



UNIVERSITI
MALAYSIA
KELANTAN

Environmental-Friendly Bio-technologies for Sustainable Agrowaste Management

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Agricultural wastes – global problem

↓
Environmental impact

↓
Recycling of wastes

↓
Disadvantages

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

↓
Physical, Chemical, Biological methods
Value added for Economic development

3R
concept
is a
must

Concept of
sustainable
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



**Malaysian golden
crop**



**Dried palm
kernel cake**



**Empty fruit
bunches**



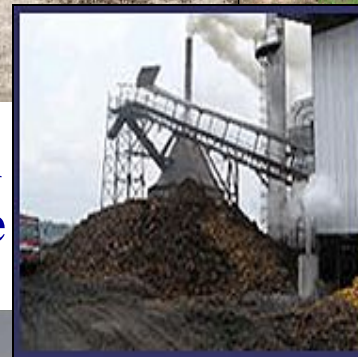
Oil palm shells



**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



Environmental impact



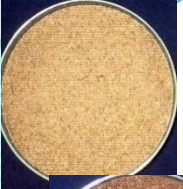
Commercializable and high value products/processes in recycling of waste materials *via* biotechnological approach

Solid State Fermentation (SSF)

Potential fungal isolates

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

SCB



RH



RW



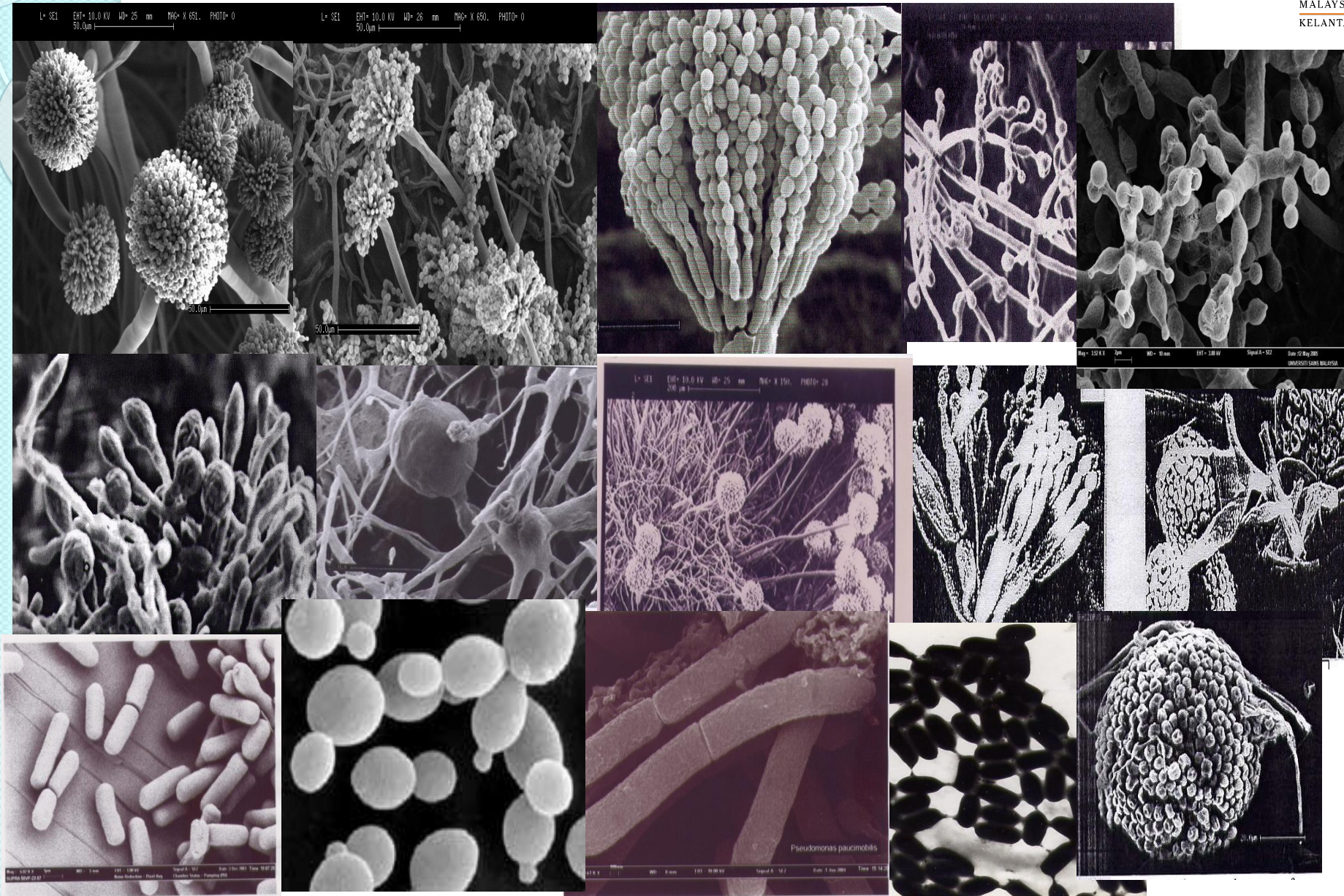
PKC



Why SSF?

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

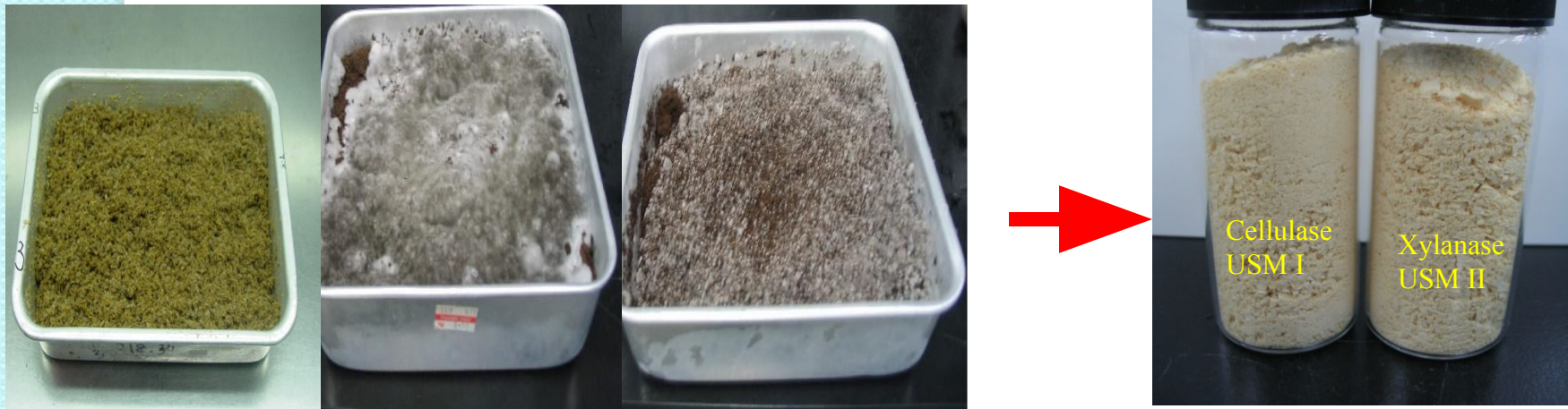
Potential microorganisms with good growth on agrowastes



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

AGRICULTURAL WASTES BIOMANAGEMENT

SSF/semi solid fermentation

Fermentable sugars

Submerged fermentation

Chemicals

SSF/enzymatic/physical

Animal feed formulation

Poultry

Submerged fermentation

Bioplastics

Aquaculture industries

Submerged fermentation

Yeast biomass

Yeast extract

SSF

Bioremediation

Nitrogenous compounds/SSF

Composting, biofertilizers, antibiosis

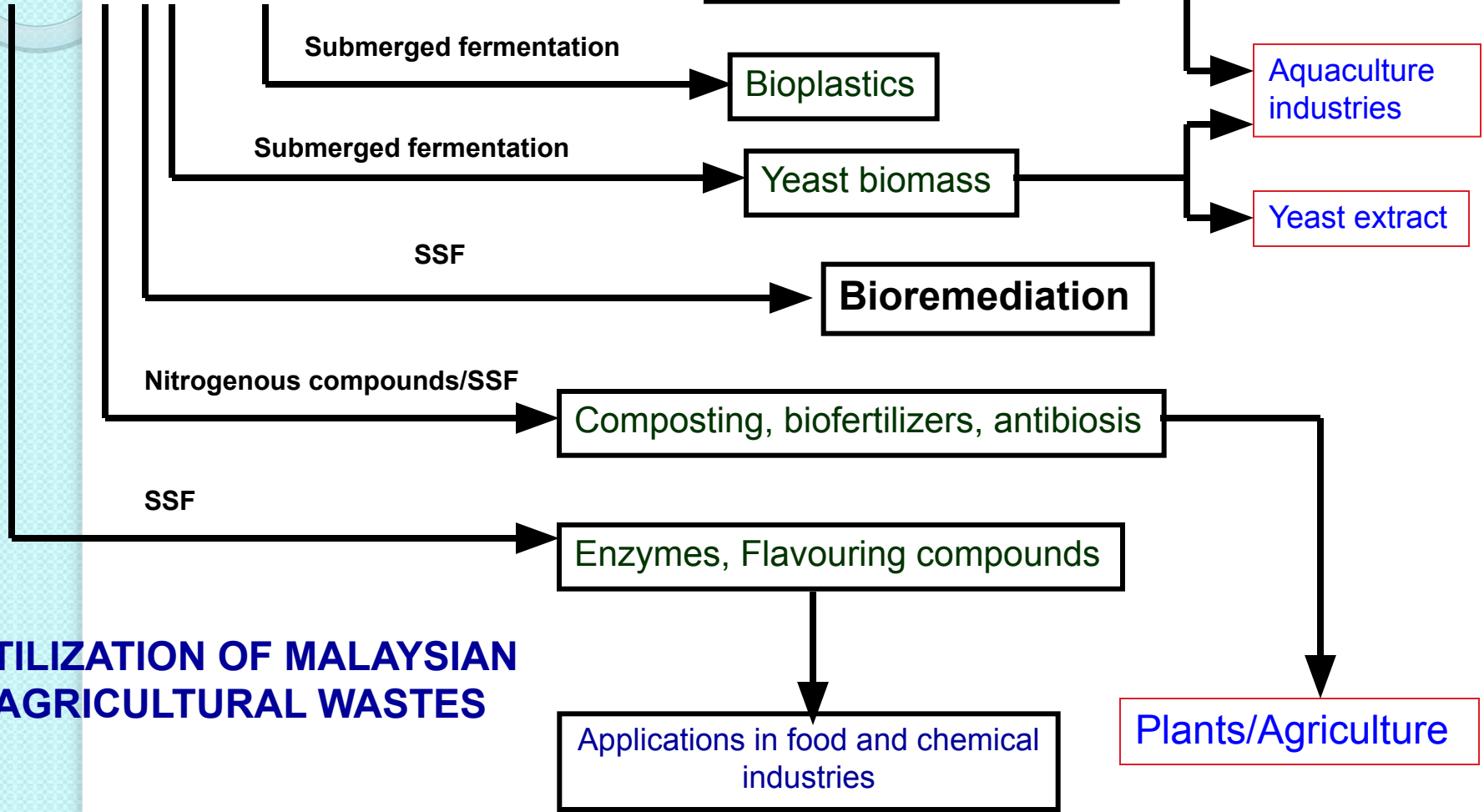
SSF

Enzymes, Flavouring compounds

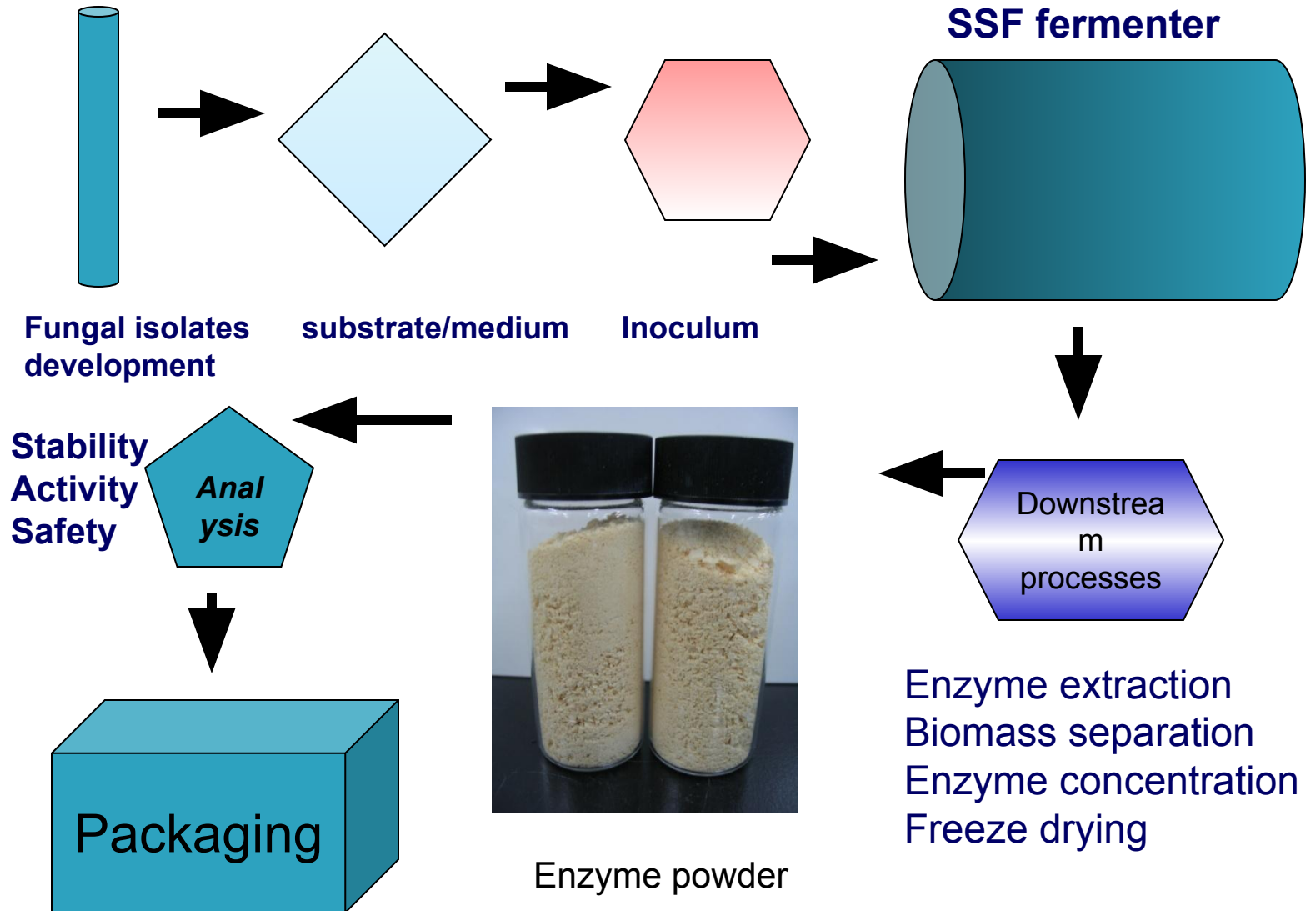
Applications in food and chemical industries

