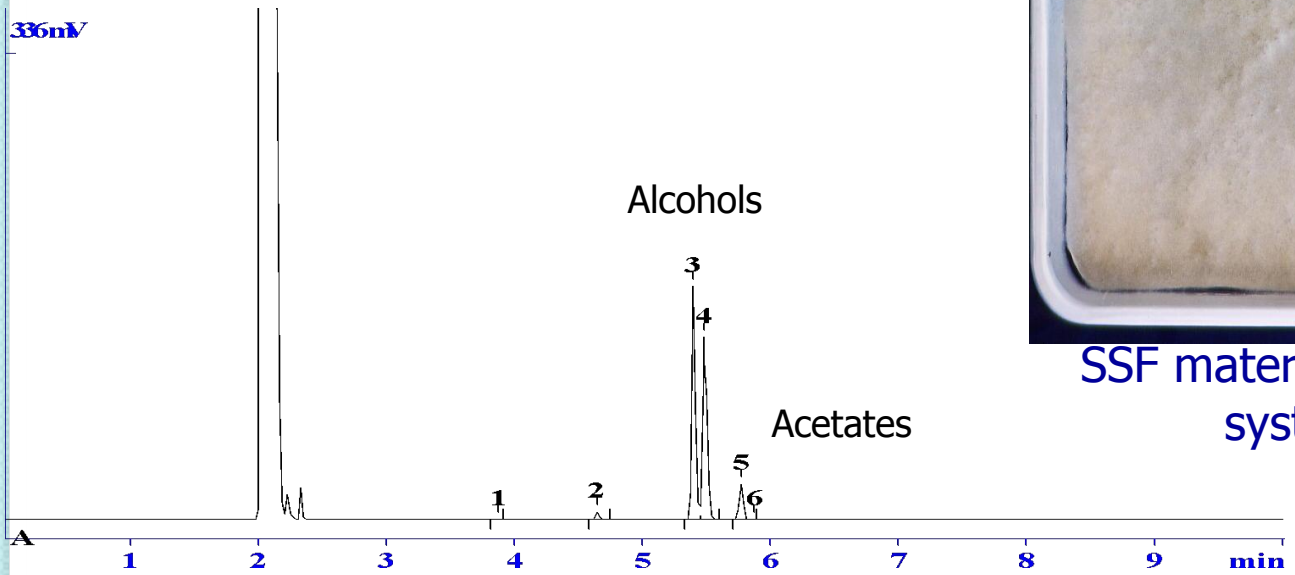

Phenylethanol

0.5 ml vinyl acetate
1.0 ml hexane
0.5 ml tetrahydrofuran
20 mg SSF materials

Room temperature, 1000 rpm




Phenylacetate




SSF materials in tray system

**Kinetic resolutions by SSF 292 (*Mucor hiemalis* with highest Ec.**

# Comparison on the use of SSF materials from different sources of lipase source in organic synthesis

| Strain | Substrate | Moisture content (%) | Inducer | Lipase activity (U/g) | Rxn time (hr) | C[b] (%) | ( R )-la ee[b] (%) | (S)-2a ee[b] (%) | E[c] |
|---|---|---|---|---|---|---|---|---|---|
| *Mucor hiemalis* NRRL 13.009 | Palm kernel cake (PKC) | 50 | - | 45 | 120 | 12 | 87 | 9 | 16 |
| *M. hiemalis* NRRL 13.009 | PKC | 50 | Olive oil | 17 | 120 | 7 | 9 | 5 | 20 |
| *M. hiemalis* NRRL 13.009 | PKC | 60 | - | 26 | 120 | 8 | 80 | 5 | 10 |
| *M. hiemalis* NRRL 13.009 | PKC | 60 | Olive oil | 21 | 120 | 8 | 92 | 6 | 26 |
| *M. hiemalis* NRRL 13.009 | Coconut waste | 67 | - | 8 | 120 | 12 | 85 | 10 | 14 |
| *M. hiemalis* NRRL 13.009 | Coconut waste | 67 | Olive oil | 17 | 120 | 7 | 58 | 3 | 4 |
| *M. hiemalis* NRRL 13.009 | Coconut waste | 75 | - | 15 | 120 | 15 | 84 | 12 | 13 |
| *M. hiemalis* NRRL 13.009 | Coconut waste | 75 | Olive oil | 5 | 120 | 11 | 73 | 22 | 7 |

# ENZYME APPLICATION IN PAPER INDUSTRY

Depletion of forest resources – global problem
(Each ton of paper making – 17 –20 trees, 31,500 L of water, 41,000 Kw/h energy, 50-70 kg chemicals)

↓

27 kg air pollution and creating 2.5 $m^3$ landfill materials

↓

**Environmental impact**

↓

**Recycling of waste paper** – conventional chemical methods
(sodium hydroxide, sodium silicate, hydrogen peroxide, hypochlorite, chelating agents and surfactants)

↓

**Environmental impact**
(cost intensive, unsafe, requires treatment for finished papers, high energy requirement, environmental unfriendly)

# PULP AND PAPER INDUSTRIES IN MALAYSIA

Demand for paper continues to be strong although in paperless global society (a state of self sufficiency)

↓

The industry is heavily dependent on imported fibre, particularly virgin pulp.

↓

> 1.0 million tons per annum (19 paper manufacturing companies)
Paper import: 1,189,120 metric tonnes per year (RM2.7 billion)

↓

Thus, a new source of fibre is needed to strengthen the industry

↙ ↘

Non-wood materials
Kenaf fibres
(***Hibiscus cannabinus***)

***Recycling of waste papers (< 5%)***
**(Conventional chemical method)**
**(No biological/enzymatic method)**

## ISSUES ON ENVIRONMENTAL IMPACTS

- Pollutions from conventional chemical methods

↓

Environmental friendly, biological methods *via* biotechnology

↓

Alternative biological methods for paper recycling using ***biocatalysts/enzymes***

**Why biological method for paper recycling?**

- Cheap, safe and effective
- Utilization of agrowastes for the production of added value and commercial products
- Viable large scale operation of enzymatic hydrolysis of pulp and ink removal with low production cost
- Environmental friendly system/process for deinked waste papers
- Reproducible, efficient, low operational cost, non-detrimental approach good quality deinked papers

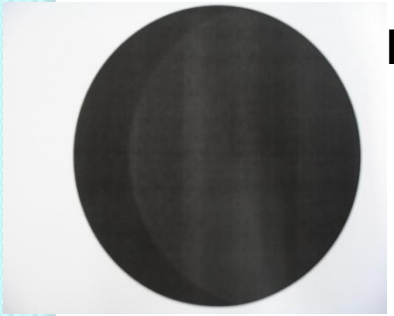
# SPECIFIC OBJECTIVES ON ENZYMATIC DEINKING SYSTEM

1. To design, construct and fabricate the enzymatic bioreactor for paper pulp hydrolysis , flotation system and pulp separation unit for continuous enzymatic deinking, ink removal, ink separation and reuse of enzymes and flotation solution.

2. To evaluate the performance of the enzymatic deinking system under continuous operation based on the quality and properties of the deinked papers.

# Application system for enzyme hydrolysis of waste papers and flotation for ink removal under optimized conditions

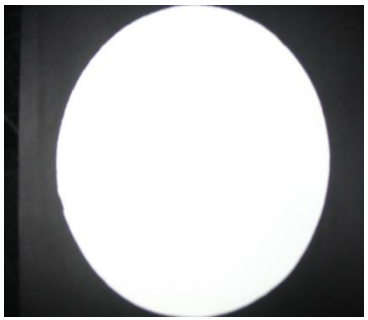

**An effective and fast prototype of the flotation system containing enzymatic hydrolysed paper pulp**



***Before***

**Enzymatic deinking**

Flotation



***After***

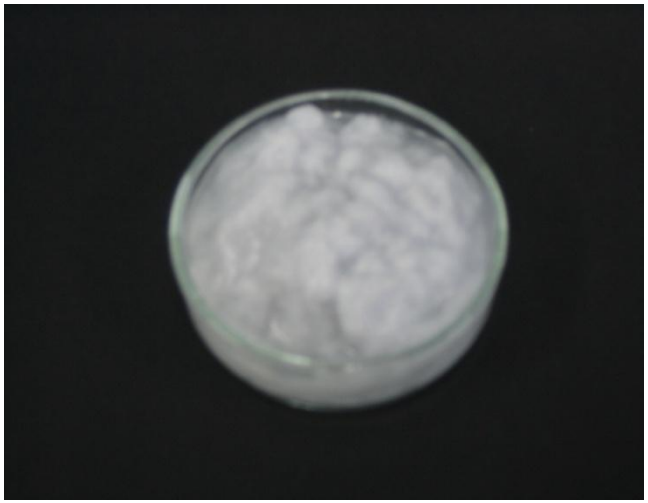
**High quality Deinked paper : Comparable properties of commercial papers or papers by conventional chemical method**

# DEINKING OF PULP – PAPER RECYCLING

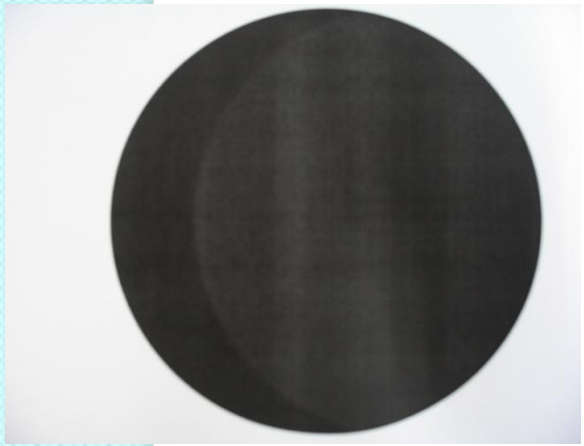
UNIVERSITI MALAYSIA KELANTAN

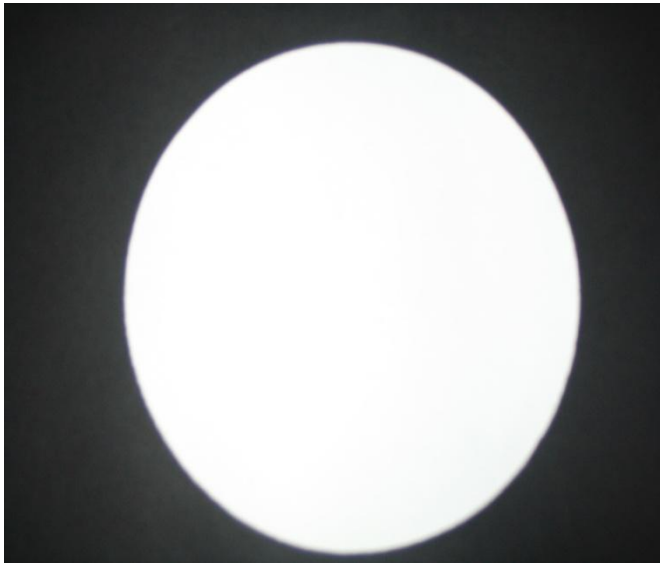
BEFORE

Enzymatic deinking and flotation process



AFTER





## Optimization of the laboratory enzymatic hydrolysis of pulp

| Parameter optimized | Optimized conditions |
|---|---|
| Pulping consistency (%,w/v) | 2 |
| Pulping time (min) | 1 |
| Temperature (°C) | 50 |
| pH (0.2 M Citrate-NaOH buffer) | 3.5 |
| Agitation rate (rpm) | 60 |
| Pulp concentration (%,w/v) | 4 |
| CH enzyme concentration (U/g dried pulp)* | 2.5 (1:1 ratio) |
| Hydrolysis time (min) | 60 |
| Deinking efficiency (%) | 73 |

*Enzymes from Indigenous sources

## Optimization of the flotation process

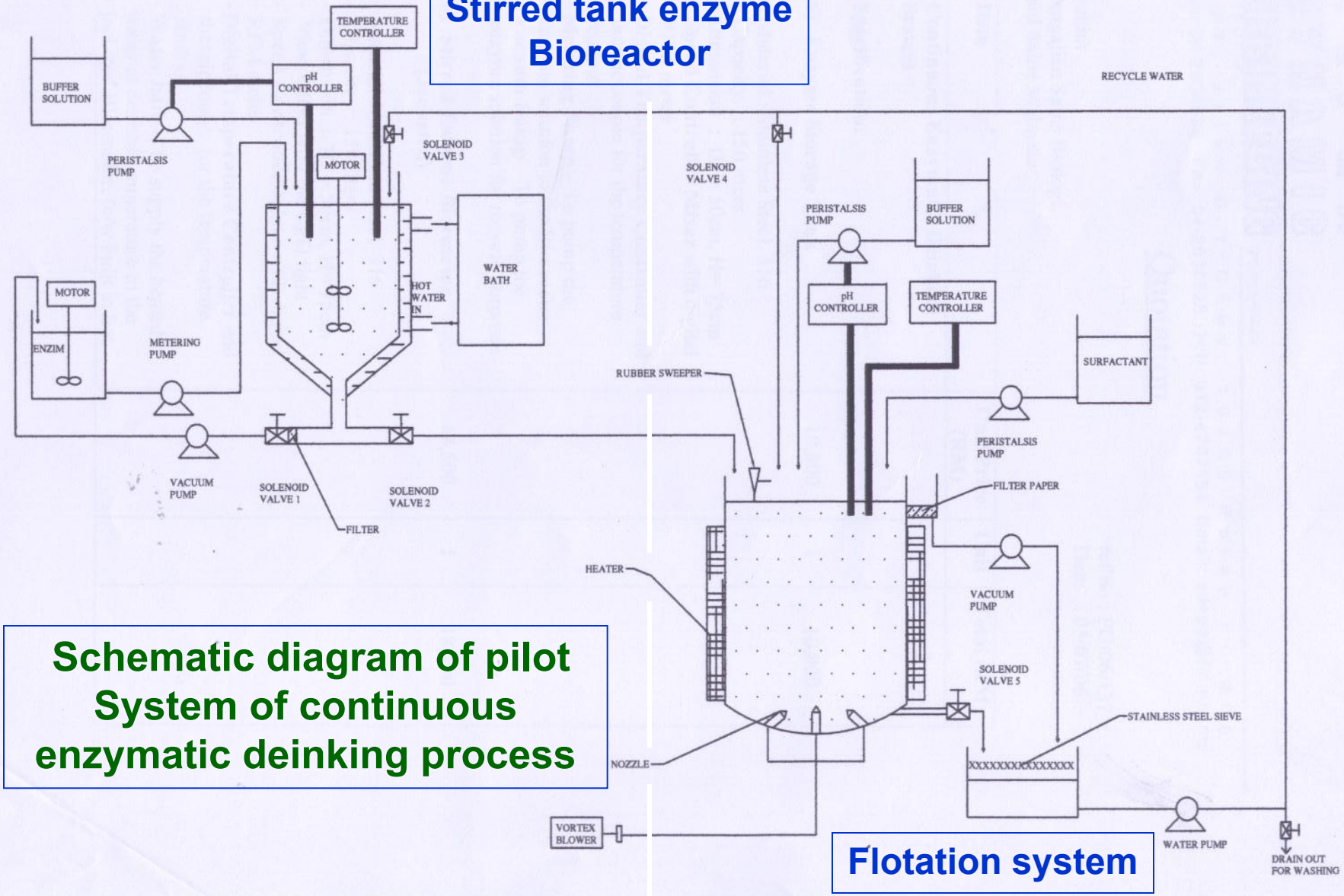
| Parameter optimized | Optimized conditions |
|---|---|
| Surfactant concentration (%,w/w dried pulp) | Tween 80, 0.5 |
| Temperature (°C) | 45 |
| pH | 6 |
| Working air flow rate (L/min) | 10 |
| Flotation time (min) | 15 |
| Deinking efficiency (%) | 95 |