Plants/Agriculture

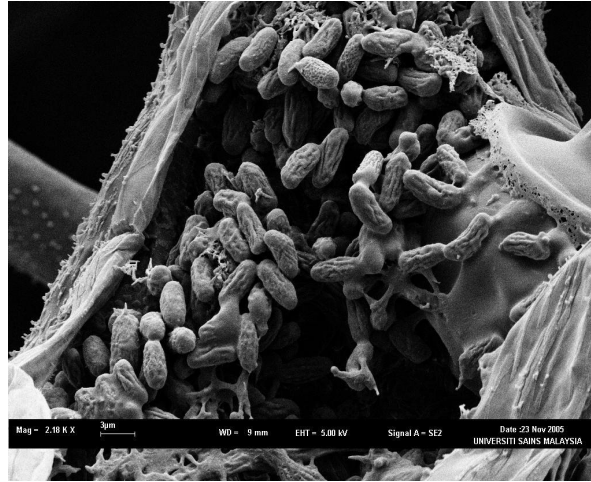
UTILIZATION OF MALAYSIAN AGRICULTURAL WASTES



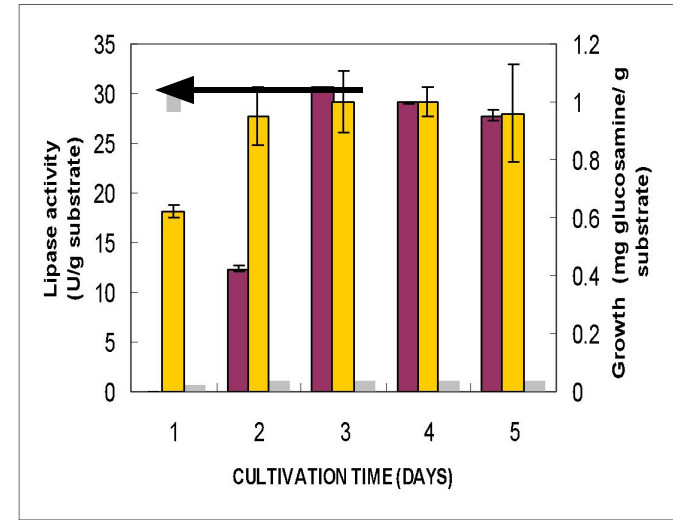
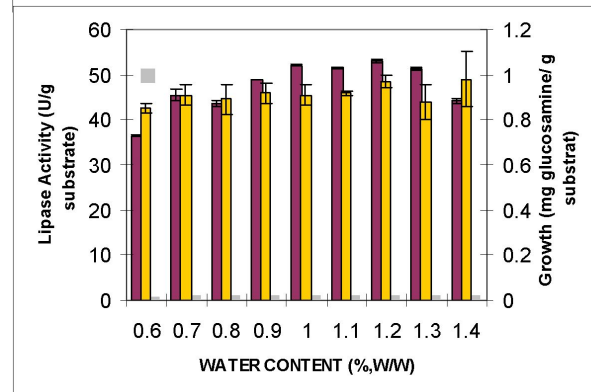
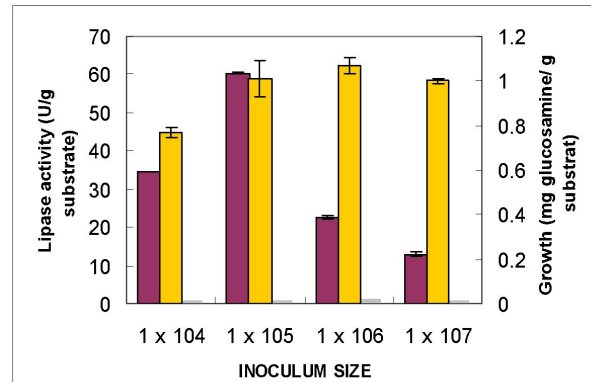
Enzyme production *via* SSF



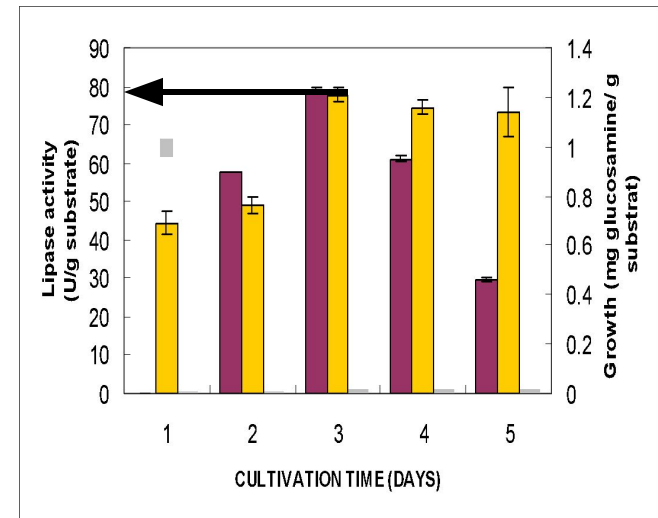
Lipase production by *Mucor miehei* by solid state fermentation



Growth of *M. miehei* in flask system

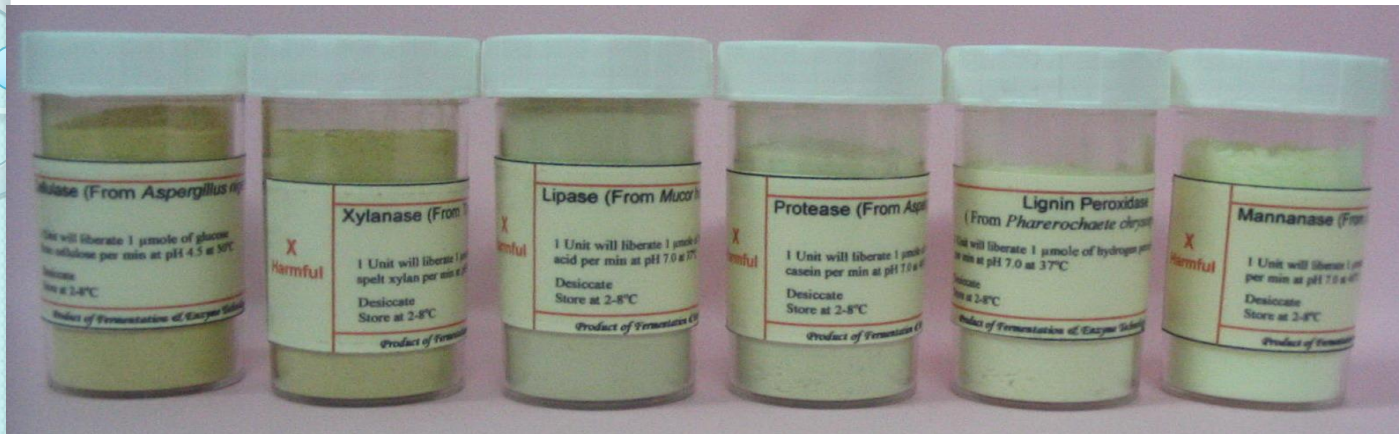


Before optimization : 10 U/g/day

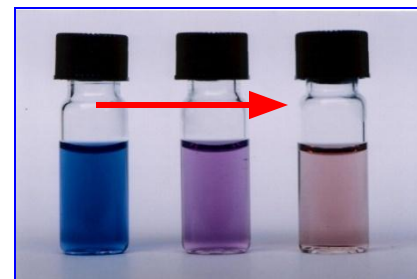


After optimization : 27 U/g/day

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 recycling, 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®

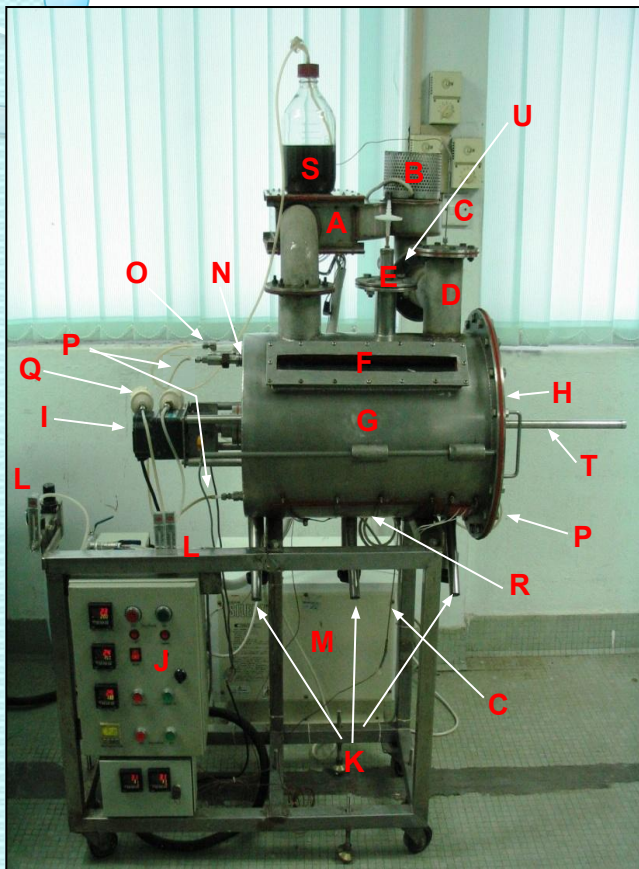


Plate 1: FERMSOSTAT with complete fermentation system.

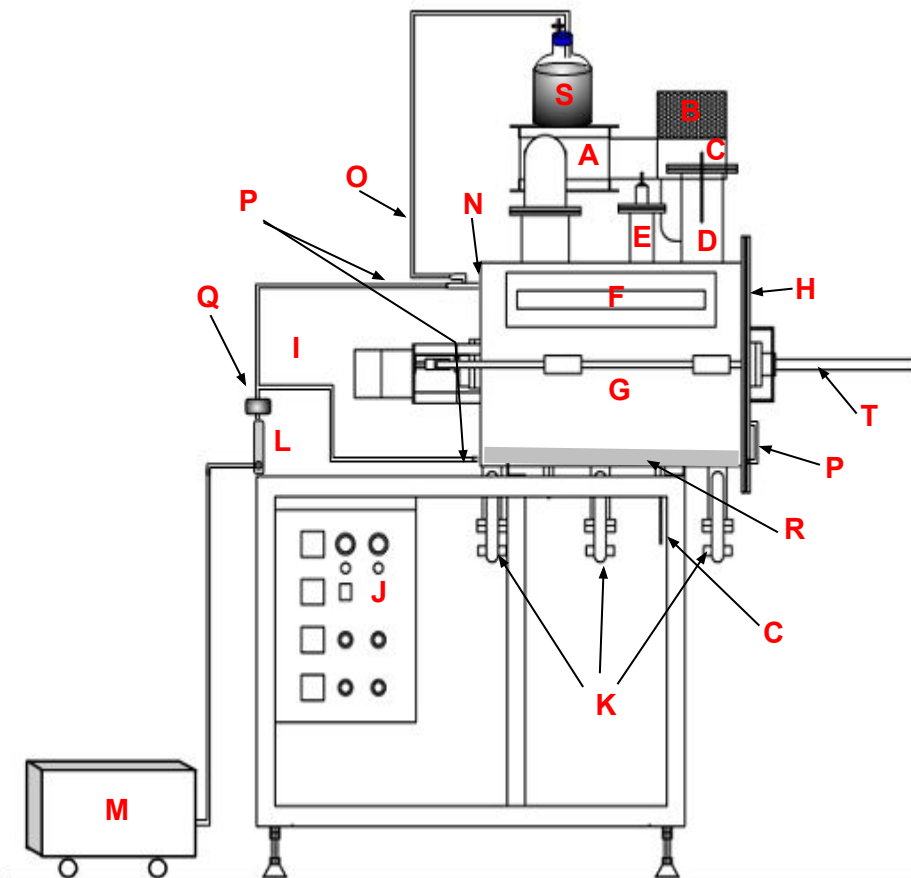
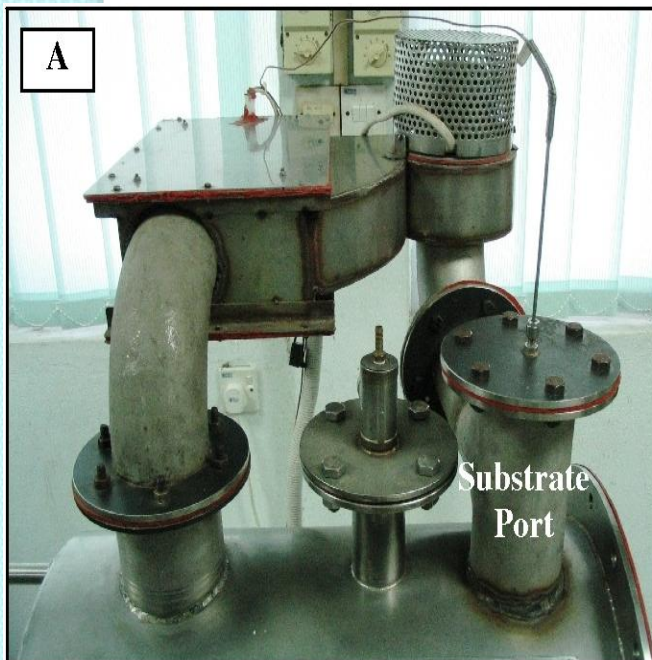
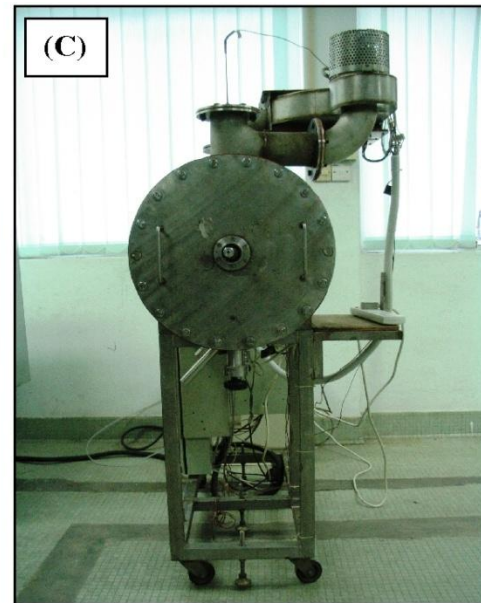
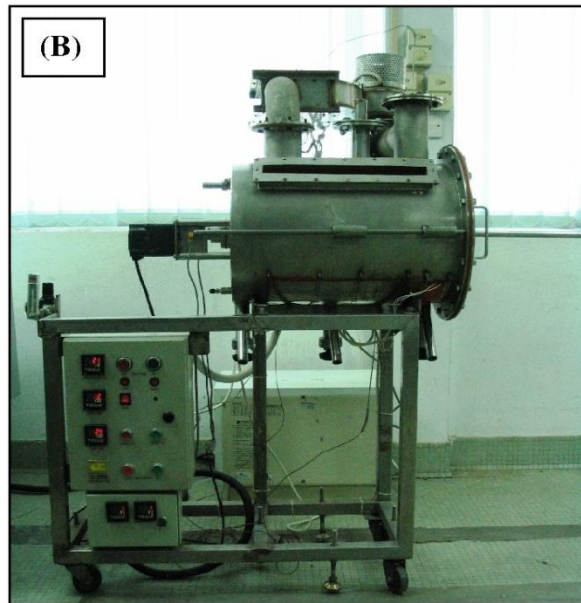
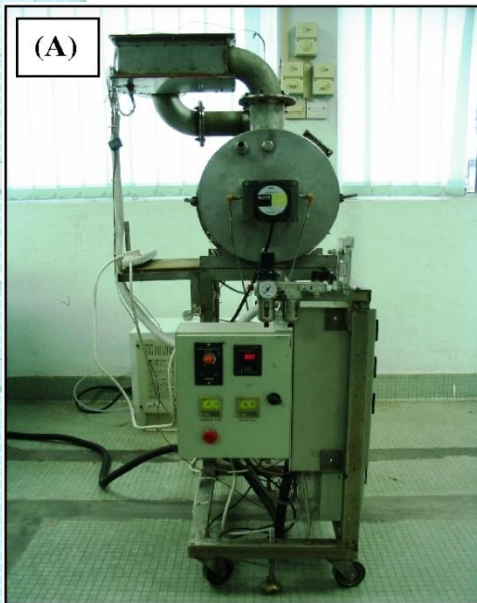
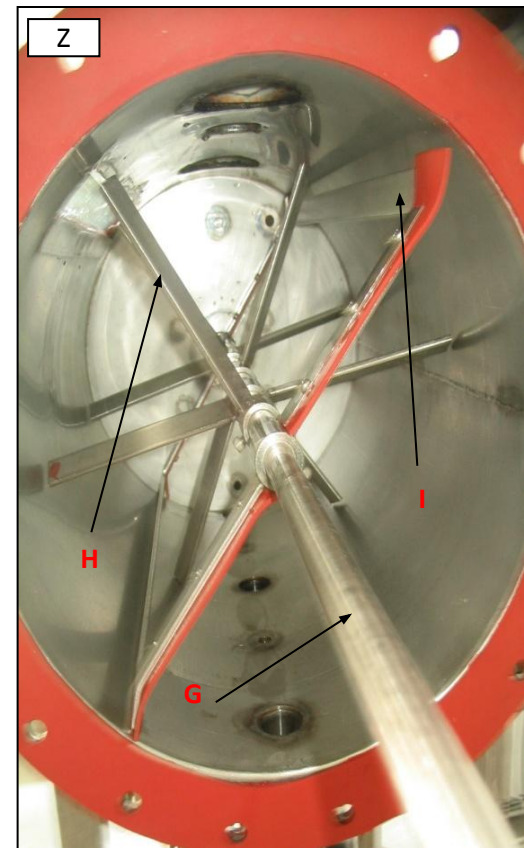


Figure 1: Schematic diagram of FERMSOSTAT with complete fermentation system.

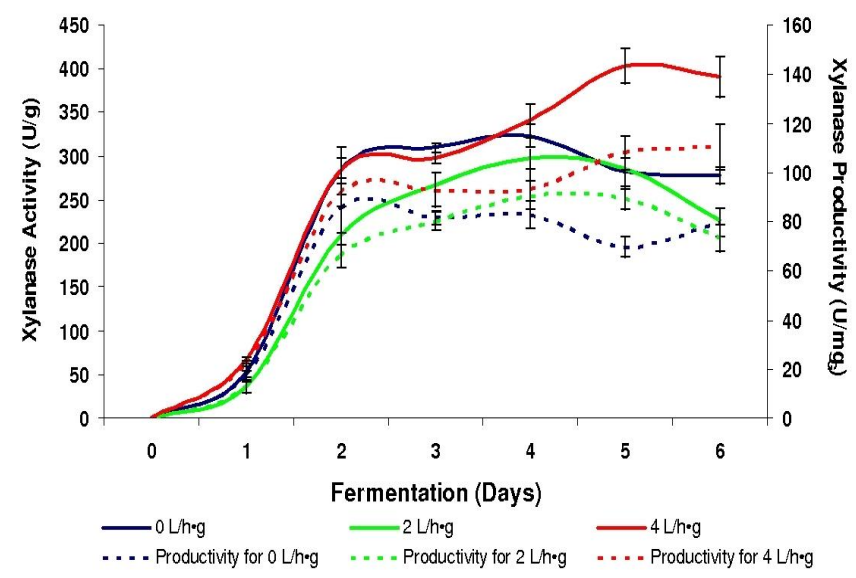
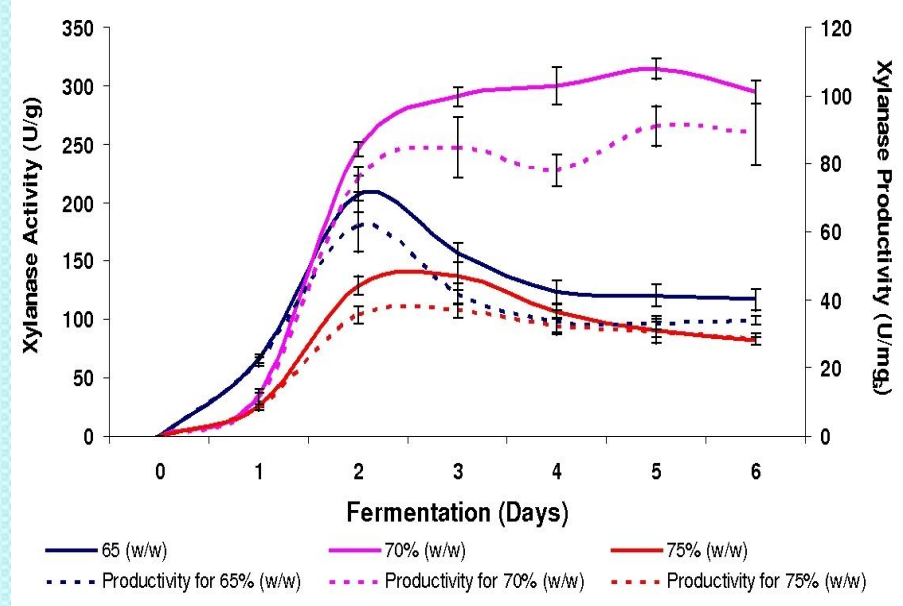
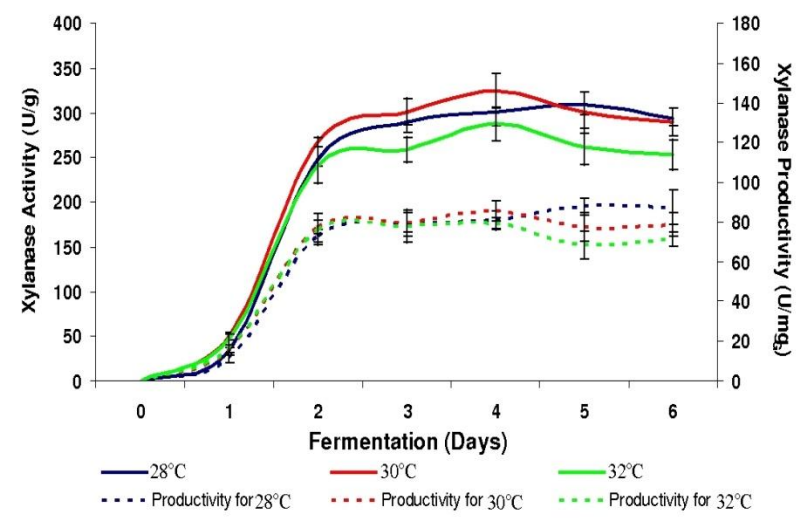
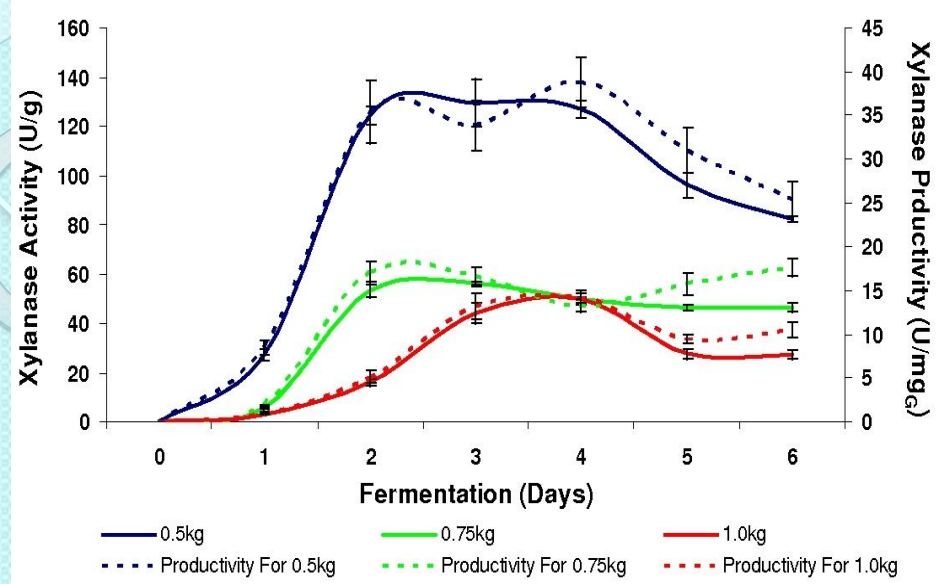


**Substrate port (A) and
Sampling port (B) provided
in FERMSOSTAT®.**



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®

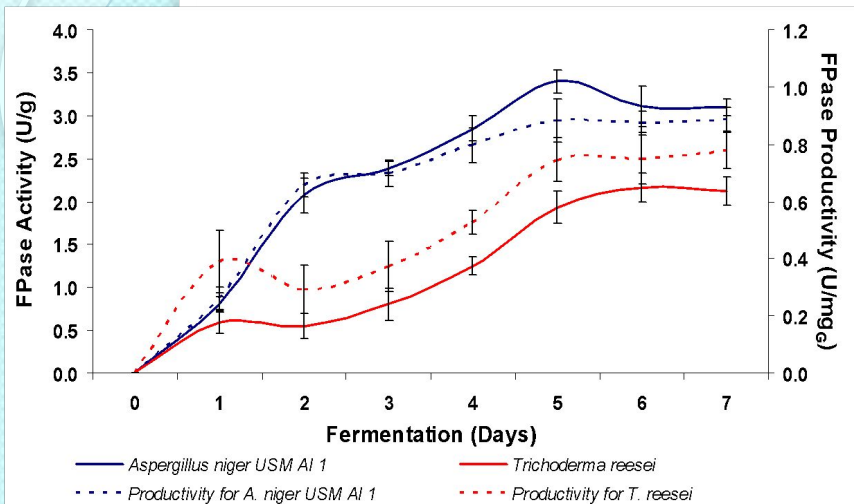


Figure 3.25: Production of FPase enzyme by *A. niger* USM AI 1 and *T. reesei* under optimized fermentation conditions.