# Physical characteristics of deinked paper

| Characteristics | Enzymatic deinked paper | Control paper |
|---|---|---|
| Grammage ($g/m^2$) | 61.19 | 60.56 |
| Thickness (mm) | 0.1400 | 0.1277 |
| Brightness (%) | 95 | 99* |
| Burst Index (kPa $m^2/g$) | 4.24 | 4.44 |
| Tensile Index (N m/g) | 26.6 | 25.56 |
| Tear Index (mN $m^2/g$) | 6.36 | 6.70 |

* Bleaching involved in treatment process

# Stirred tank enzyme Bioreactor




## Schematic diagram of pilot System of continuous enzymatic deinking process

## Flotation system

# Details of the continuous enzymatic deinking process

## I : Enzyme bioreactor

### *a. Features*

i. The bioreactor is a stirred tank reactor, which will be equipped with impeller for agitation
ii. The motor will be used for agitation with controlled agitation rate
iii. The impeller will be designed as blades to prevent clumpings of pulp.
iv. The temperature of the bioreactor will be controlled by temperature controlled jacket
v. Upon completion of reaction, the pulp will be pump into the flotation system while the enzyme solution will be recycled into the bioreactor the next batch of pulp.
vi. Equipped with probes for temperature and pH

### *b. Capacity*

i. Volume : 150 L (estimated to be 10 kg of pulp at 1% pulp consistency per cycle*)
ii. 75 cm (ID) and 120 cm height

Pulp consistency can be varied, more pulp at higher consistency and higher agitation rate and enzyme concentration. At 1%, per cycle 30 – 40 min or 36 - 40 cycles per day or 360 – 400 kg pulp deinked per day

## II. Flotation system

### *a. Features*

i. Equipped with a motor for agitation with controlled agitation rate
ii. Equipped with multiblade impeller to disperse the hydrolysed pulp
iii. Temperature control via heating coil
iv. Equipped with sparger connected to flowmeter for air flow rate from compressor
v. Ink removed via ink trap and collected in the ink reservoir
vi. Upon completion deinked pulp drain in pulp collection container via a siever. The flotation solution will be recycled into the flotation system for next batch of pulp.
vii. Place vertically supported with pillars
vii. Power supply : 240 V

### *b. Capacity*

i. Volume : 750 L (estimated to be 10 kg of pulp at 1% pulp consistency*)
ii. Flotation column: 200 cm height, total height 300 cm, ID 90 cm
iii. Pulp collecting container: 100 cm height
iv. Ink reservoir : ID 20 cm and 30 cm height

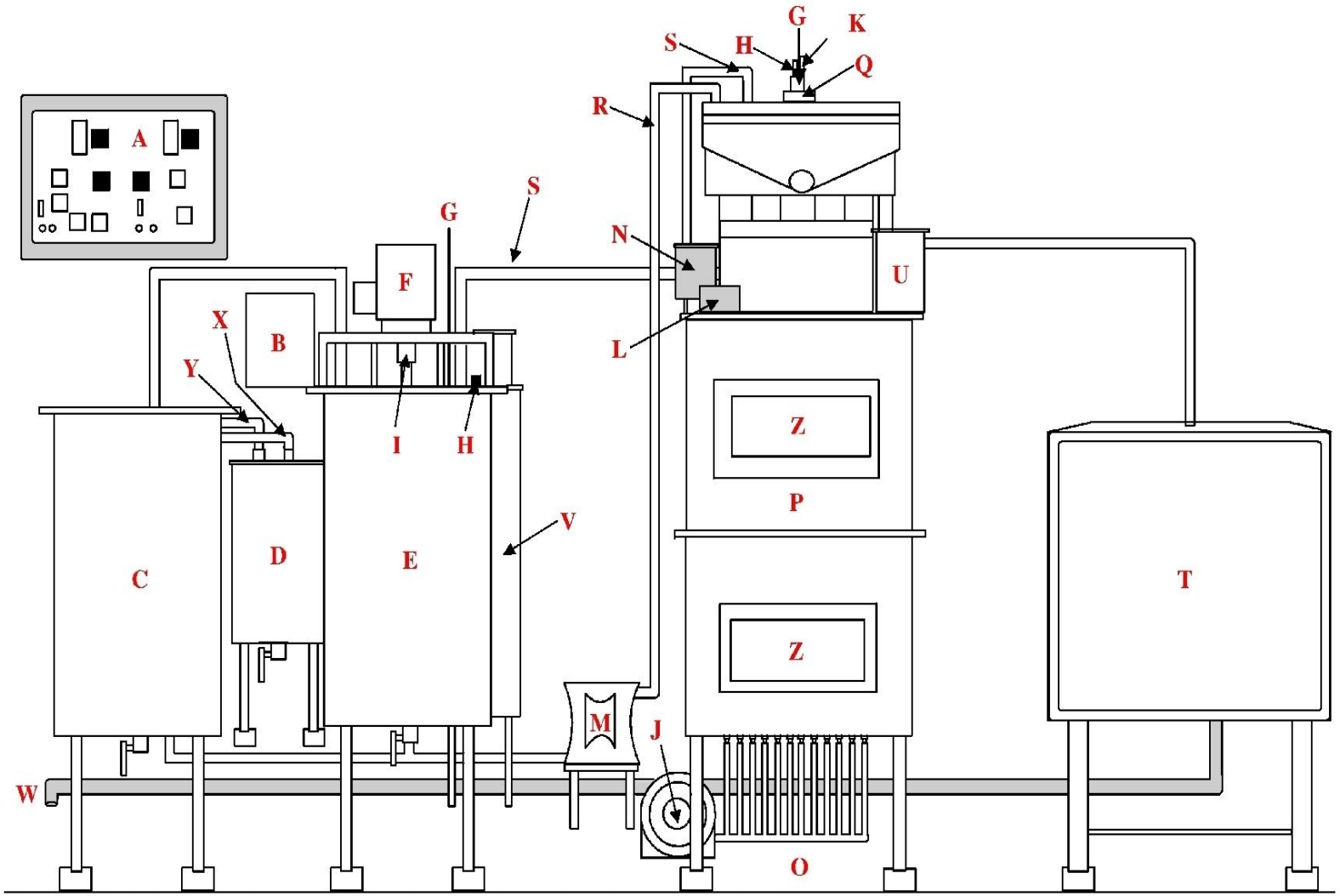
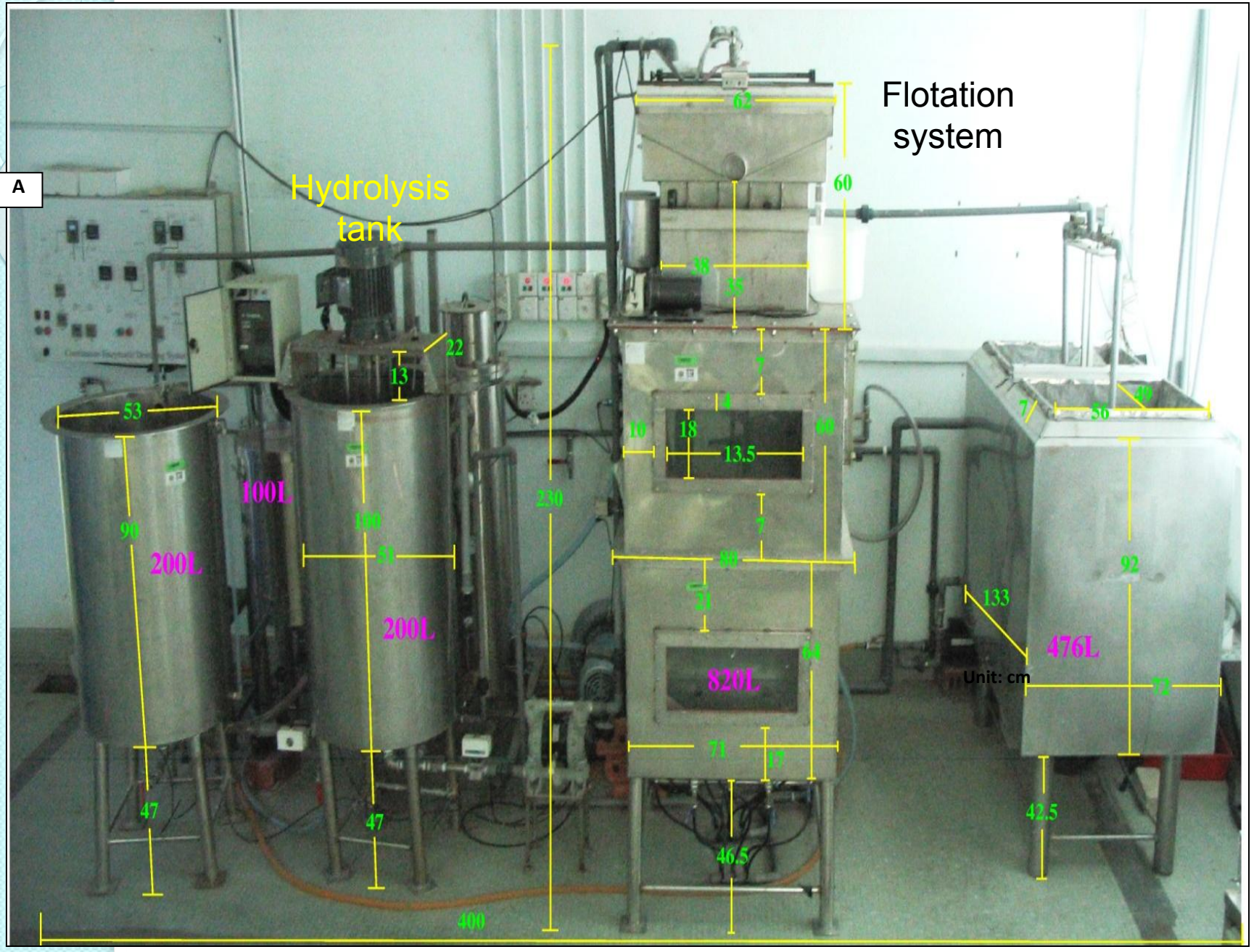
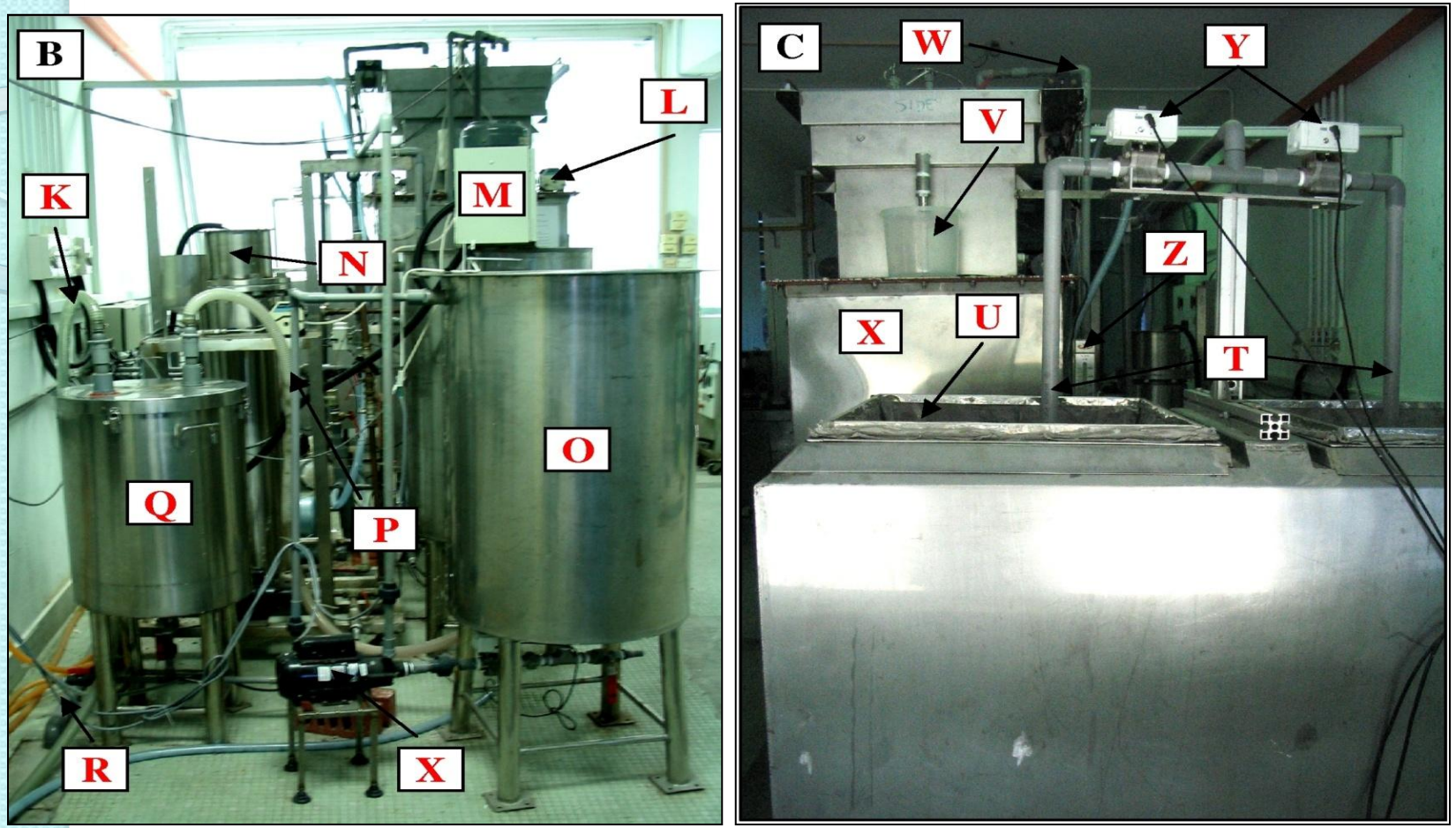



**Figure 4.1:** Schematic diagram of developed pilot scale of enzymatic deinking system.

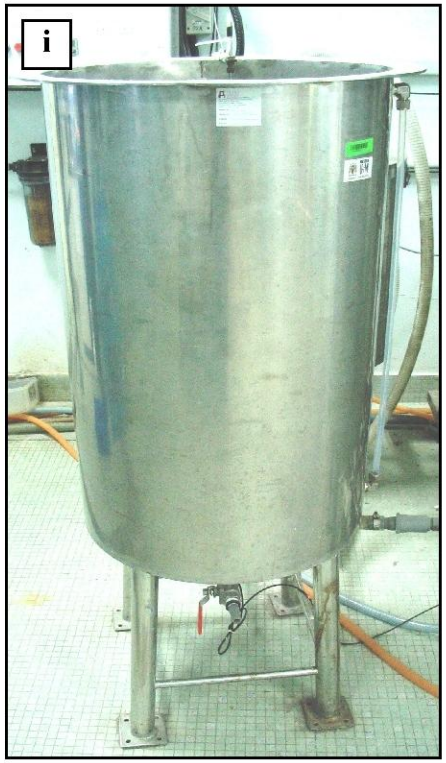
*Notes:* **A**: Control panel; **B:** Mixer controller box; **C:** Enzyme storage vessel; **D**: Enzyme/water recycling vessel; **E**: Bioreactor; **F**: Mixer; **G**: Thermocouple; **H**: pH probe; **I**: impeller shaft; **J**: Blower; **K**: Water level sensor; **L**: Peristaltic pump; **M**: Diaphragm pump; **N**: surfactant/acids/base reservoir; **O**: Nozzles unit; **P** Flotation vessel; **Q**: Scraper unit; **R**: Pulp transfer line; **S**: Water line; **T**: Pulp collecting vessel; **U**: Ink reservoir; **V**: Heater; **W**: Drain pipe; **X**: Vacuum pipe; **Y**: Enzyme/water recycling pipe; **Z**: Viewing glass.