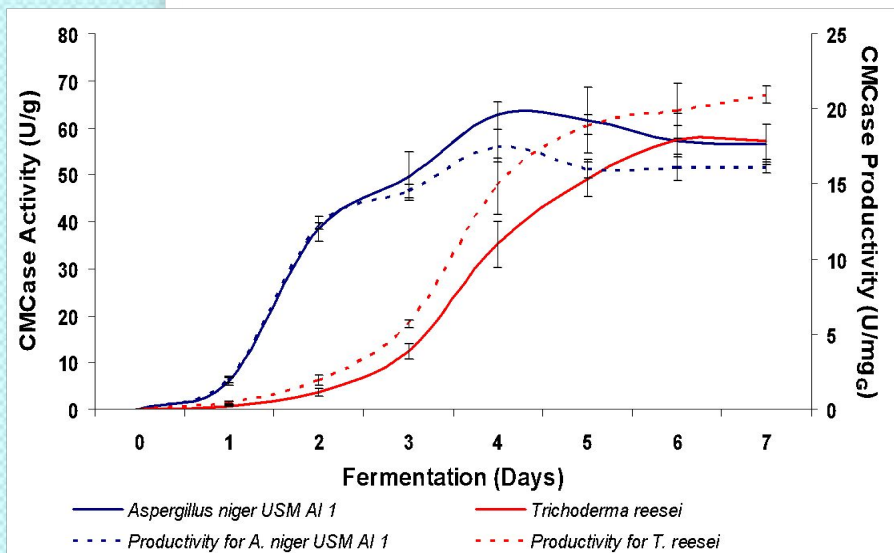


Figure 3.23: Production of CMCase enzyme by *A. niger* USM AI 1 and *T. reesei* under optimized fermentation conditions.

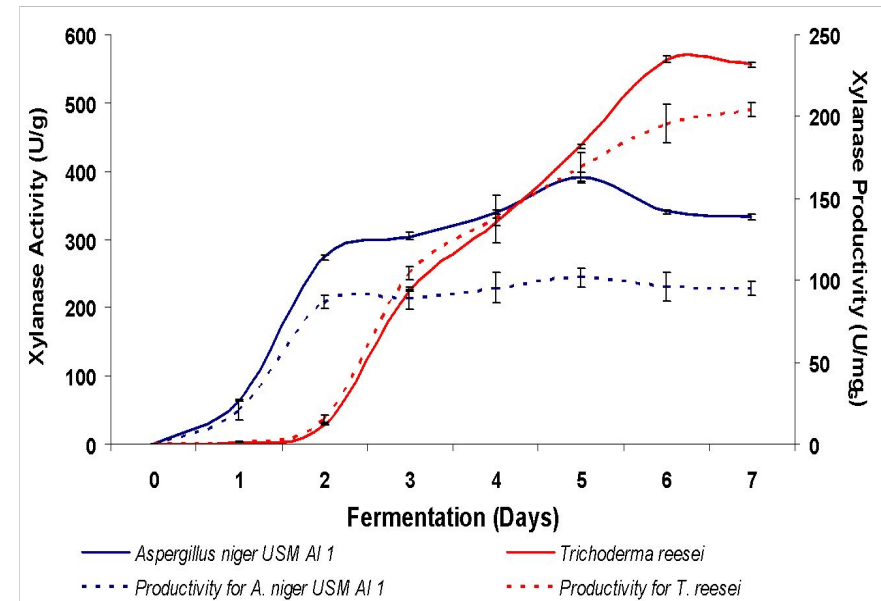


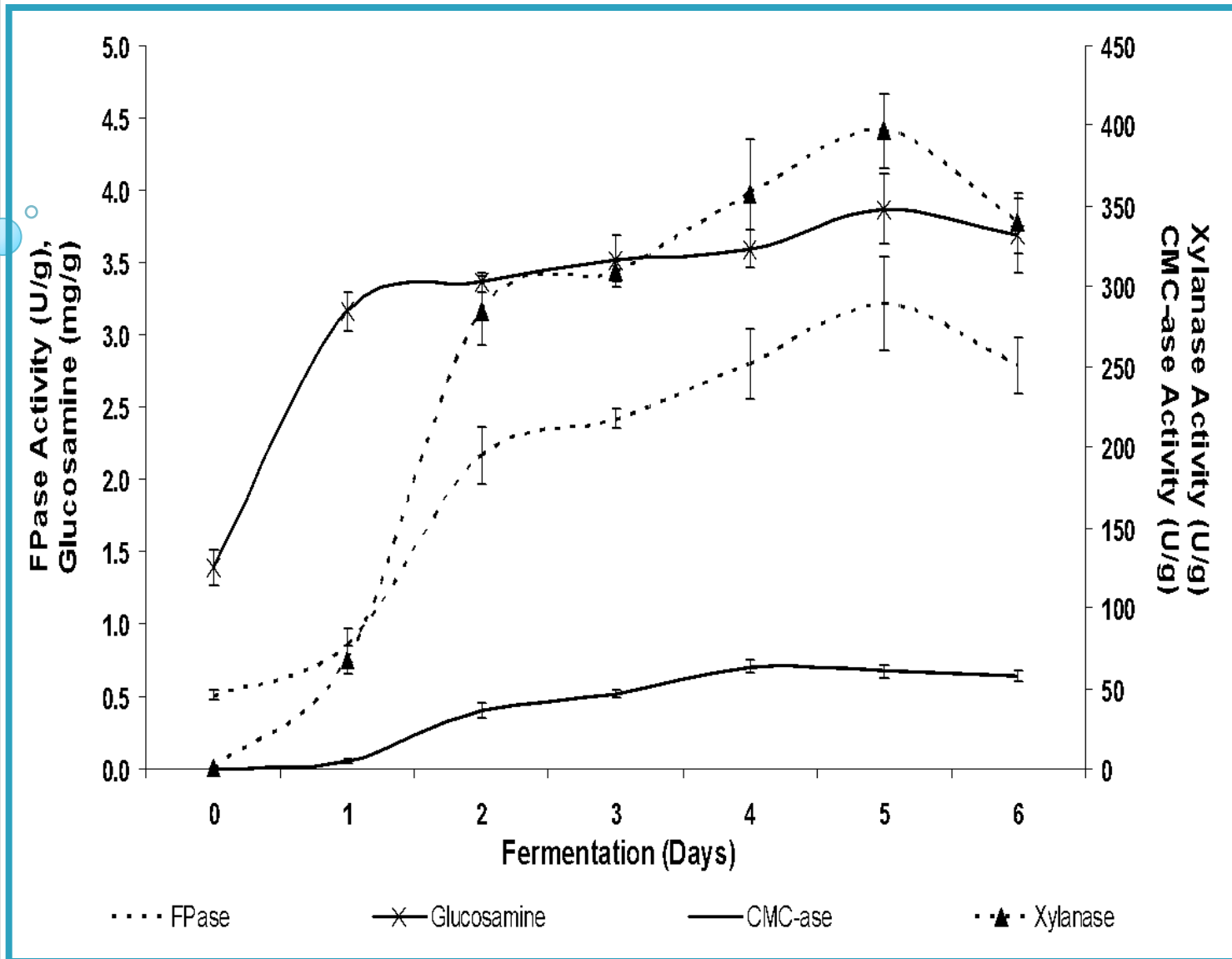
Figure 3.24: Production of xylanase enzyme by *A. niger* USM AI 1 and *T. reesei* under optimized fermentation conditions.

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	30°C	
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



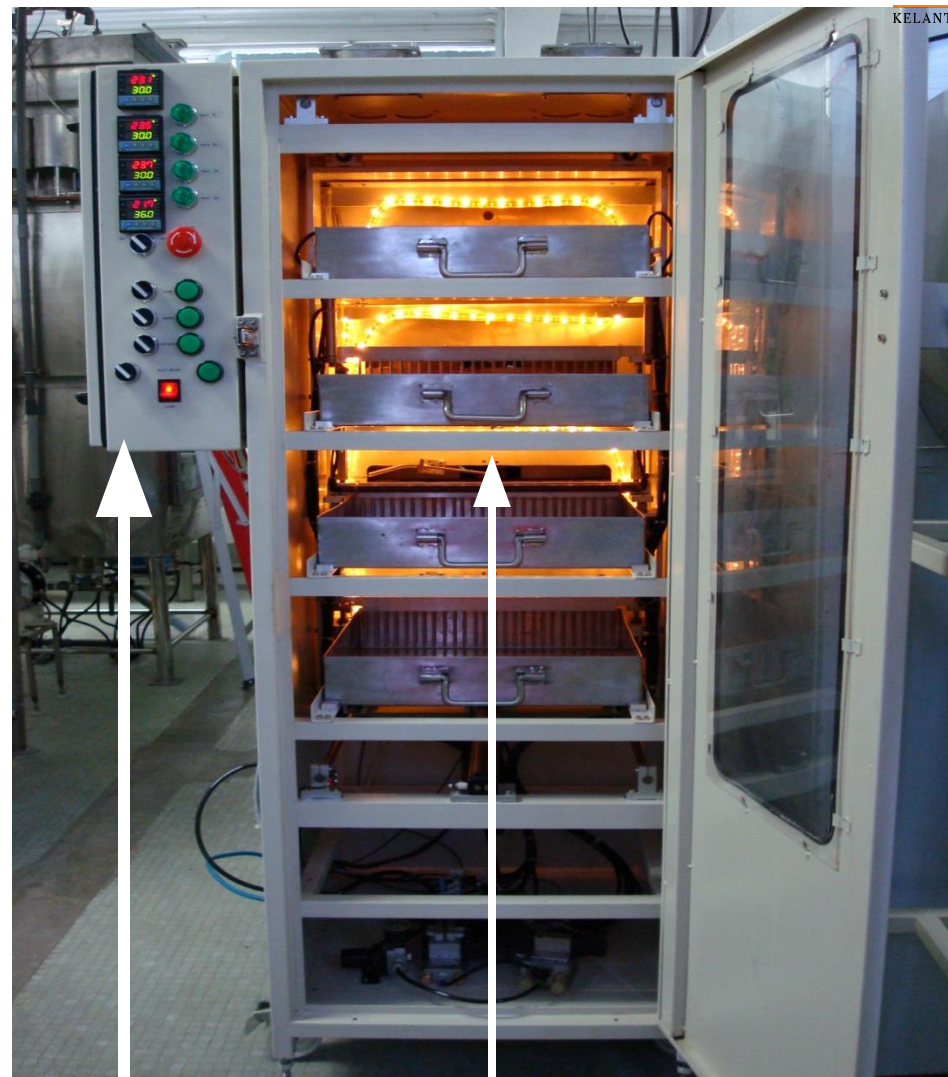


Profiles of batch production of enzymes using the FERMSOSTAT®

SSF INTELLIGENT FERMENTER



Water/inoculum storage



Control panel

Tray system

Optimization of enzymes production via solid state fermentation using PKC

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

Application of lipase for esterification reaction of acetone glycerol acyl esters

1,2-O-isopropylidene glycerol + fatty acids

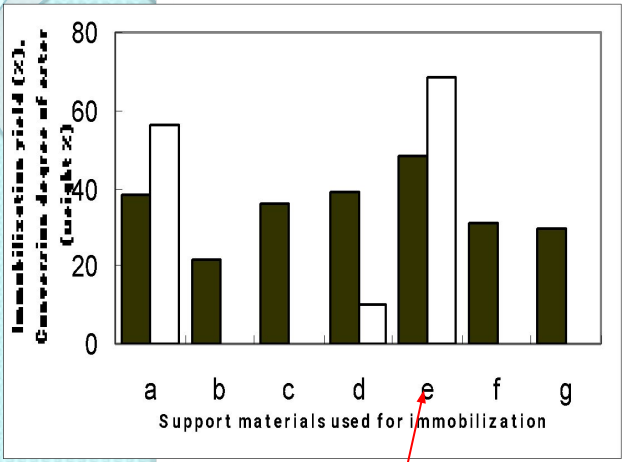


1,2-O-isopropylidene acyl glycerol + water

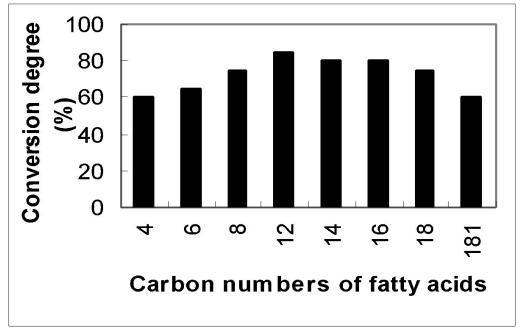


Mild hydrolysis

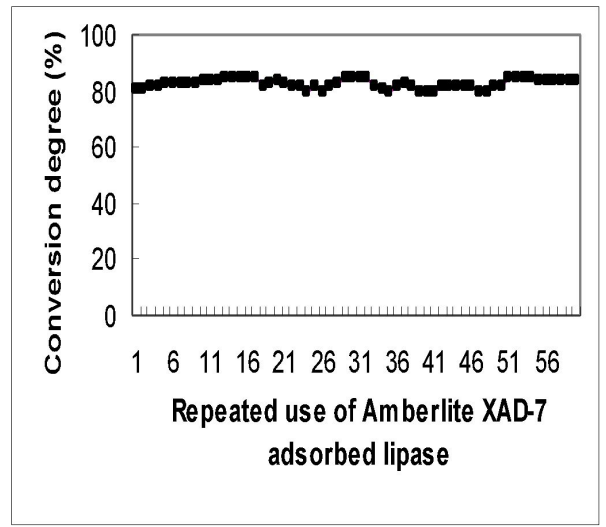
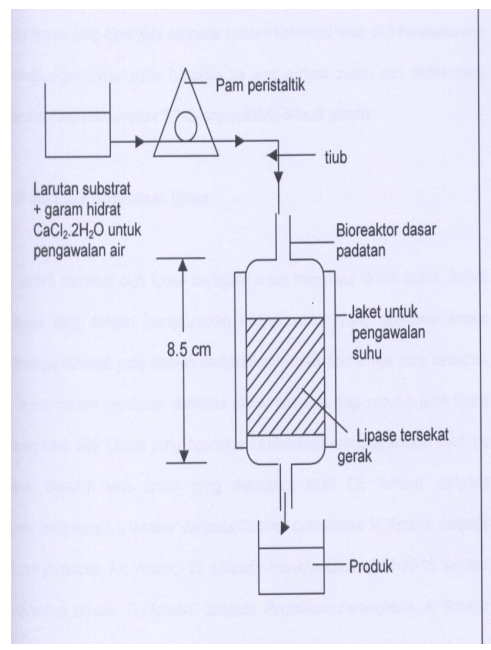
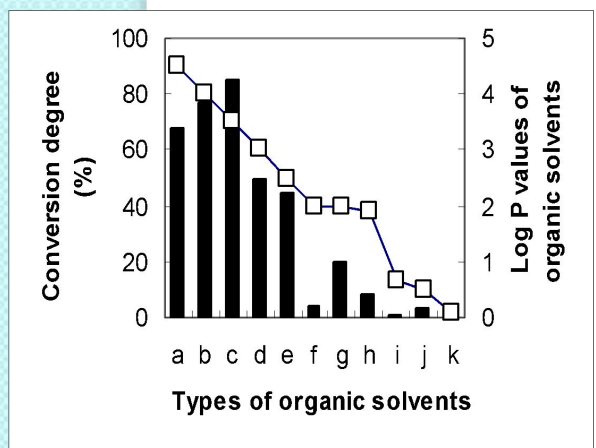
monoacylglycerol + acetone



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)

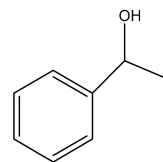
The use of SSF materials as enzyme source in organic synthesis



2-Cl-cyclohexanol
2-Cl-cycloacetate



2-I-cyclohexanol
2-I-cycloacetate

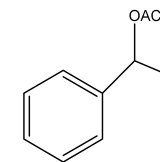


Phenylethanol

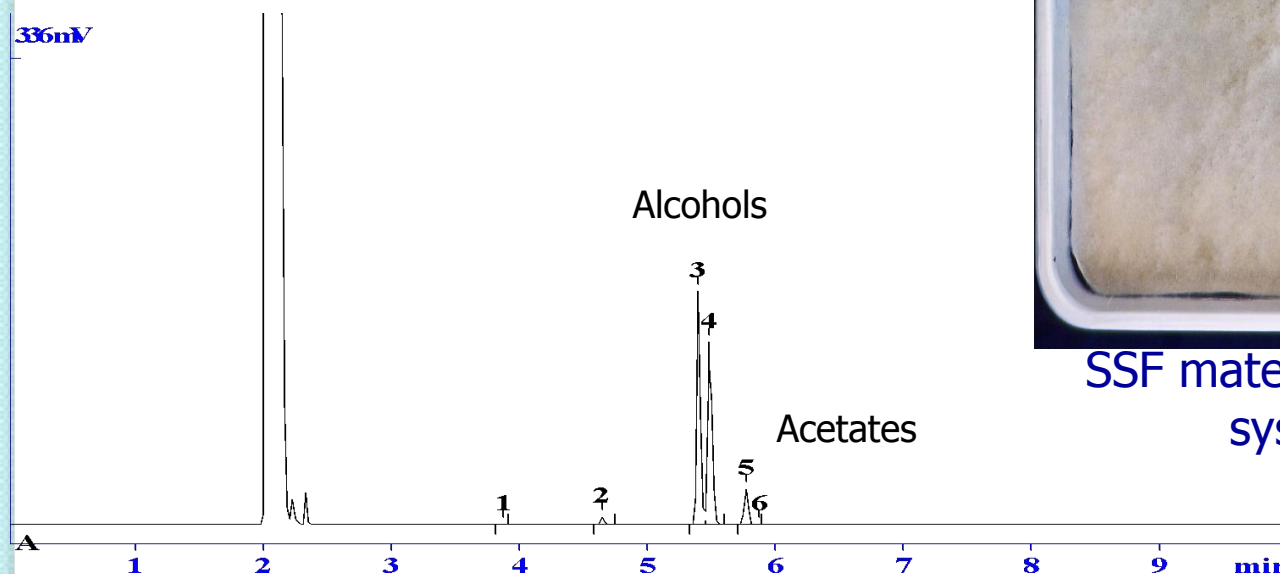
0.5 ml vinyl acetate
1.0 ml hexane
0.5 ml tetrahydrofuran
20 mg SSF materials



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)-1a ee ^b (%)	(S)-2a ee ^b (%)	E ^c
<i>Mucor hiemalis</i> NRRL 13.009	Palm kernel cake (PKC)	50	-	45	120	12	87	9	16
<i>M. hiemalis</i> NRRL 13.009	PKC	50	Olive oil	17	120	7	9	5	20
<i>M. hiemalis</i> NRRL 13.009	PKC	60	-	26	120	8	80	5	10
<i>M. hiemalis</i> NRRL 13.009	PKC	60	Olive oil	21	120	8	92	6	26
<i>M. hiemalis</i> NRRL 13.009	Coconut waste	67	-	8	120	12	85	10	14
<i>M. hiemalis</i> NRRL 13.009	Coconut waste	67	Olive oil	17	120	7	58	3	4
<i>M. hiemalis</i> NRRL 13.009	Coconut waste	75	-	15	120	15	84	12	13
<i>M. hiemalis</i> 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 m³ 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.



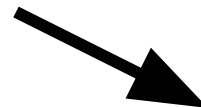
> 1.0 million tons per annum (19 paper manufacturing companies)
Paper import: 1,189,120 metric tonnes per year (RM2.7 billion)



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

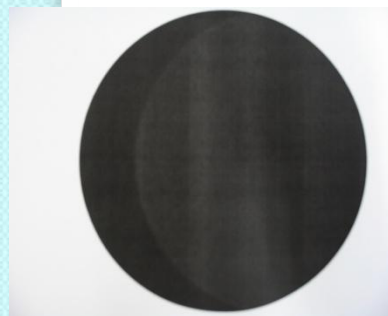
SPECIFIC OBJECTIVES ON ENZYMATIC DEINKING SYSTEM

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

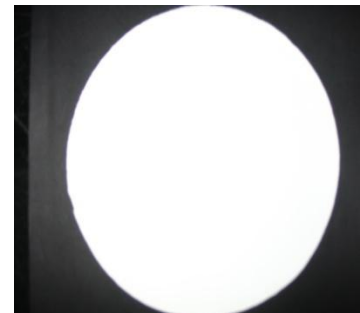


Before

Enzymatic deinking



Flotation



After

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

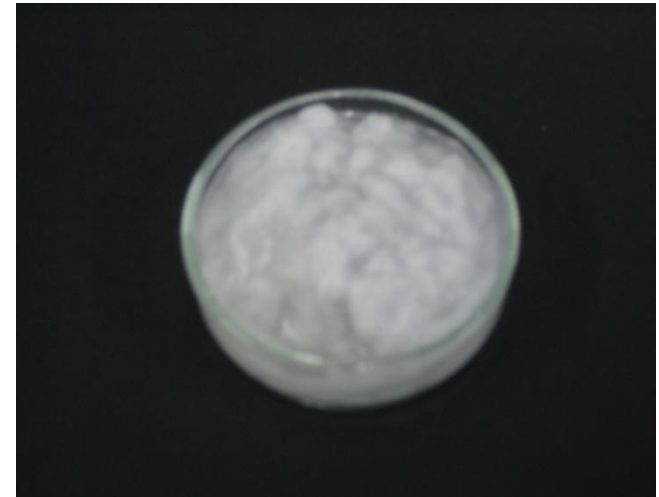
DEINKING OF PULP – PAPER RECYCLING



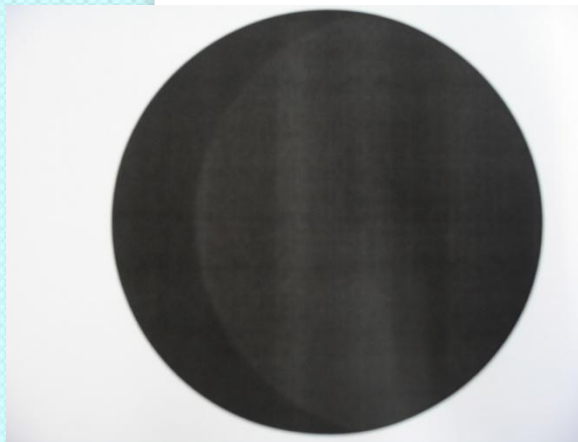
BEFORE



**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

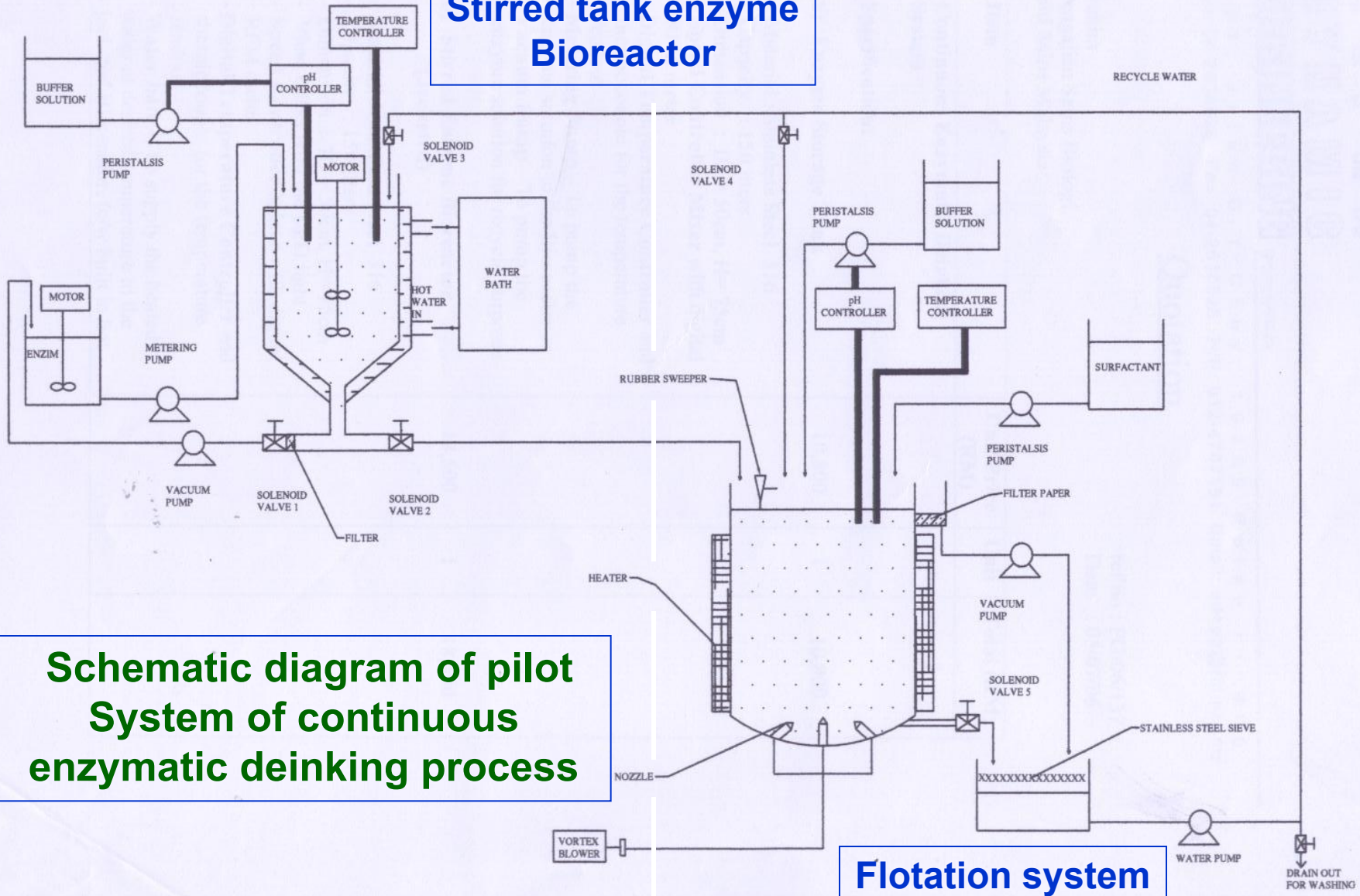
Parameter optimized	Optimized conditions
Surfactant concentration (% w/w dried pulp)	Tween 80, 0.5
Temperature (°C)	45
pH	6
Working air flow rate (L/min)	10
Flotation time (min)	15
Deinking efficiency (%)	95