A
Hydrolysis tank
Flotation system
100L
200L
200L
820L
476L
Unit: cm
62
60
38
35
22
13
53
7
4
10
18
13.5
60
7
49
56
230
90
100
51
7
80
92
21
133
64
72
71
17
47
47
46.5
42.5
400

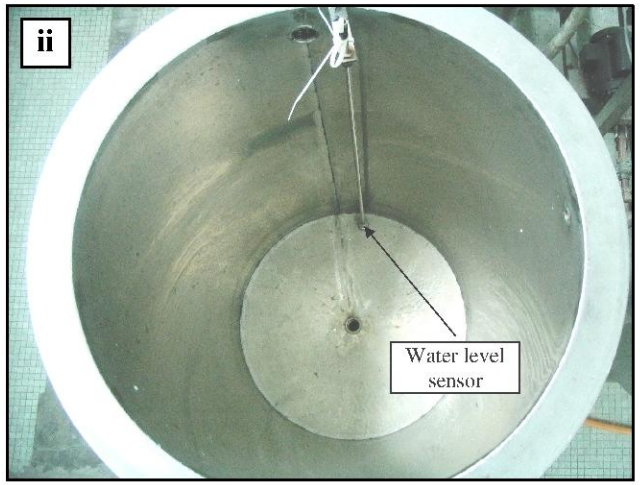


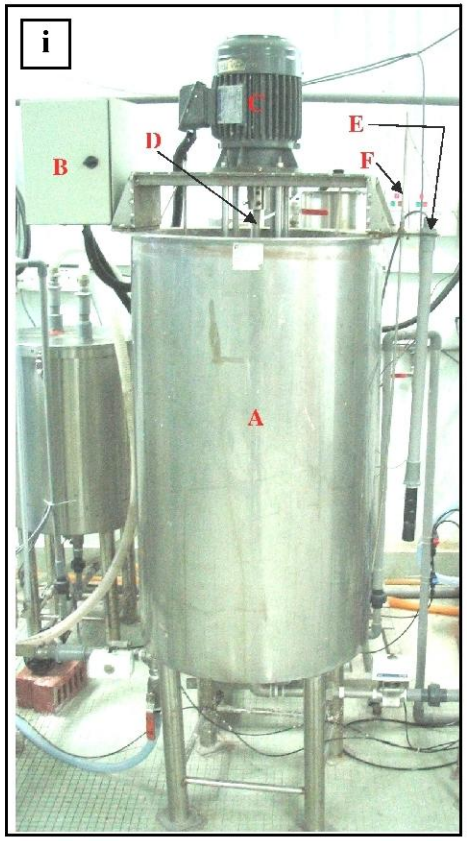

Developed pilot scale of enzymatic deinking system; (A) Front view, (B) Left view and (C) Right view.

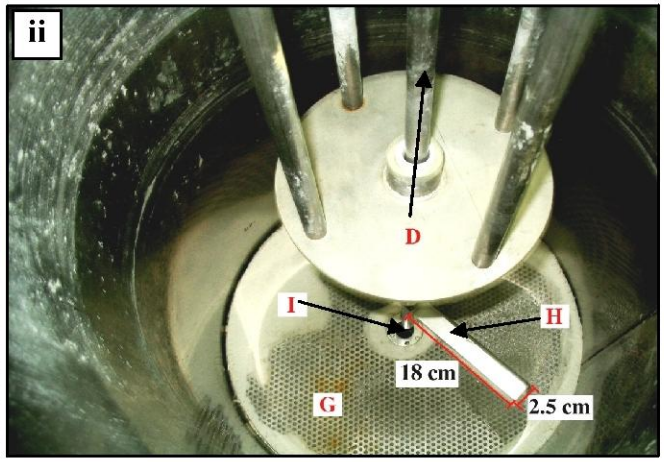
*Notes:* **K**: Vacuum pipe; **L**: Peristaltic pump; **M**: Mixer controller box; **N**: Heater; **O**: Enzyme storage vessel; **P**: Enzyme/water recycling pipe; **Q**: Enzyme/water recycling vessel; **R**: Drain pipe; **S**: Water pump; **T**: Deinked pulp outlet; **U:** Sieve; **V**: Ink reservoir; **W**: Water inlet; **X**: Flotation vessel; **Y**: Solenoid valves; **Z**: Flowmeter.



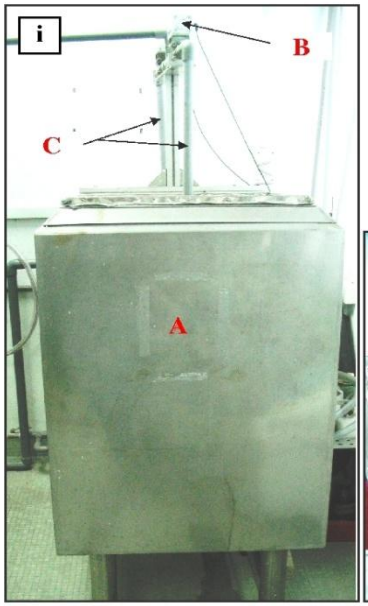

Enzyme storage vessel; Front view (i) and Top view ii).

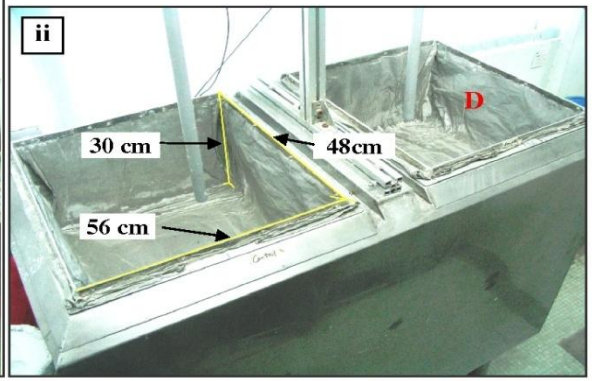


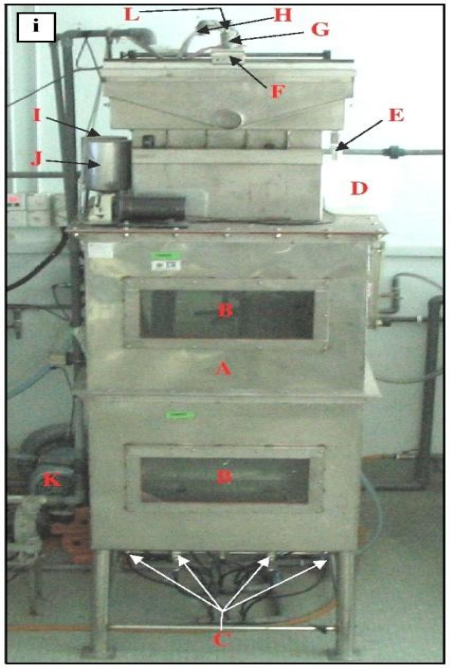

Bioreactor equipped with mixer; Front view (i) and Top view (ii).
***Note:*** **A**: Enzymatic hydrolysis vessel; **B**: Mixer controller box; **C**: Mixer, **D**: Impeller shaft, **E**: pH probe; **F**: Thermocouple; **G**: Sieve; **H**: Impeller tips; **I**: Pulp slurry outlet.

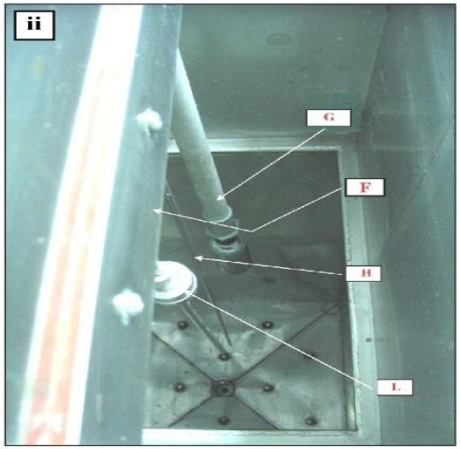



Pulp collecting vessel; Front view (i) and Top view (ii)

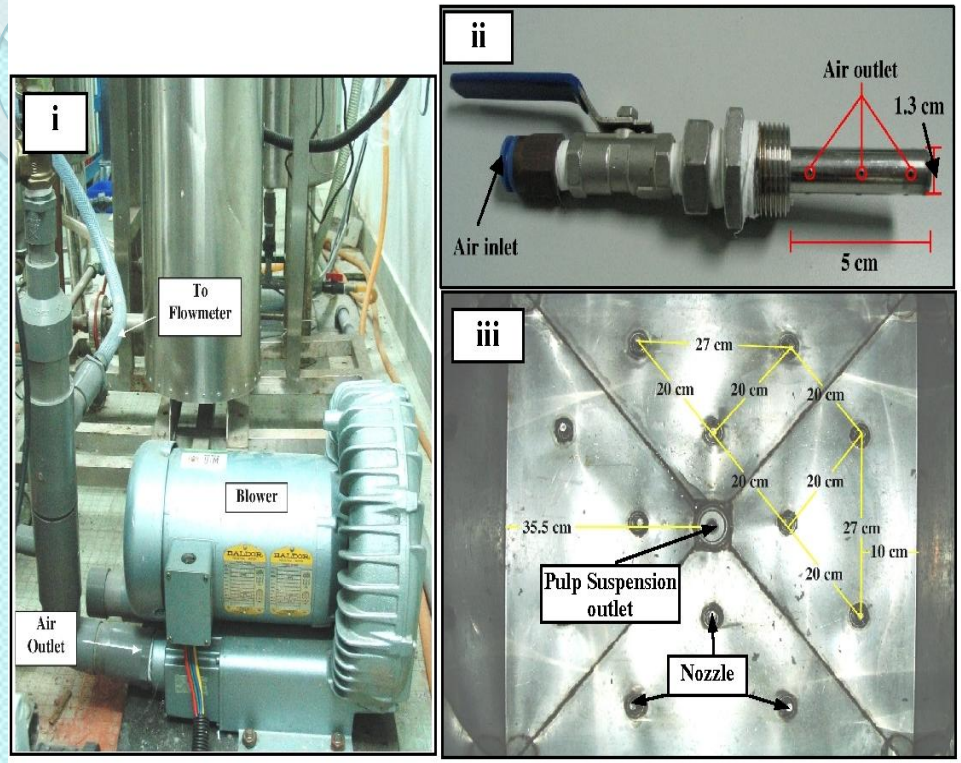
***Note:*** **A**: Pulp collecting vessel; **B**: Solenoid valve; **C**: Pulp suspension outlet; **D**: Sieve.



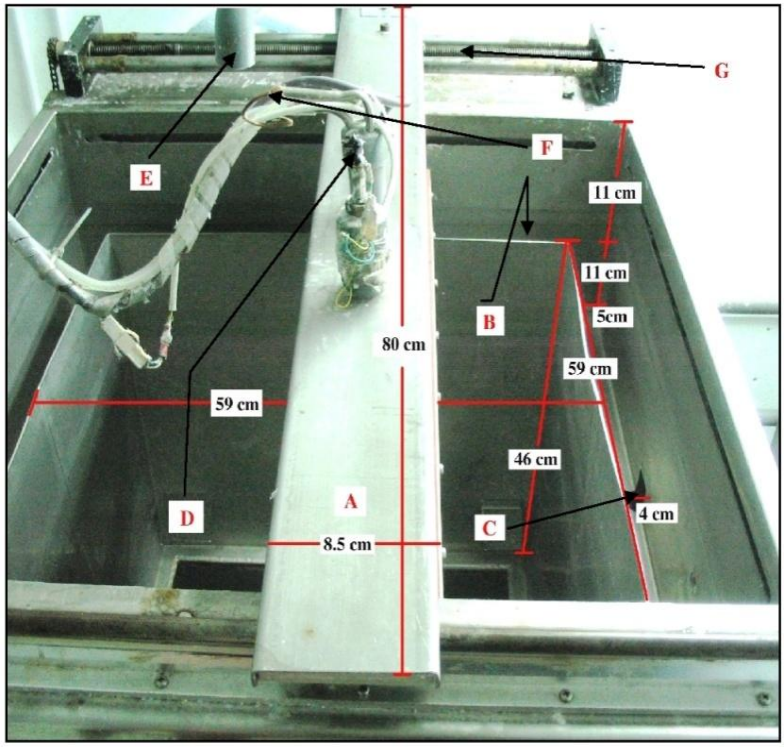




Flotation vessel; Front view (i) and Top view (ii).