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 sieve. 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

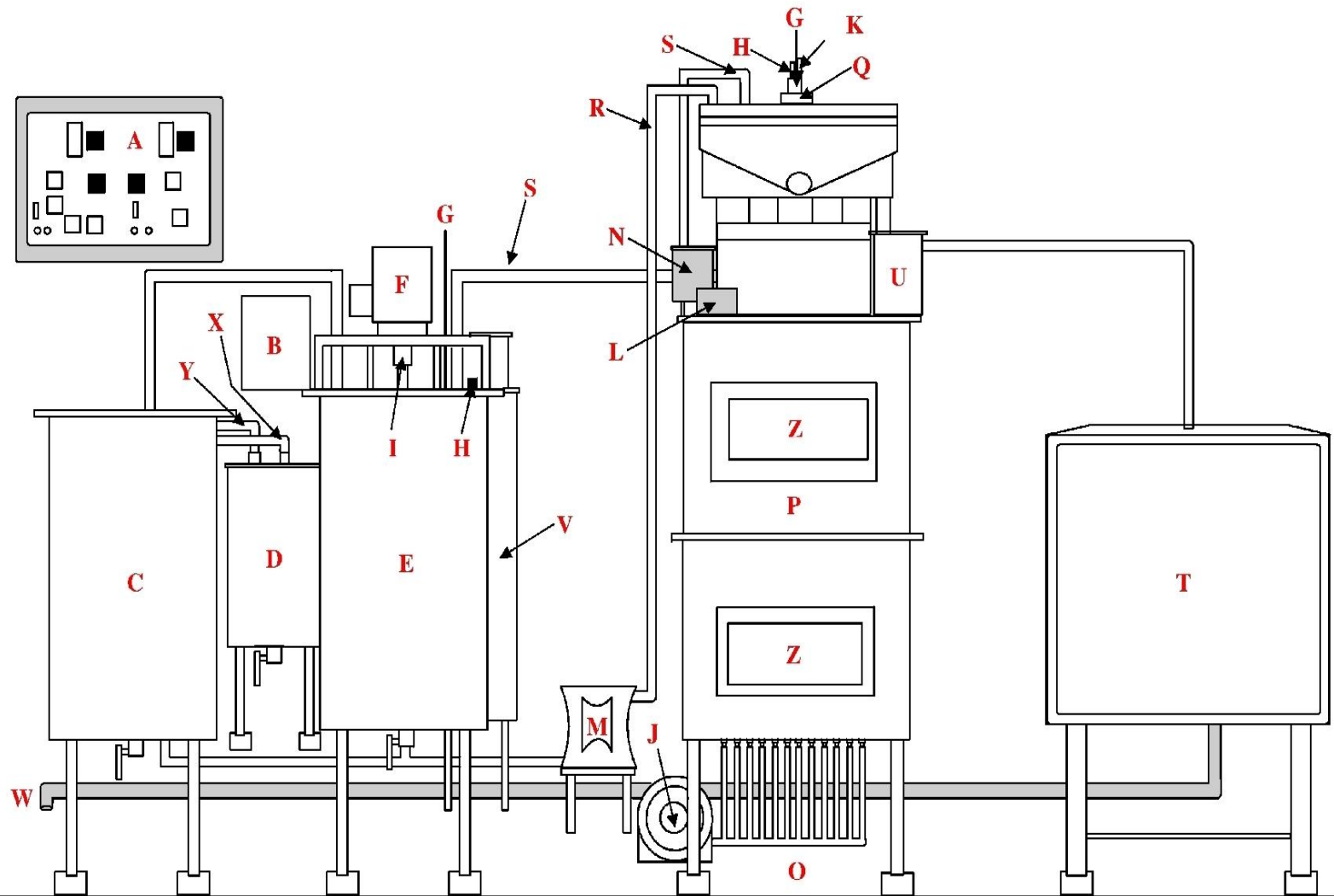
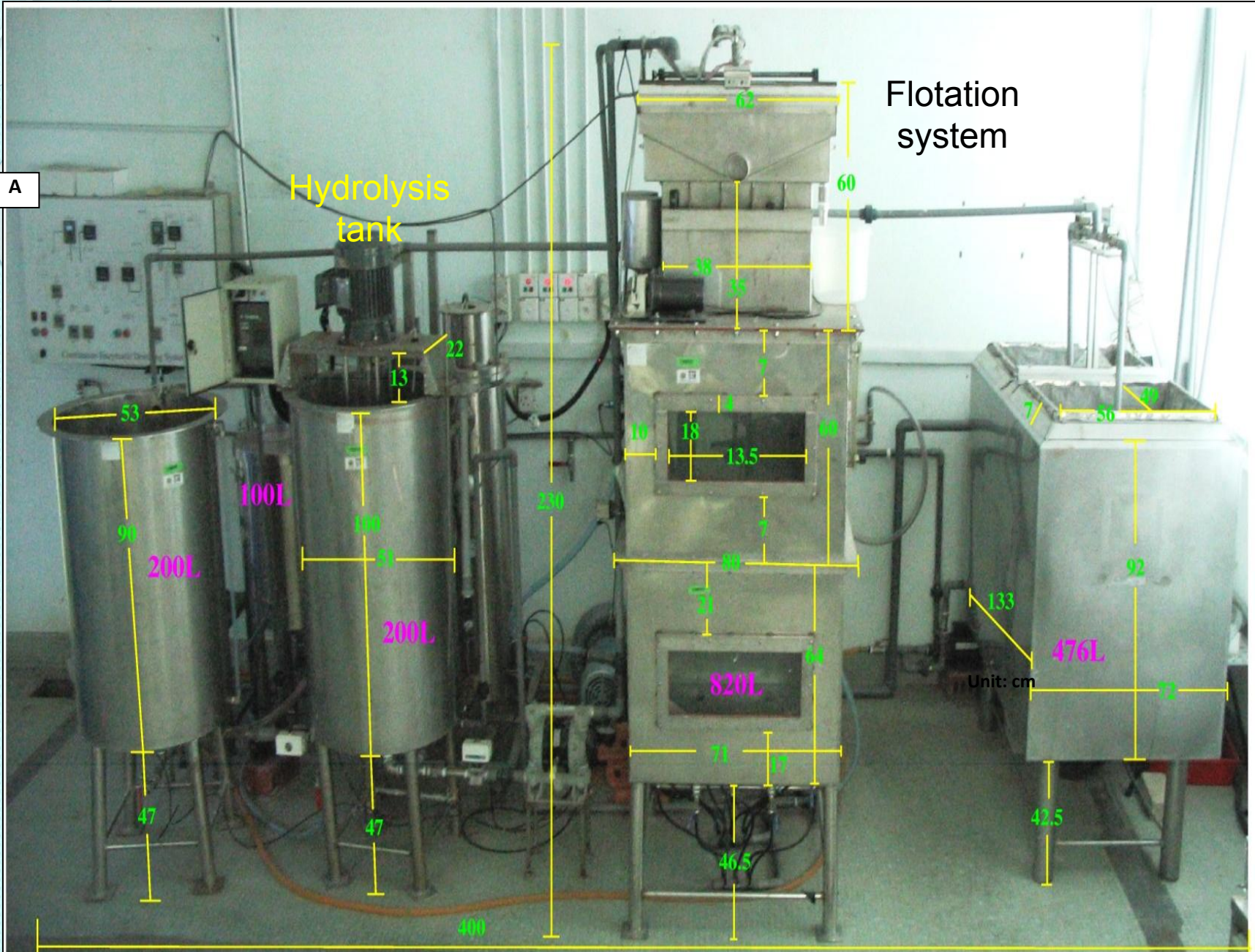
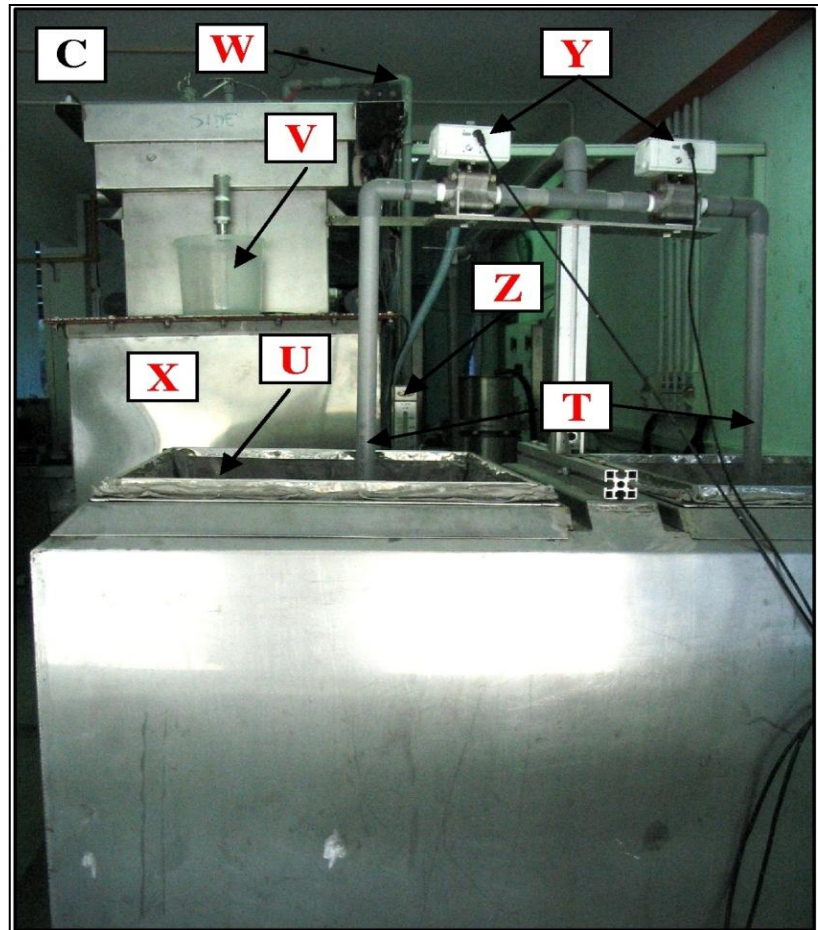
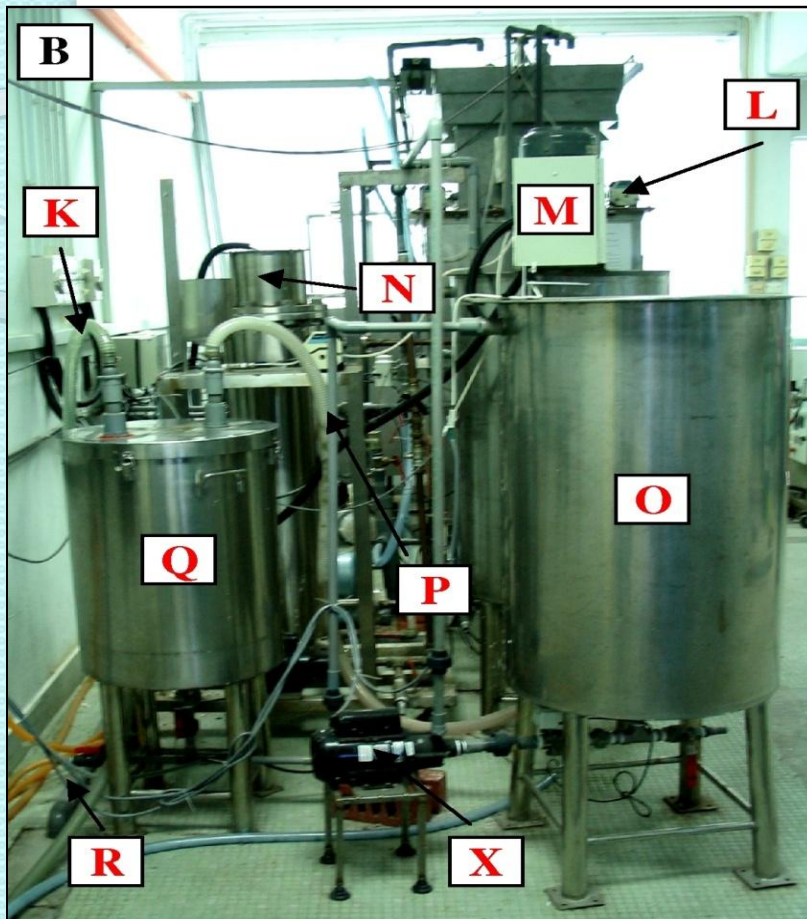


Figure 4.1: Schematic diagram of developed pilot scale of enzymatic deinking system.