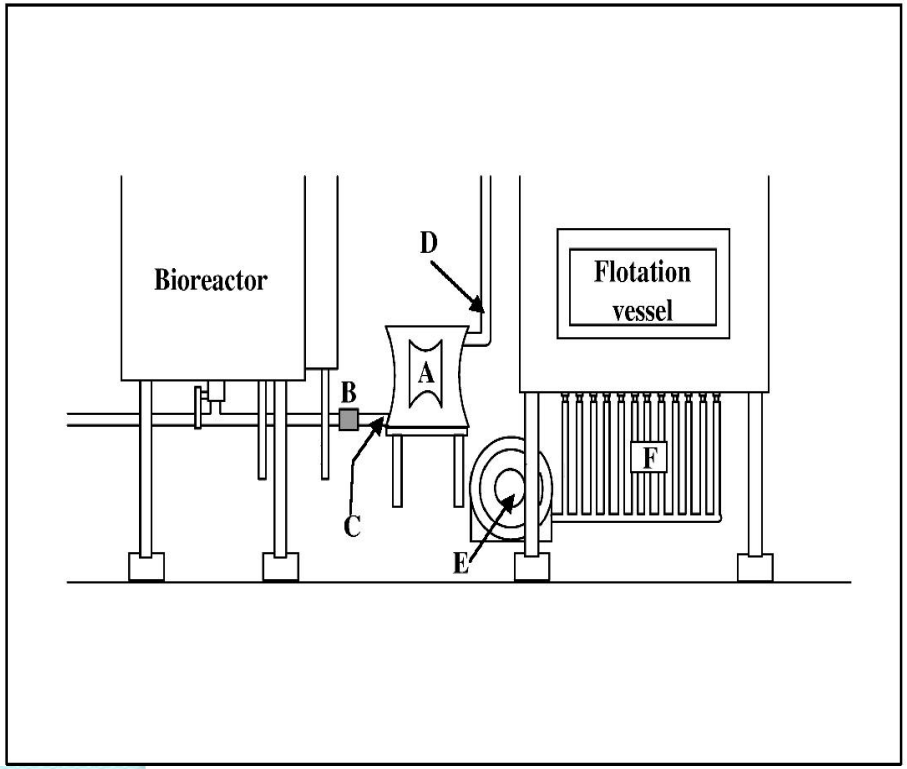
***Note:*** **A**: Flotation vessel; **B**: Viewing glass; **C**: Nozzles; **D**: Ink reservoir; **E**: Foam outlet; **F**: Scraper; **G**: pH Probe; **H**: Thermocouple; **I**: acid/base reservoir; **J**: surfactant reservoir; **K**: Blower; **L**: water level sensor.




Blower (i), single nozzle (ii) and Nozzles unit (iii) used in the flotation system

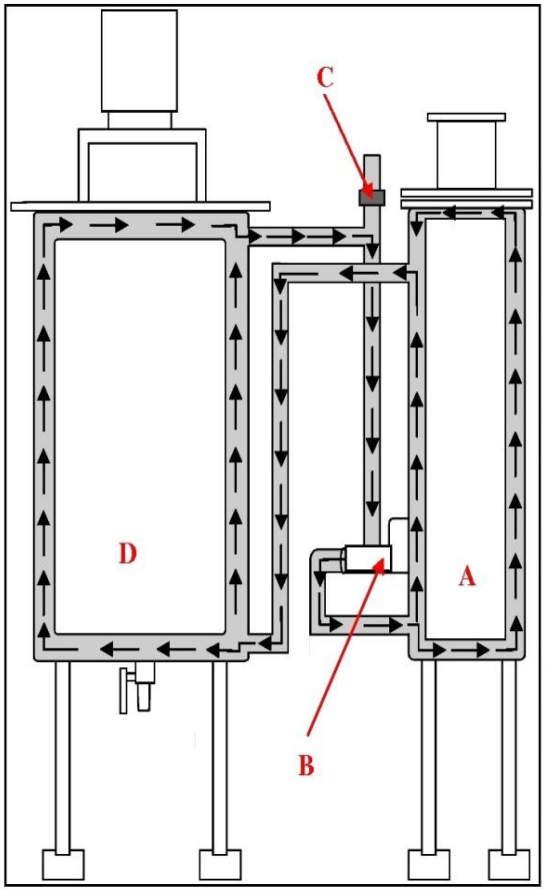


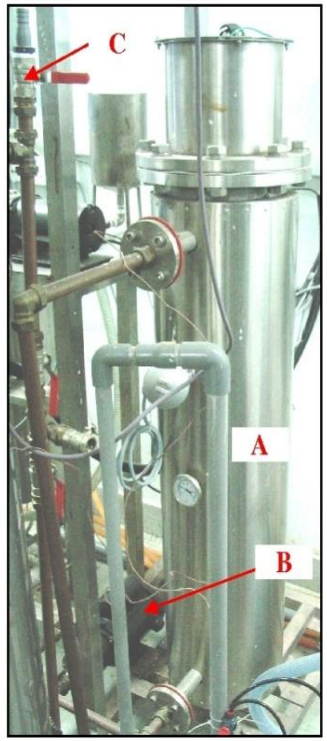

Foam scraping unit used in flotation process
***Note:*** **A**: Scraper; **B**: Foam drain; **C**: Foam/ink particle outlet; **D**: Water level sensor; **E**: Water inlet; **F**: Thermocouple; **G**: Scraper carrier




: Schematic diagram of diaphragm pump used in the deinking process

***Note:*** **A**: Diaphragm pump; **B**: Solenoid valve; **C**: pulp suspension from bioreactor; **D:** pulp suspension to flotation vessel; **E:** Blower; **F:** Nozzles unit.




Heater used in deinking process.

Circulation of water (control temperature) around bioreactor and heater.

***Note:*** **A:** Heater; **B:** Metering pump; **C:** Stopper; **D:** Bioreactor vessel.

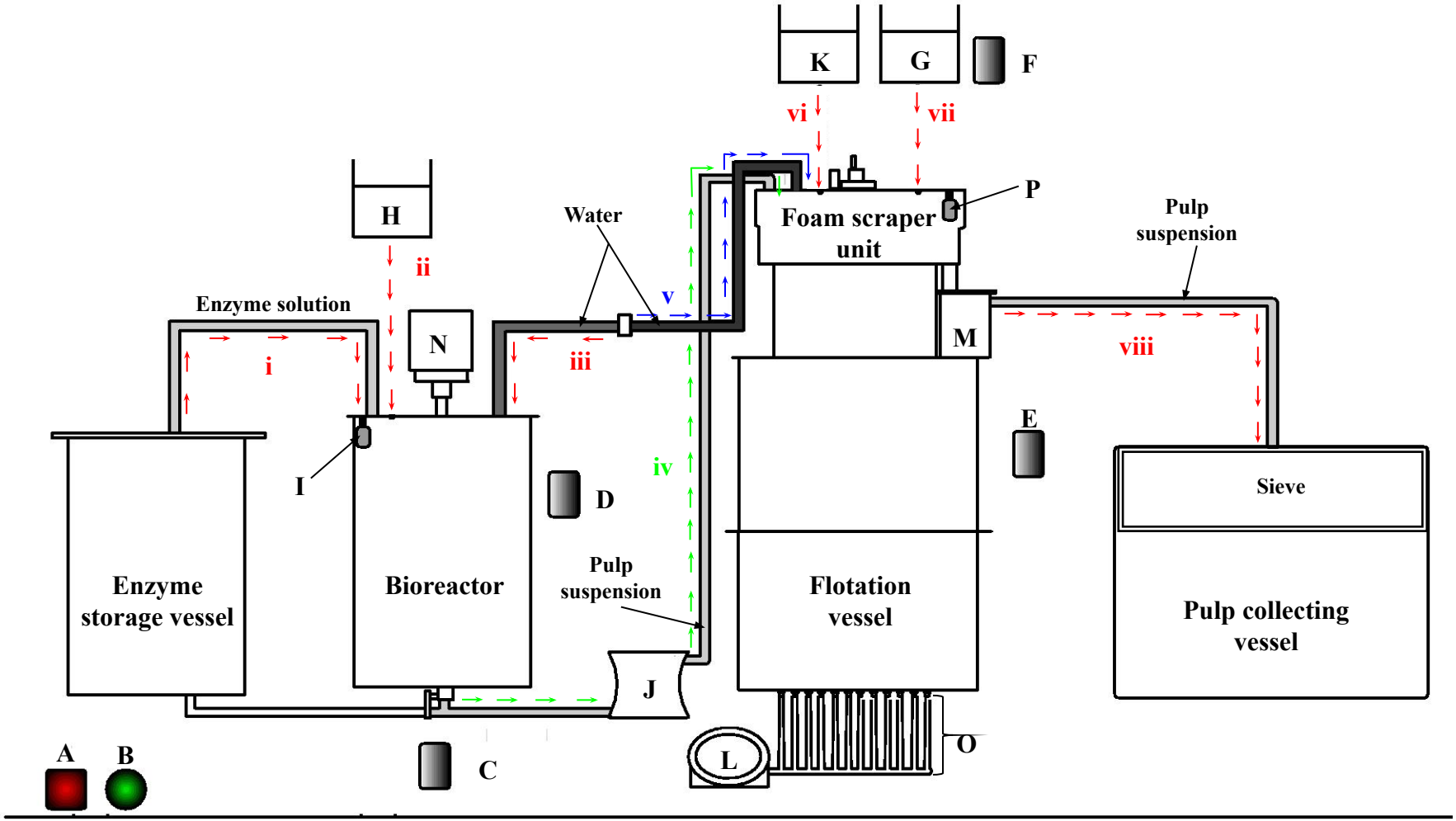



**Figure 4.4:** Control panel for the operational of enzymatic deinking system.

***Note:*** Process sequence (start: i; end: viii) A: Main switch; B: Process sequence switch; C: Timer for pulp hydrolysis; D: Bioreactor temperature; E: Timer for flotation process; F: Timer for surfactant; G: Surfactant; H,K: Acid/base solution; I,P: Water level sensor; J: Diaphragm pump; L: Blower; M: Ink reservoir; N: Mixer; O: Nozzles unit.

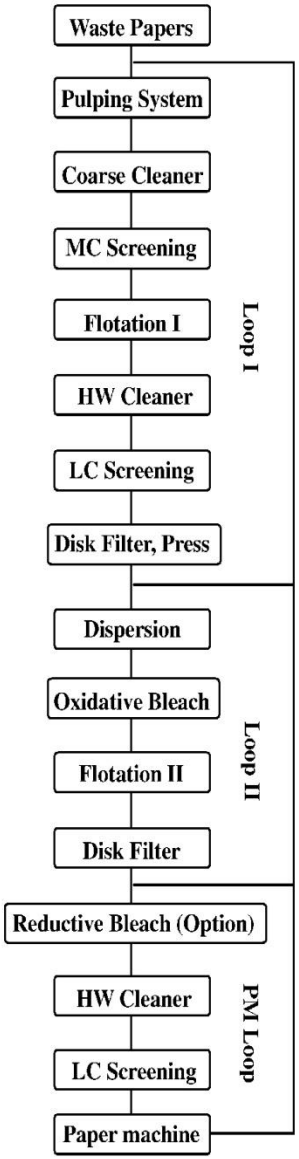



**Figure 4.5:** Plant concept for newsprint and improved paper grades (Schwarz, 2000)

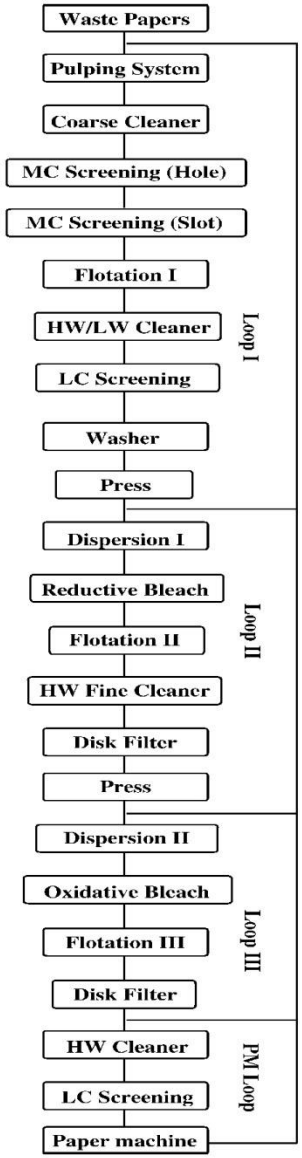
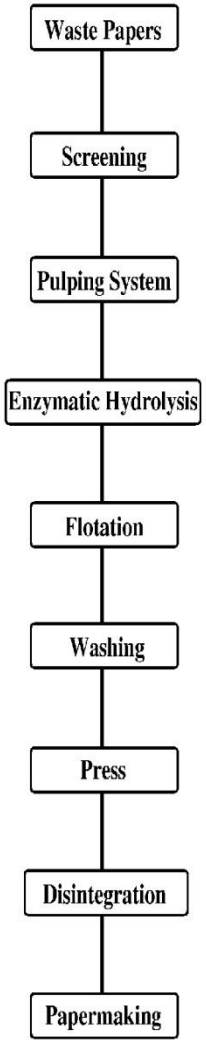



**Figure 4.6:** Plant concept for DIP from mixed office wastepaper (Schwarz, 2000)




enzymatic deinking process used in present work

**Table 5.1:** Initial conditions set for the enzymatic hydrolysis of pulp (MOW and ONP)

| Parameter | Value |
|---|---|
| Hydrolysis temperature | 55°C |
| pH | 5.5 |
| Enzyme concentration | 1.2 U per gram of air dried pulp |
| Hydrolysis time | 45 min |

**Table 5.2:** Initial conditions set for the flotation process

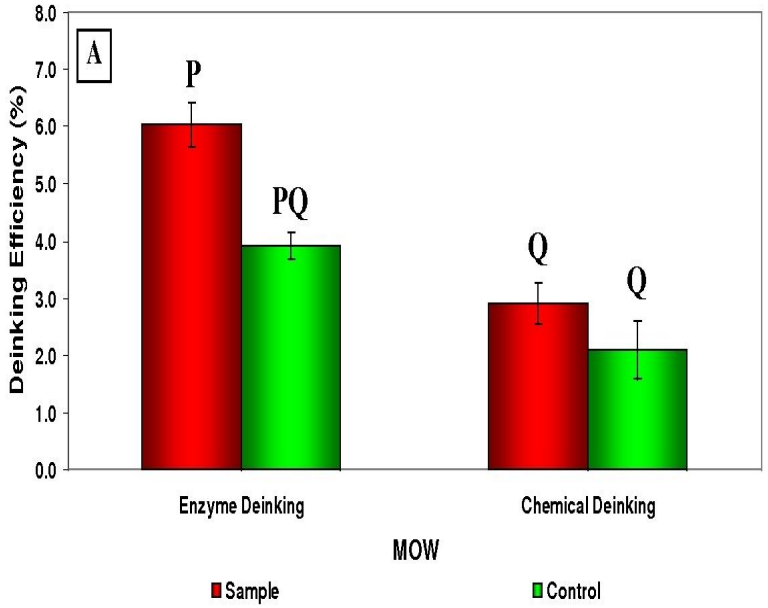
| Parameter | Value |
|---|---|
| pH | 8.0 |
| Tween 80 | 0.200 % (w/w) of air dried pulp (for MOW)<br>0.575% (w/w) of air dried pulp (for ONP) |
| Flotation time | 5 min |

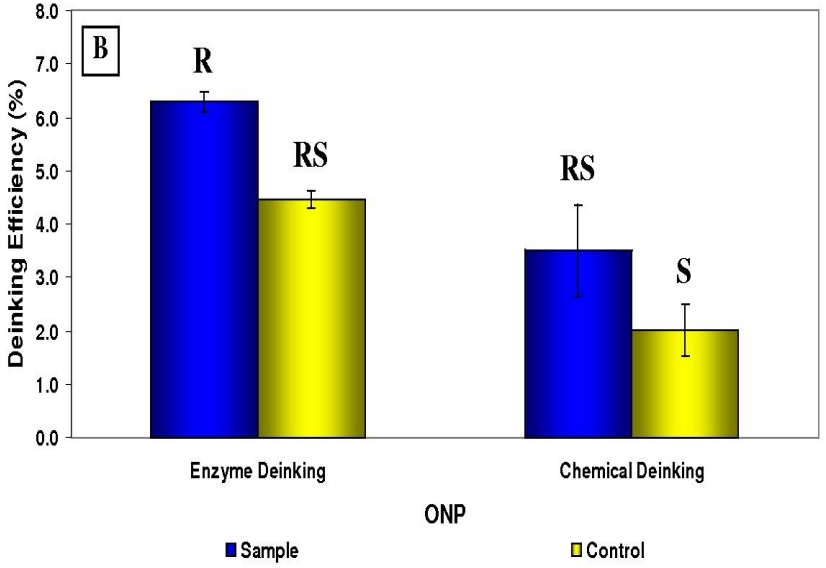
**Table 5.3**: Summary of selected optimum enzymatic hydrolysis of MOW and ONP

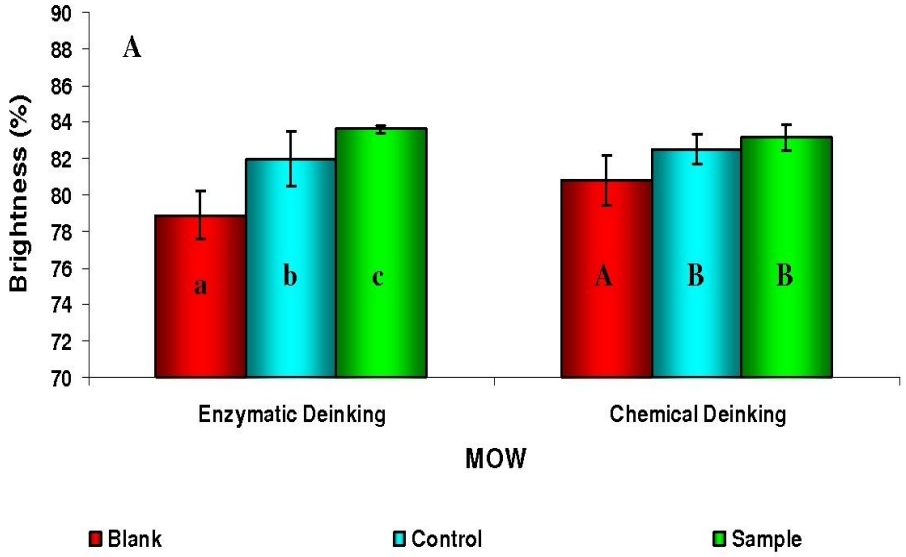
| Pulping process | MOW | ONP |
|---|---|---|
| Pulping consistency | 2% | 3% |
| Pulping time | 60 min | 45 min |
| **Enzymatic hydrolysis process** | | |
| Temperature | 50°C | 50°C |
| pH | 5.5 | 5.5 |
| Enzyme concentration | 4.8 U/g air-dry pulp | 2.4 U/g of air dry pulp |
| Hydrolysis time | 60 min | 45 min |
| Deinking efficiency | 5.00% | 4.74% |
| Total reducing sugar obtained | 40.76 mmol | 18.71 mmol |

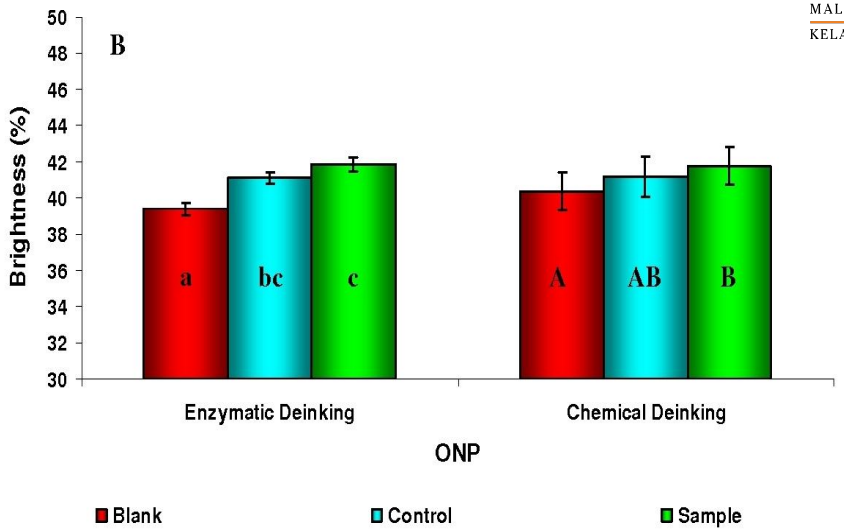
Mixed Office wastes (MOW)

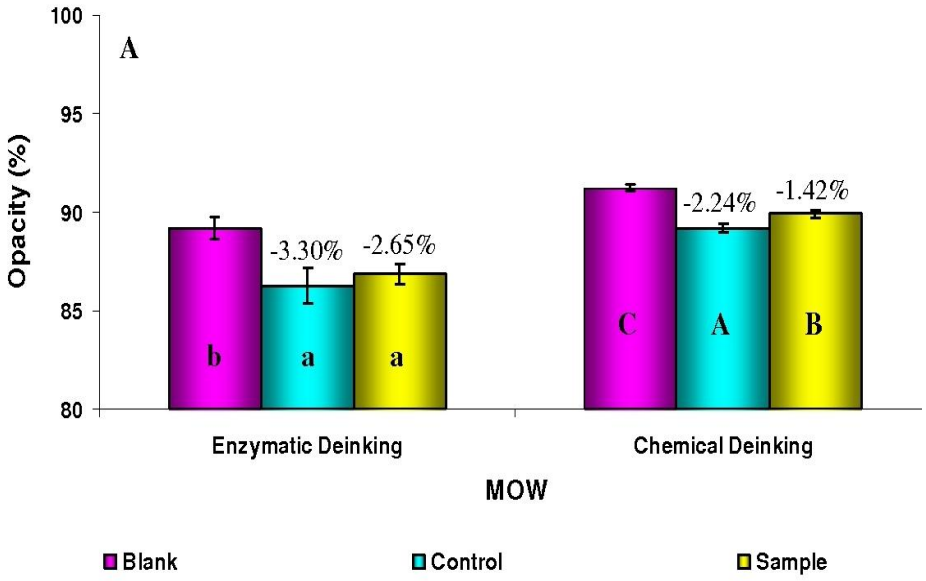
Old newspapers (ONP)

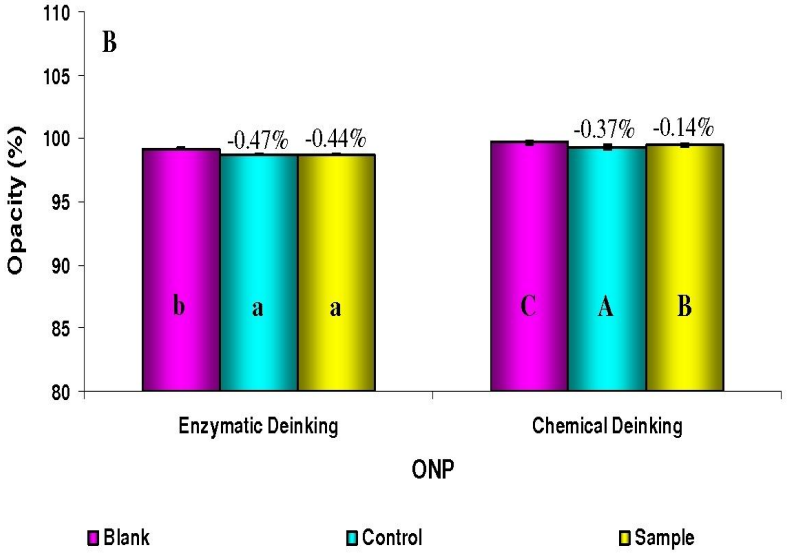


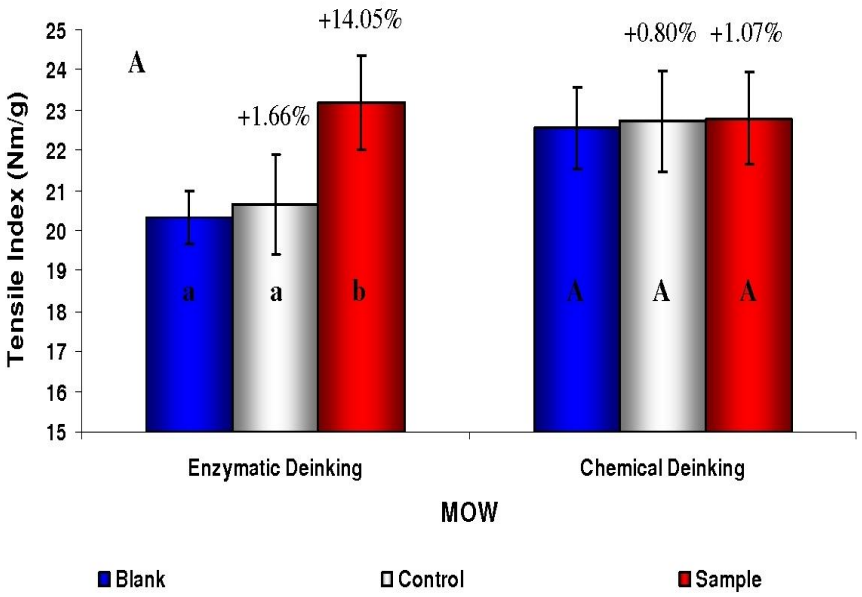



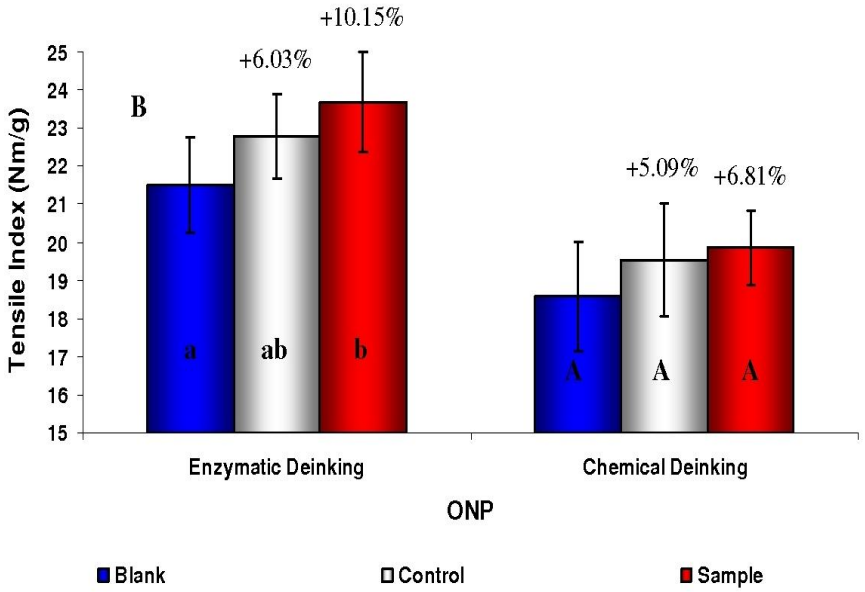

A
Brightness (%)
90
88
86
84
82
80
78
76
74
72
70
a
b
c
A
B
B
Enzymatic Deinking
Chemical Deinking
MOW
Blank
Control
Sample

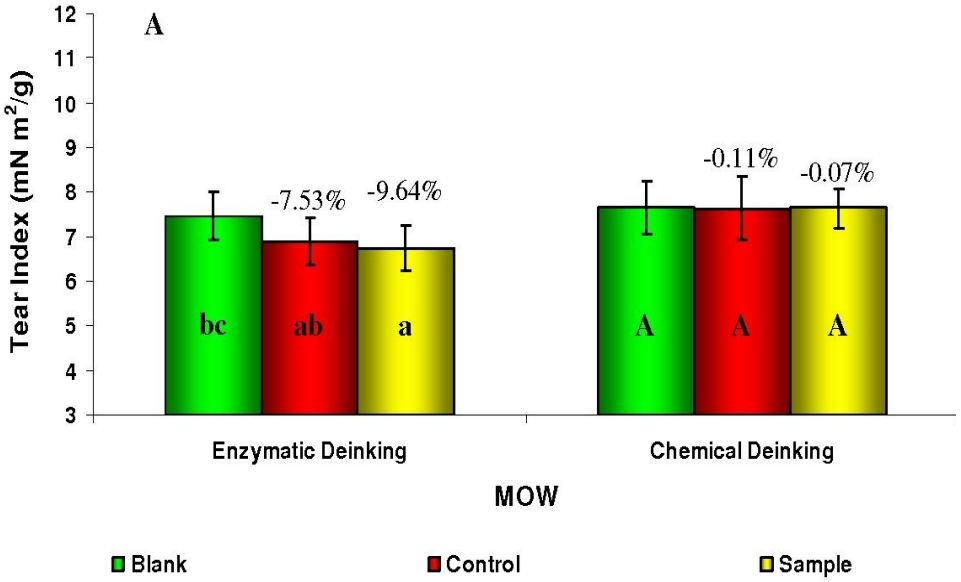

B
Brightness (%)
50
48
46
44
42
40
38
36
34
32
30
a
bc
c
A
AB
B
Enzymatic Deinking
Chemical Deinking
ONP
Blank
Control
Sample