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.



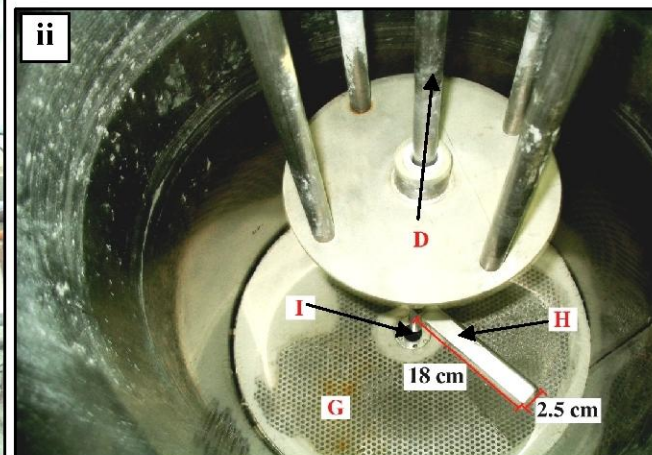
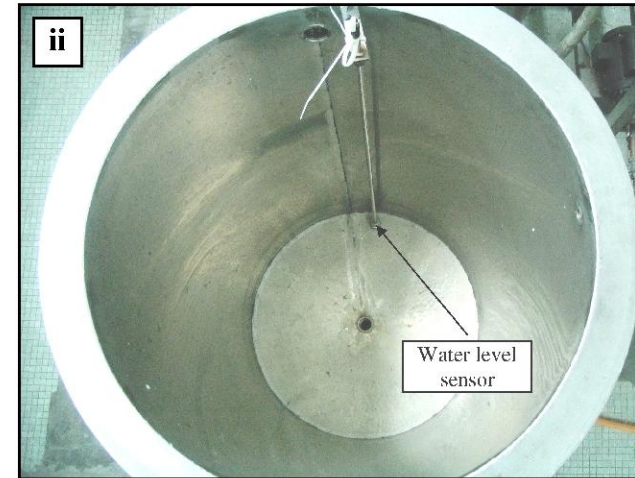


Developed pilot scale of enzymatic deinking system; (A) Front view, (B) Left view and (C) Right view.

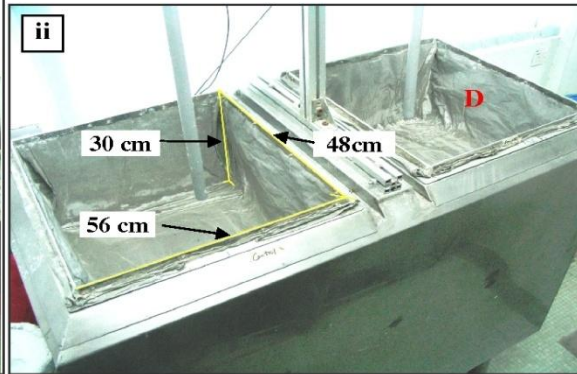
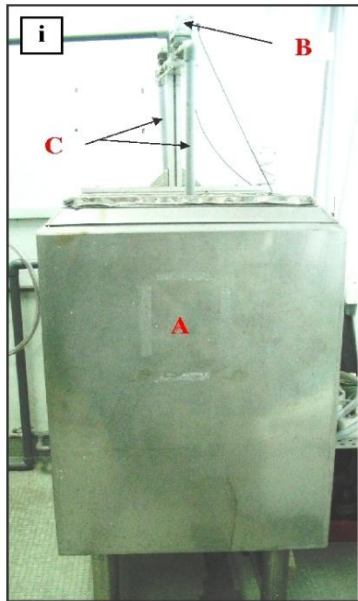
Notes: **K:** Vacuum pipe; **L:** Peristaltic pump; **M:** Mixer controller box; **N:** Heater; **O:** Enzyme storage vessel; **P:** Enzyme/water recycling pipe; **Q:** Enzyme/water recycling vessel; **R:** Drain pipe; **S:** Water pump; **T:** Deinked pulp outlet; **U:** Sieve; **V:** Ink reservoir;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



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



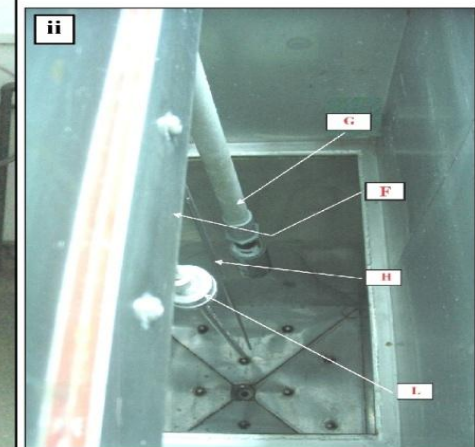
Bioreactor equipped with mixer; Front view (i) and Top view (ii).
Note: A: Enzymatic hydrolysis vessel; B: Mixer controller box; C: Mixer; D: Impeller shaft; E: pH probe; F: Thermocouple; G: Sieve; H: Impeller tips; I: Pulp slurry outlet.

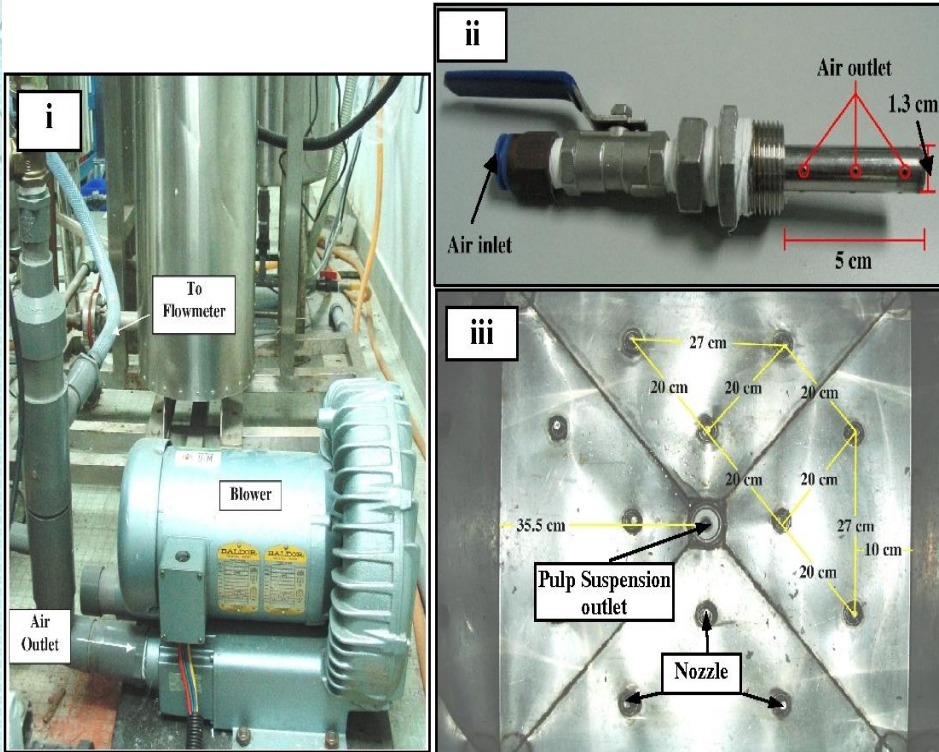


Pulp collecting vessel; Front view (i) and Top view (ii)
Note: **A:** Pulp collecting vessel; **B:** Solenoid valve; **C:** Pulp suspension outlet; **D:** Sieve.

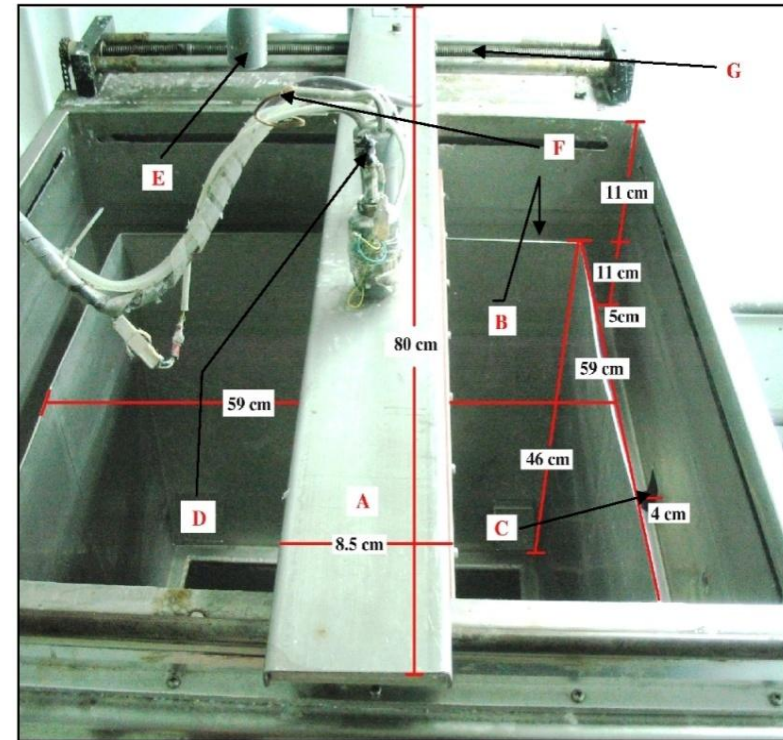
Flotation vessel; Front view (i) and Top view (ii).

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.

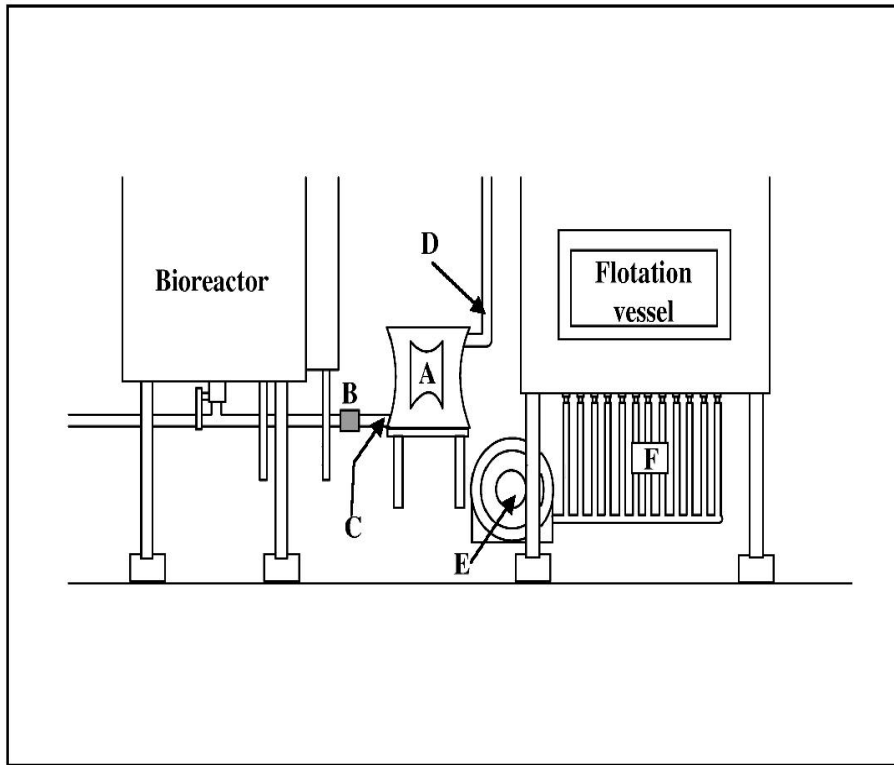




Blower (i), single nozzle (ii) and Nozzles unit (iii) used in the flotation system



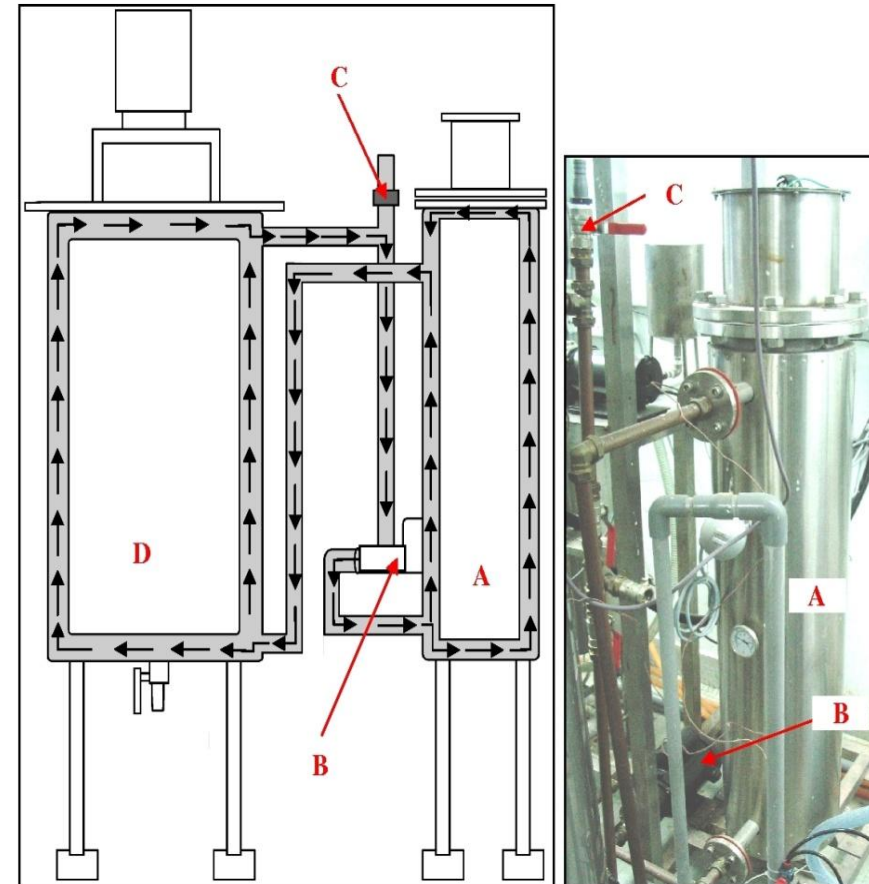
Foam scraping unit used in flotation process
Note: **A:** Scraper; **B:** Foam drain; **C:** Foam/ink particle outlet; **D:** Water level sensor; **E:** Water inlet; **F:** Thermocouple; **G:** Scraper carrier



: 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.