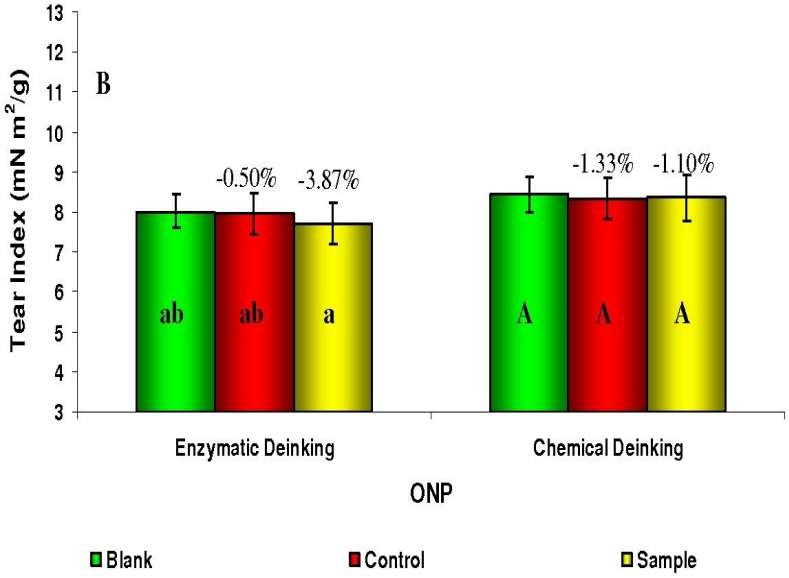

A
Opacity (%)
100
95
90
85
80
-3.30%
-2.65%
-2.24%
-1.42%
b
a
a
C
A
B
Enzymatic Deinking
Chemical Deinking
MOW
Blank
Control
Sample

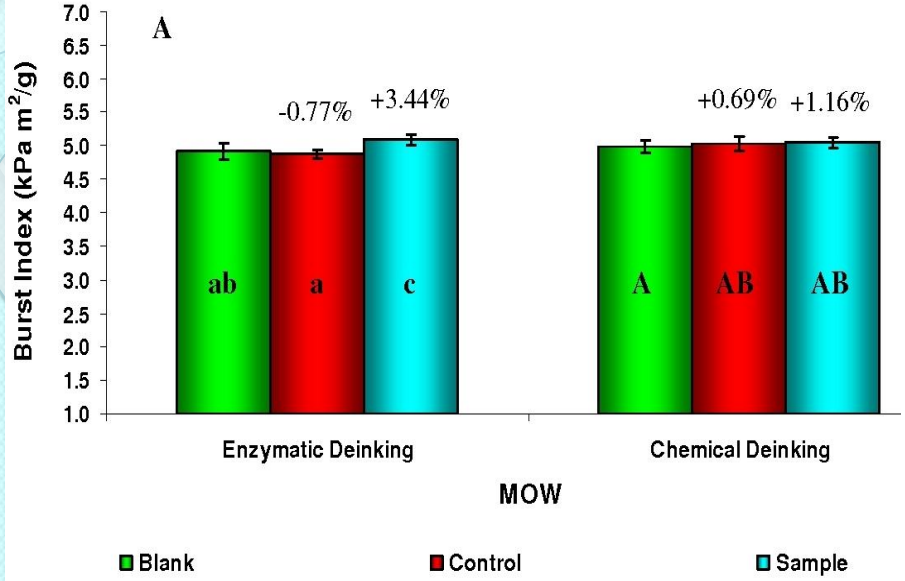

B
Opacity (%)
110
105
100
95
90
85
80
-0.47%
-0.44%
-0.37%
-0.14%
b
a
a
C
A
B
Enzymatic Deinking
Chemical Deinking
ONP
Blank
Control
Sample

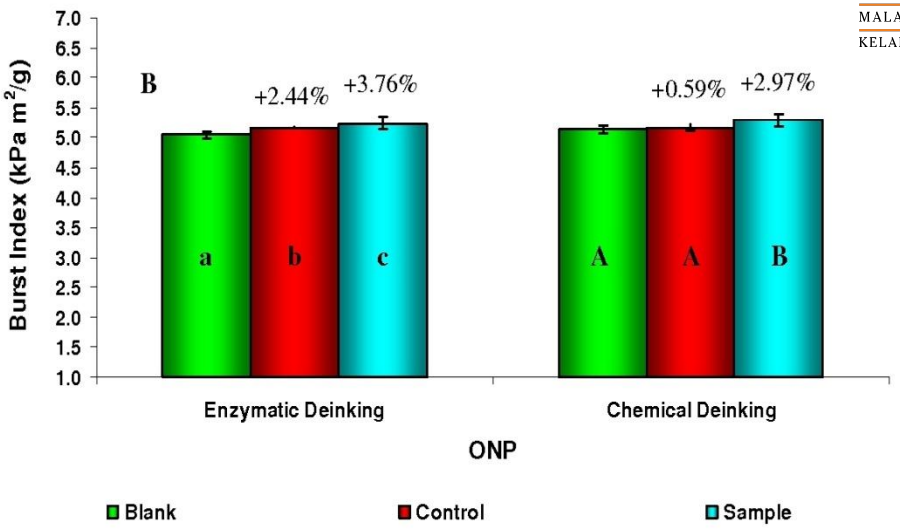

A
Tensile Index (Nm/g)
+14.05%
+1.66%
+0.80%
+1.07%
a
a
b
A
A
A
Enzymatic Deinking
Chemical Deinking
MOW
Blank
Control
Sample

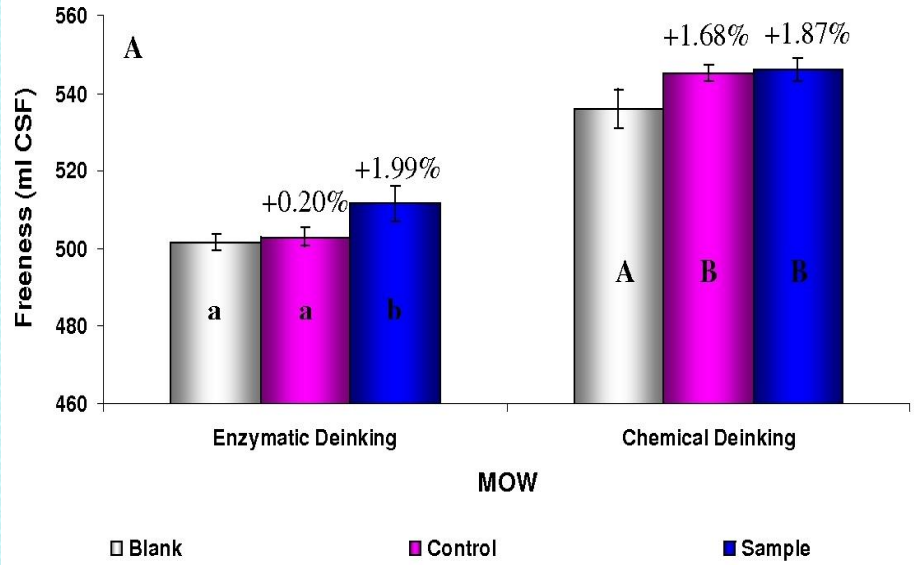

B
Tensile Index (Nm/g)
+10.15%
+6.03%
+5.09%
+6.81%
a
ab
b
A
A
A
Enzymatic Deinking
Chemical Deinking
ONP
Blank
Control
Sample

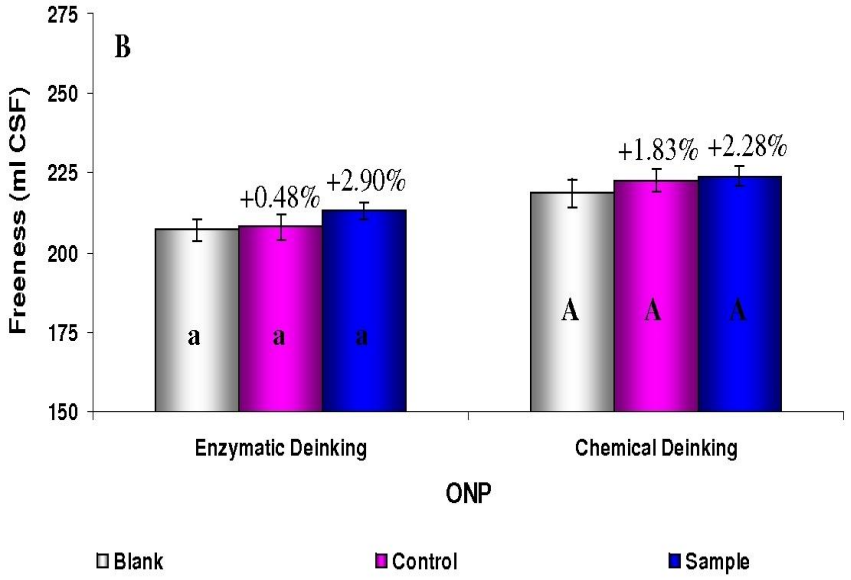

A
Tear Index (mN m²/g)
-7.53%
-9.64%
-0.11%
-0.07%
bc
ab
a
A
A
A
Enzymatic Deinking
Chemical Deinking
MOW
Blank
Control
Sample

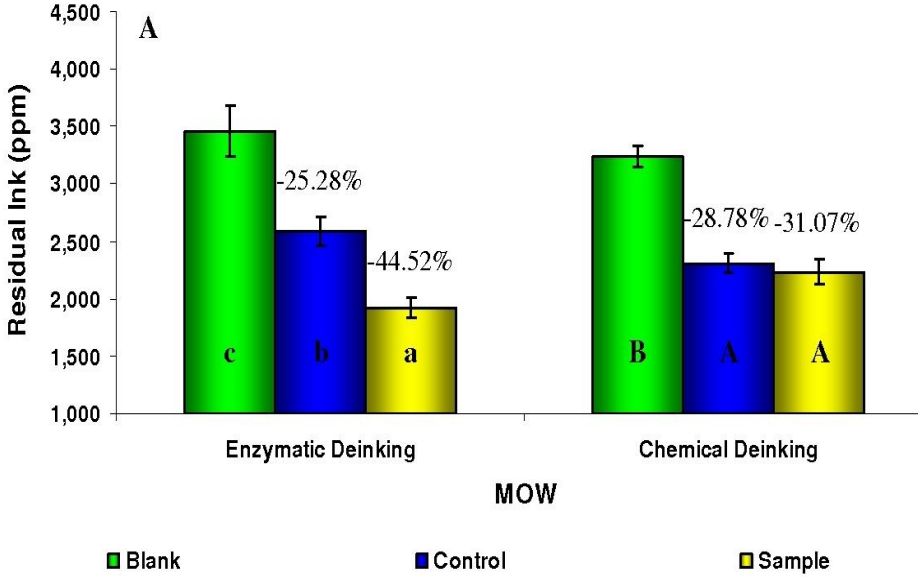

B
Tear Index (mN m²/g)
-0.50%
-3.87%
-1.33%
-1.10%
ab
ab
a
A
A
A
Enzymatic Deinking
Chemical Deinking
ONP
Blank
Control
Sample

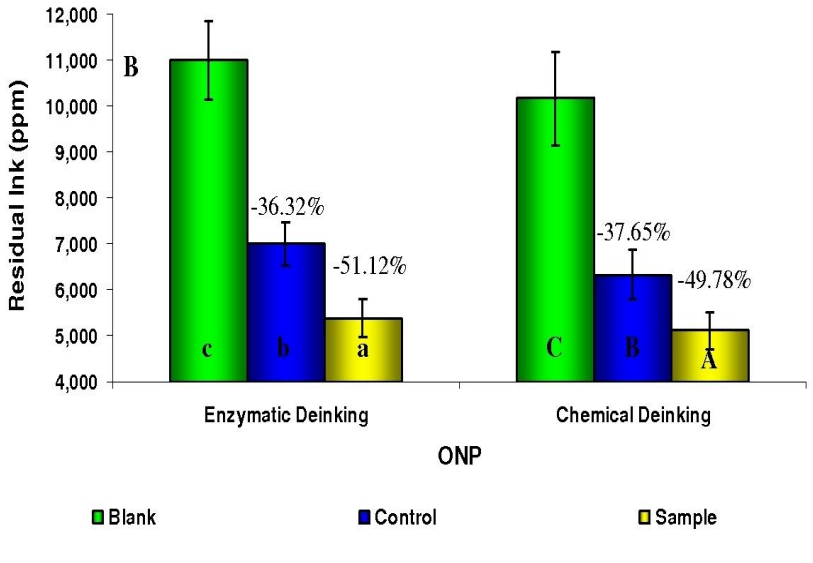

A
Burst Index (kPa m2/g)
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
-0.77%
+3.44%
+0.69%
+1.16%
ab
a
c
A
AB
AB
Enzymatic Deinking
Chemical Deinking
MOW
Blank
Control
Sample

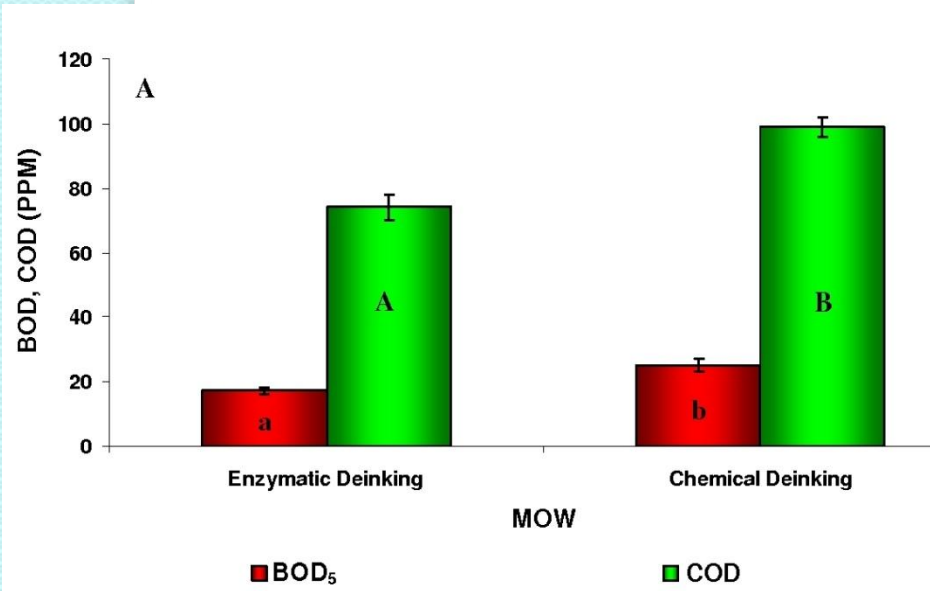

B
Burst Index (kPa m2/g)
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
+2.44%
+3.76%
+0.59%
+2.97%
a
b
c
A
A
B
Enzymatic Deinking
Chemical Deinking
ONP
Blank
Control
Sample


A
Freeness (ml CSF)
560
540
520
500
480
460
+0.20%
+1.99%
+1.68%
+1.87%
a
a
b
A
B
B
Enzymatic Deinking
Chemical Deinking
MOW
Blank
Control
Sample


B
Freeness (ml CSF)
275
250
225
200
175
150
+0.48%
+2.90%
+1.83%
+2.28%
a
a
a
A
A
A
Enzymatic Deinking
Chemical Deinking
ONP
Blank
Control
Sample


A
Residual Ink (ppm)
4,500
4,000
3,500
3,000
2,500
2,000
1,500
1,000
-25.28%
-44.52%
-28.78%
-31.07%
c
b
a
B
A
A
Enzymatic Deinking
Chemical Deinking
MOW
Blank
Control
Sample


B
Residual Ink (ppm)
12,000
11,000
10,000
9,000
8,000
7,000
6,000
5,000
4,000
-36.32%
-51.12%
-37.65%
-49.78%
c
b
a
C
B
A
Enzymatic Deinking
Chemical Deinking
ONP
Blank
Control
Sample


A
BOD, COD (PPM)
120
100
80
60
40
20
0
A
B
a
b
Enzymatic Deinking
Chemical Deinking
MOW
BOD5
COD

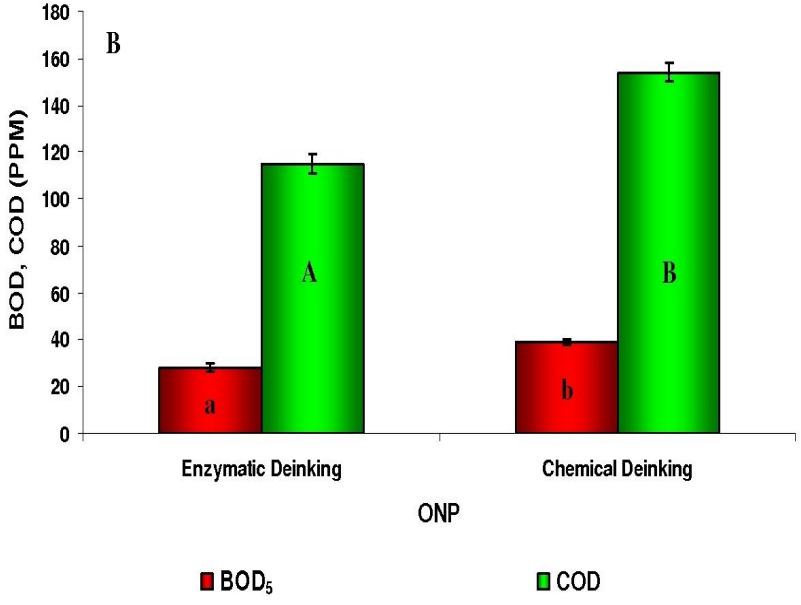

B
BOD, COD (PPM)
180
160
140
120
100
80
60
40
20
0
A
B
a
b
Enzymatic Deinking
Chemical Deinking
ONP
BOD5
COD

**Table 6.1:** Summary of pulp and paper properties after deinking process.

| Pulp/Paper properties | Deinking Method | MOW | ONP |
|---|---|---|---|
| Brightness | Enzymatic | + 4.73 units | + 2.47 units |
| | Chemical | + 2.30 units | + 1.35 units |
| Opacity | Enzymatic | - 2.56% | - 0.44% |
| | Chemical | - 1.42% | - 0.14% |
| Tensile Index | Enzymatic | + 14.05% | + 10.15% |
| | Chemical | + 1.07% | + 6.81% |
| Tear Index | Enzymatic | - 9.64% | - 3.87% |
| | Chemical | - 0.07% | - 1.10% |
| Burst Index | Enzymatic | + 3.44% | + 3.76% |
| | Chemical | + 1.16% | + 2.97% |
| Freeness | Enzymatic | + 1.99% | + 2.90% |
| | Chemical | + 1.87% | + 2.28% |
| Residual Inks | Enzymatic | -44.52% | - 51.12% |
| | Chemical | -31.07% | - 49.78% |

*Notes:* Means with the symbol (+, -) are different (sample) in percentage relative to its blank.

# MARKET POTENTIAL (SIZE)

**Global market**

The application of enzymes in pulp and paper industries large : Capacity
Indonesia : 10 million tonnes per annum
Thailand : 4.5 million tonnes per annum

**10-20 kg enzyme per tonne**

**Malaysia**

Capacity : 1.0 million tonnes, Import : 1.2 million tonnes, Export :waste paper ; 50,000 tonnes (Year 2000), < 5 % recycled by chemicals
(Malaysia is working towards self sufficiency for papers, reduce import and encourage foreign capital inflow)

~ RM 4.5 billion

**2.2 million tonnes** of papers available for enzymatic deinking per year

**Environmental issues and sustainable development**

**Advancement of Industrial/Env Biotechnology and bioengineering**

Enzymatic processes cover large market potential

# INVESTMENT COMPARISON

## Enzymatic deinking

- Installation and setting up of fermentation unit : RM40,000.00
- Installation and setting of deinking system : RM 60,000.00
- Enzyme production cost (1000kg substrate) - 150 kg crude enzyme : RM7,200.00
  (With 150 kg enzymes, a total of **7.5 tonnes** of pulp can be deinked)
- Energy cost and maintenance : RM7,000.00
- Cost of Pulp and pulp processing (7.5 tonnes) : RM3,750.00

**Total cost: RM 117,950.00**

## Chemical deinking (for 7.5 tonnes pulp)

- Installation of flotation chemical system/facilities : RM120,000.00
- Cost of pulp and pulp processing : RM3,750.00
- Cost of chemicals/bleaching agents : RM13,500.00
- Processing of finished product : RM22,500.00
- Energy cost, operation and maintenance of system : RM18,500.00
- Effluent treatment of chemicals and facilities : RM30,000.00

**Total cost : RM 208,250.00**

**Operational cost: (for 7.5 tonnes pulp per first run)**

Enzymatic deinking : **RM 117,950.00**

Chemical deinking : **RM 208,250.00 ++++**

# INOCULUM DEVELOPMENT FOR ENVIRONMENTAL MANAGEMENT

- Bioremediation – hydrocarbon degradation
- Organic domestic waste decomposition
- Degradation of dyes from batik (textiles) efffluent

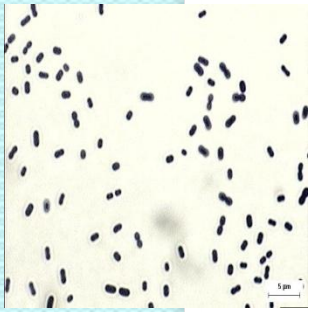
## PRODUCTION OF BIOPRODUCTS FROM WASTES

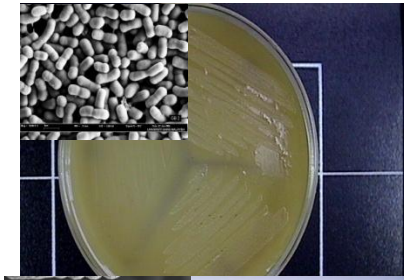
- Bioplastics
- Fermentable sugars (Ethanol)

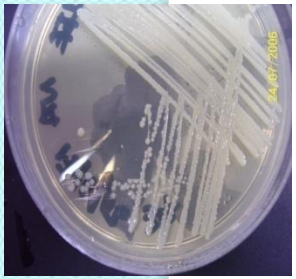
# Inoculum development for hydrocarbon bioremediation

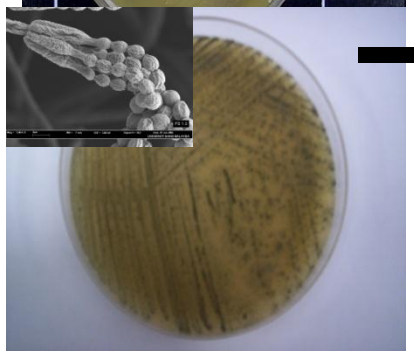

Isolation of hydrocarbon degrading microorganisms:
Oil contaminated soil/water (Penang, Kedah)
Soils at oil refineries (Melaka and Kerteh, Terengganu)

Potential isolates for consortia development



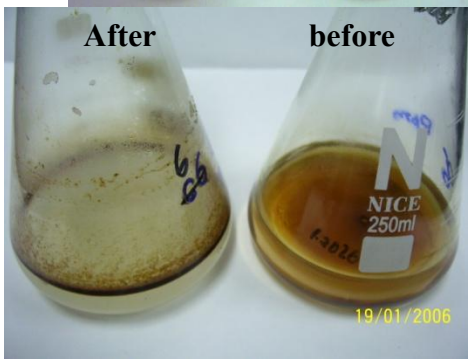




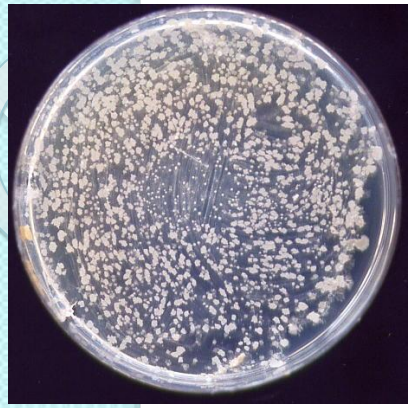


Slow degradation capability of inoculum preparation

- Improvement via enriching with N and P content of soils
- **BIOSURFACTANTS**

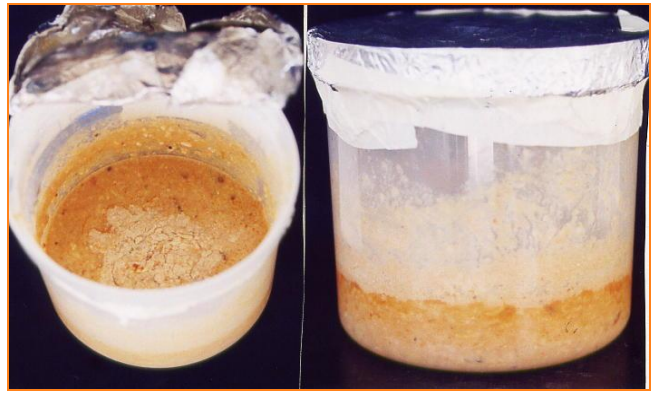


# Development of inoculum for domestic wastes decomposition



Microbial isolates

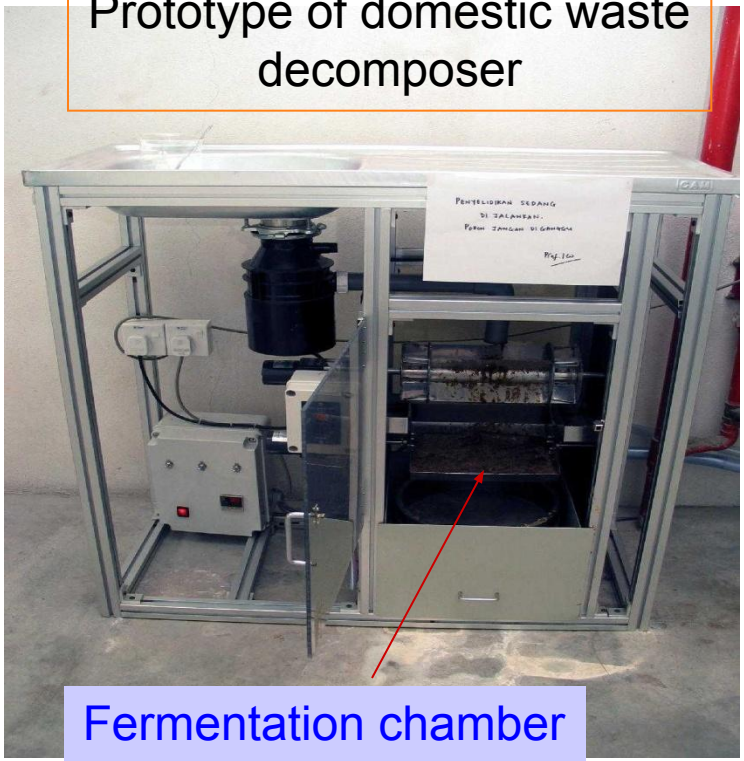
Inoculum in rice husk as binder



| Types of absorber | odour absorption (%) |
|---|---|
| AC1 | 86.6 |
| HP1 | 78.9 |
| HP2 | 70.6 |
| HP3 | 90.2 |
| HP4 | 92.8 |
| HP5 | 79.9 |
| Commercial activated carbon | 58.8 |

| Parameter for optimal decomposition | Conditions |
|---|---|
| Amount of domestic wastes | 120 g |
| Inoculum | 1 g in 10 ml of water |
| Temperature | 30°C |
| Frequency of agitation | Every 24 hr |
| Maximum decomposition time | 3 days |

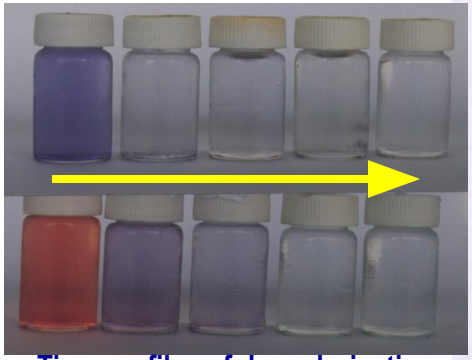
Prototype of domestic waste decomposer



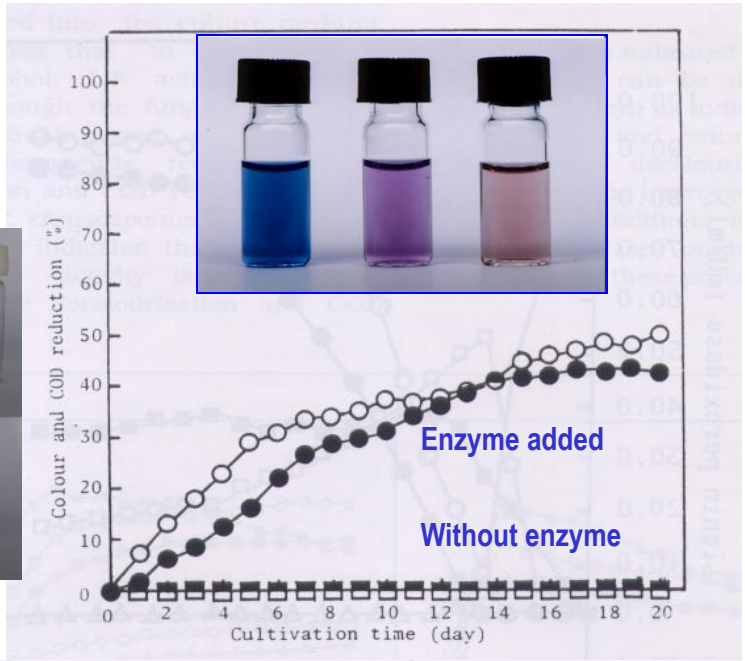
Fermentation chamber

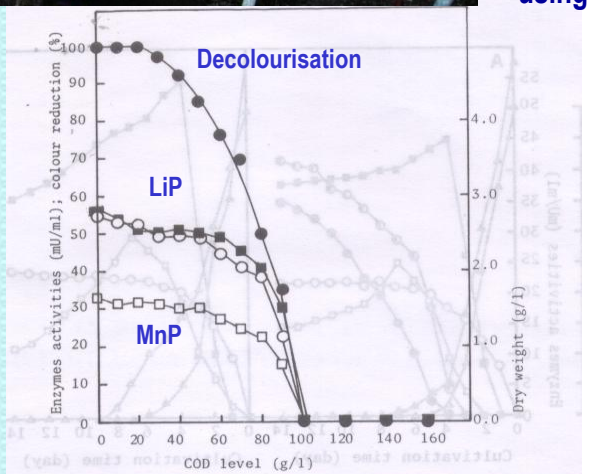
# Decolourisation of dyes from effluent of batik industries

**Enzymes involved**: Lignin peroxidase (LiP)
manganese peroxidase (MnP)
**Source**: ***Phanerochaete chrysosporium.***
**Waste water**: batik effluent

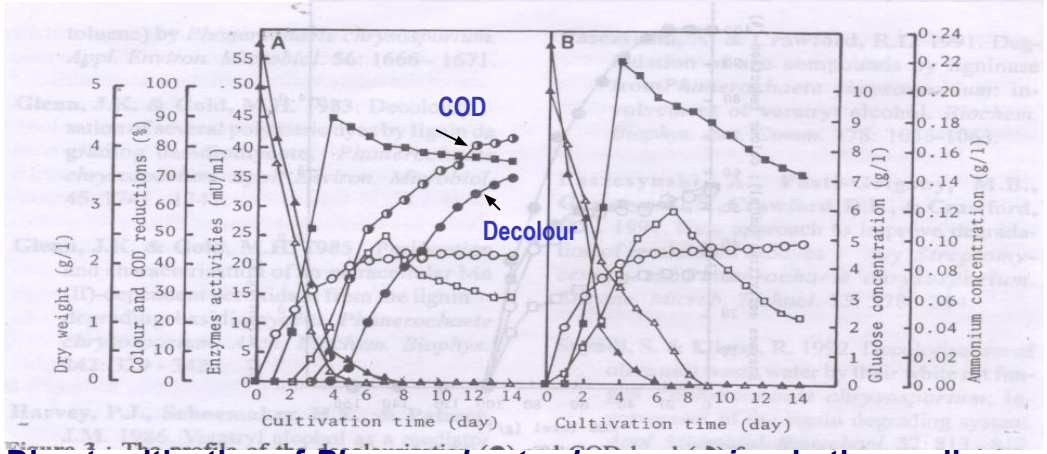


Time profiles of decoulorisation using lignin degrading enzymes




Decolourisation of wastes with the addition of enzyme preparations of LiP and MnP




Direct cultivation of *Phanerochaete chrysosporium* in the medium containing waste water
A. In the presence of waste water, B: absence of waste water.

# Physical Method

## Decolourisation process by biological method

Pump system

Wastewater from textile factories

I: Tubular filtration/absorber system


SSF of absorber materials

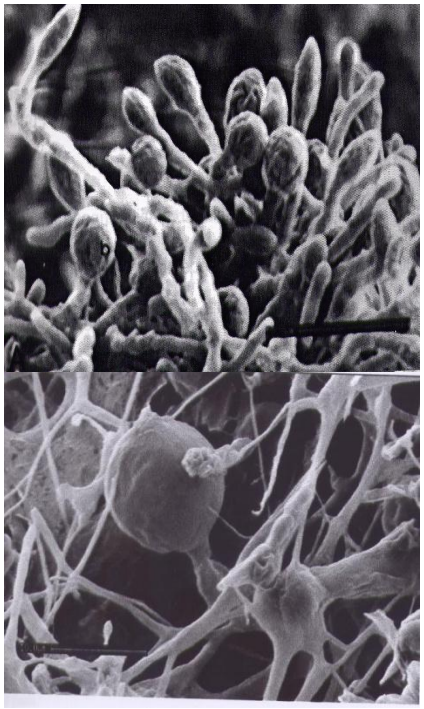
Filtration system

Wastewater tank + Fungal growth medium

II : Continuous loop bioreactor system (Pneumatic type)

Aeration

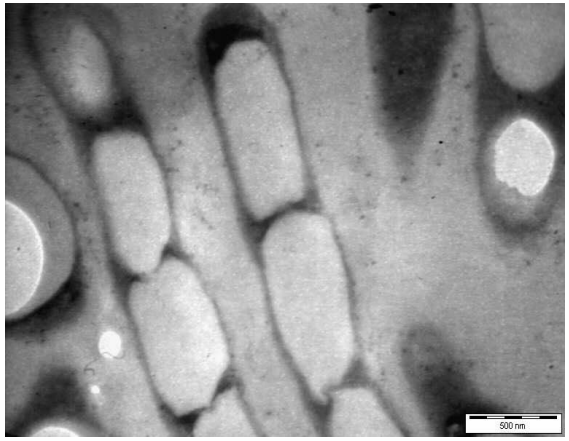
Release of water to river/recycle



Dye degrading fungi

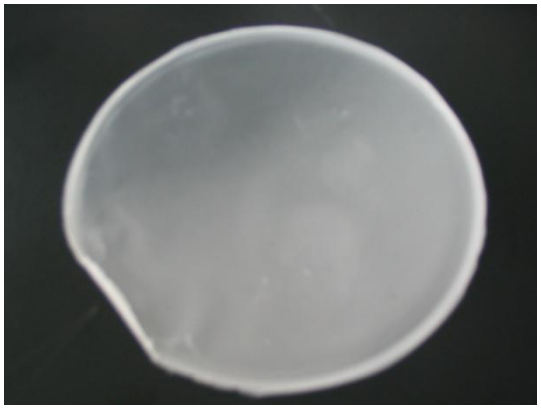
Oils

Fermentation



PHA in bacterial cells

Extraction



Fabrication

Biodegradable bottles

$CO_2$ and $H_2O$

Renewable plant resources (palm oil)

**Ecologically sustainable 'green' processes**

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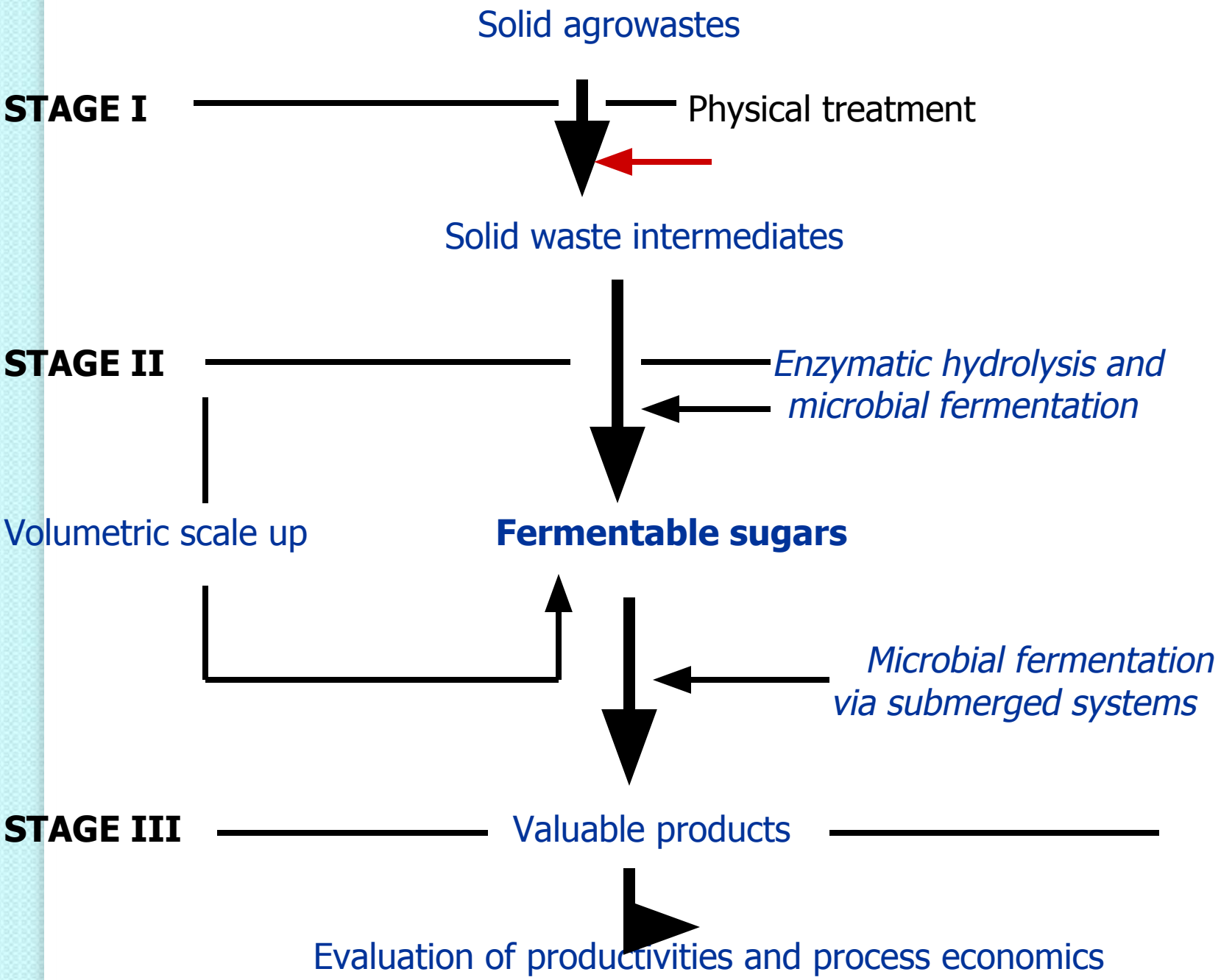
# Biodegradation Test (30 days)


Solid agrowastes
STAGE I
Physical treatment
Solid waste intermediates
STAGE II
Enzymatic hydrolysis and microbial fermentation
Volumetric scale up
Fermentable sugars
Microbial fermentation via submerged systems
STAGE III
Valuable products
Evaluation of productivities and process economics

# Valuable products from microorganisms *via* submerged fermentation processes from fermentable sugars

Microbial fermentation
using single culture of *Aspergillus niger*
USM AI 1

| Agricultural wastes | Maximum amount of fermentable sugars (mg/g substrate) |
|---|---|
| Paddy husk | 0.433 |
| Coconut fibres | 2.129 |
| Wood dust | 0.049 |
| Coconut meal | 0.586 |
| Palm kernel cake | 0.361 |
| Sugar cane baggase | 0.441 |
| Tapioca meal | 57.016 |
| Oil palm trunk | 6.503 |
| Oil palm frond | 29.677 |

**Enzyme production**

- Lipase (*Mucor* sp, *Pseudomonas* sp)
- Xylanase (*Aspergillus* sp.)
- Cellulase (*Aspergillus* sp.)
- Protease (*Bacillus* sp.)
- Wide applications

**Yeast biomass**

- *Candida utilis*
- *Saccharomyces cerevisiae*
- *Xanthophyllomyces dendrorhous*
- 30°C, aerobic
- Applications in aquaculture industries as feeds

**Ethanol fermentation (Biofuel/Bioenergy)**

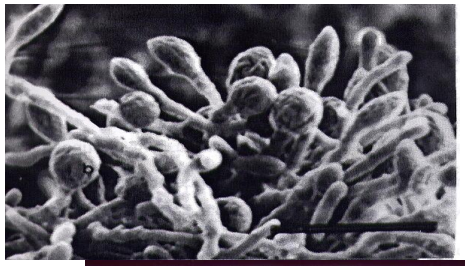
- *Saccharomyces cerevisiae*
- 30°C, optimized medium
- 20%(v/v) ethanol concentration
- Membrane system for separation of solvent
- Evaluation as energy source

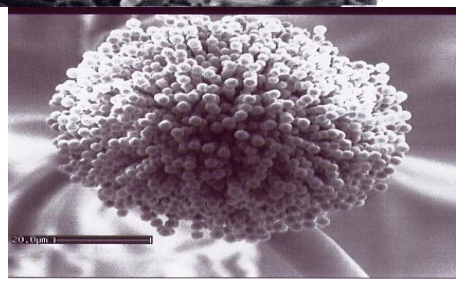
# Ethanol production as fuel

## Agrowastes

Delignification by fungi
Solid state fermentation (SSF)

*Phanerochaete chrysosporium*
*Aspergillus niger*
*Aspergillus* sp.



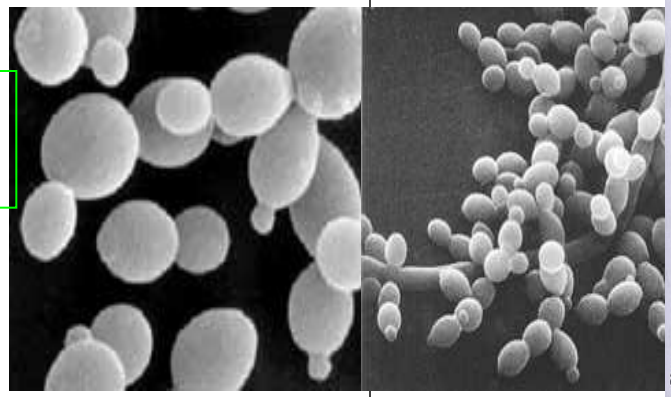


## Fermentable sugars

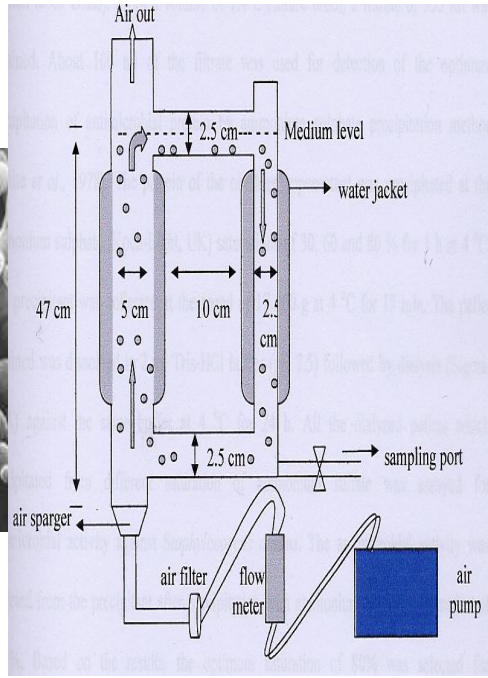
Optimization of SSF

## Yeast fermentation

*Saccharomyces cerevisiae*



Optimization of ethanol fermentation




## Ethanol fermentation

Development of separation system

## Separation/concentration/distillation

**ETHANOL**
**10-12% (v/v)**

# Conclusion

- Environmental Issues : Global problem
- Multidisciplinary strategies : Biotechnology, Industrial Chemistry, bio-engineering, environmental engineering, bioremediation, biosorption, microbial degradation of wastes
- Development of innovative bio-technologies for environmental management
- Industrial applications for large scale waste management
- Future direction : Biological approaches supported by innovative technologies and engineering
- Sustainable development with minimum impact on environment by wastes

# Thank you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