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

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



Heater used in deinking process.

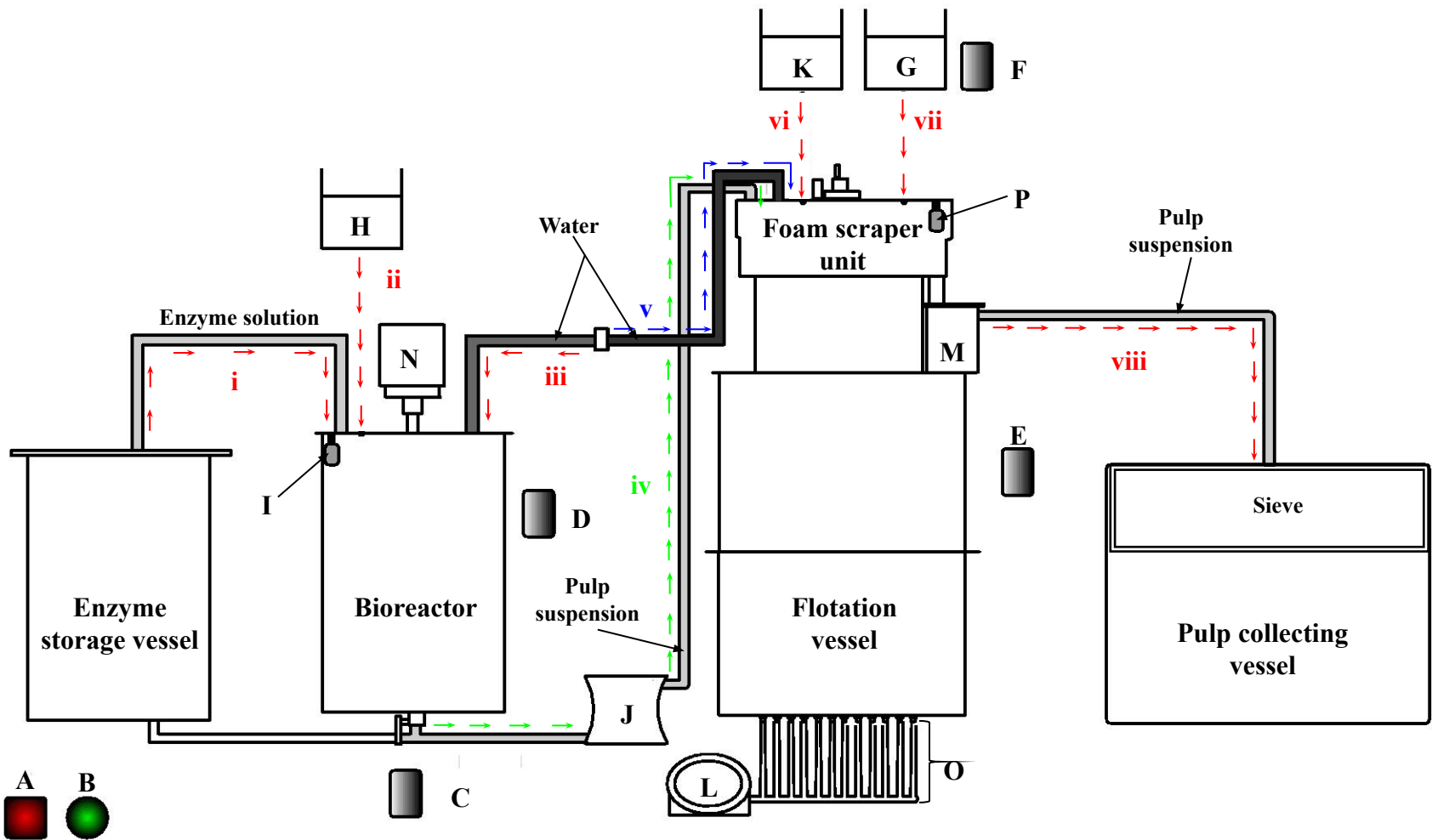


Figure 4.4: Control panel for the operational of enzymatic deinking system.

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.

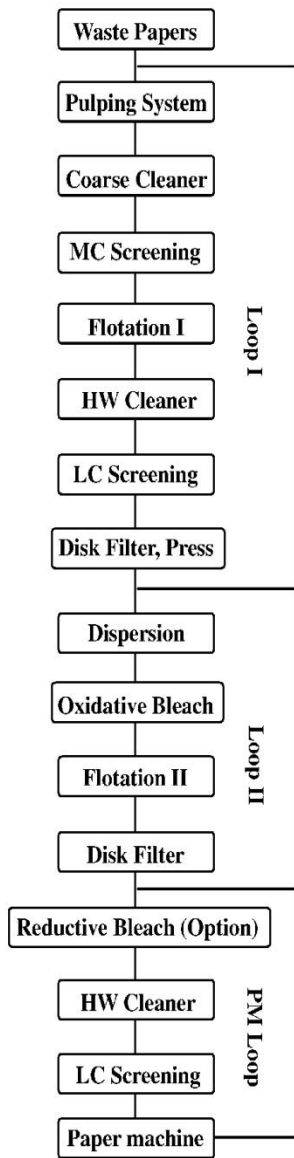


Figure 4.5: Plant concept for newsprint and improved paper grades (Schwarz, 2000)

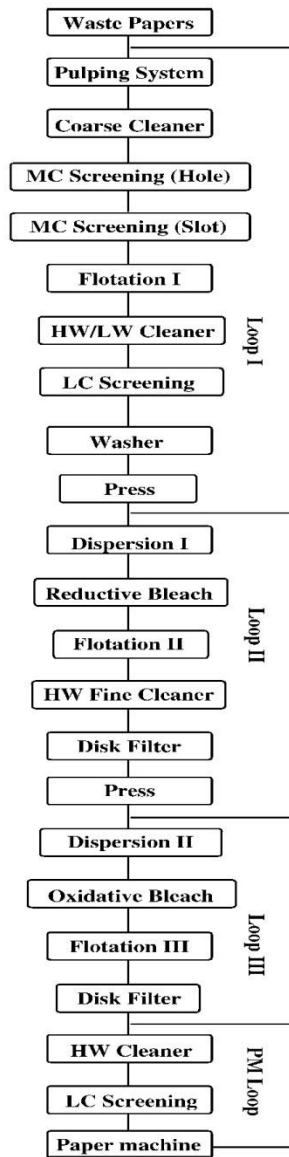
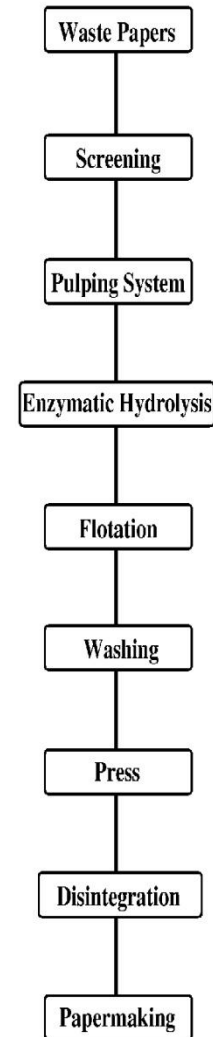


Figure 4.6: Plant concept for DIP from mixed office wastepaper (Schwarz, 2000)



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) 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

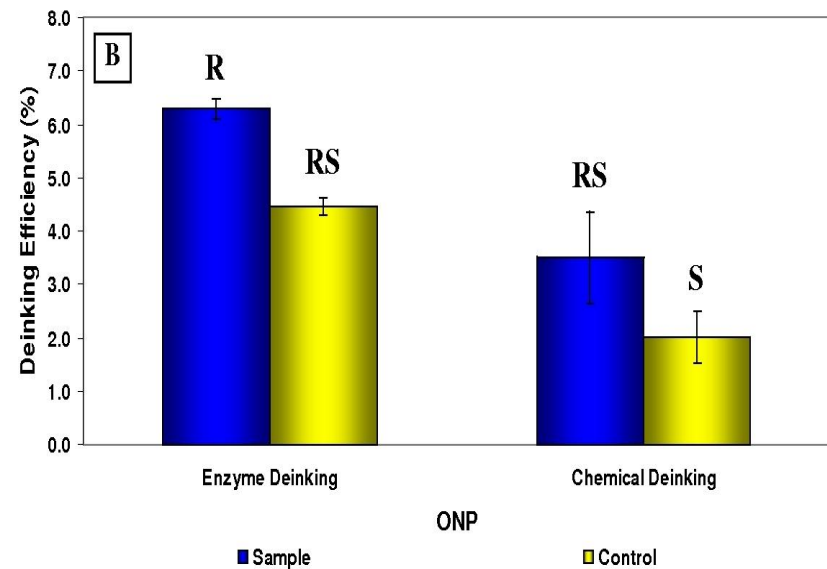
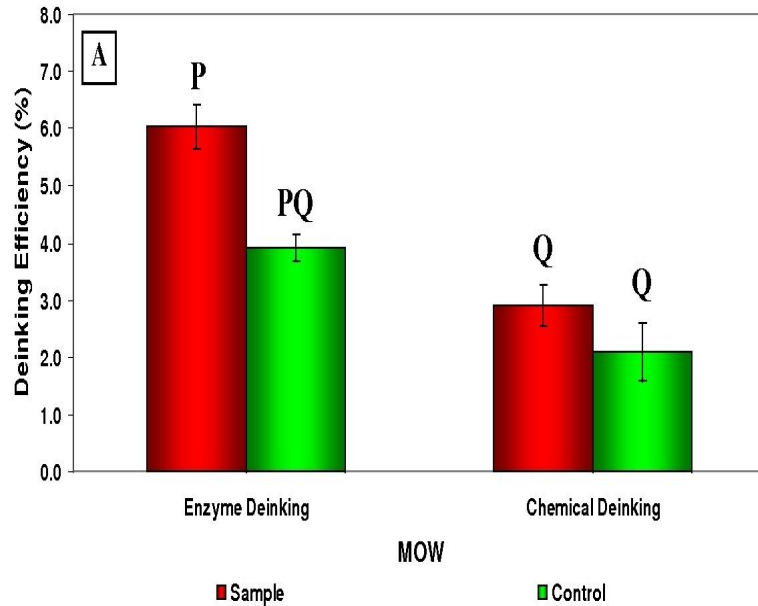
Pulping process	MOW	ONP
Pulping consistency	2%	3%
Pulping time	60 min	45 min
Enzymatic hydrolysis process		
Temperature	50°C	50°C
pH	5.5	5.5
Enzyme concentration	4.8 U/g air-dry pulp	2.4 U/g of air dry pulp
Hydrolysis time	60 min	45 min
Deinking efficiency	5.00%	4.74%
Total reducing sugar obtained	40.76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

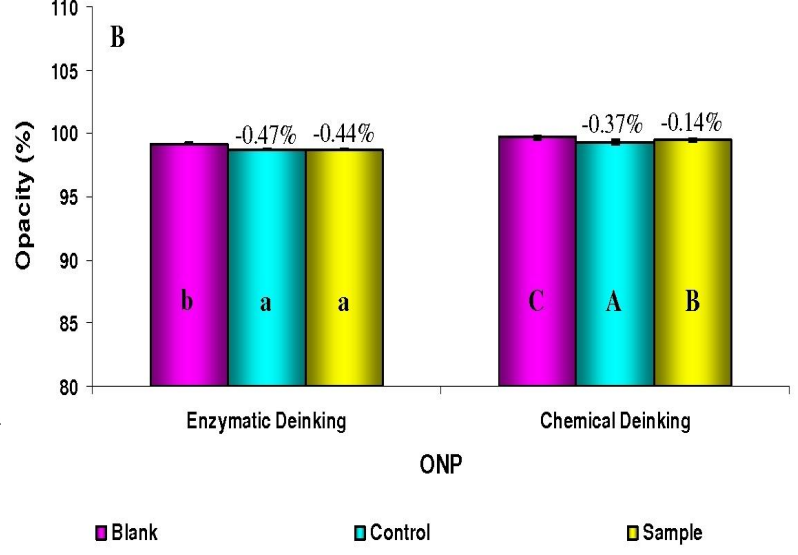
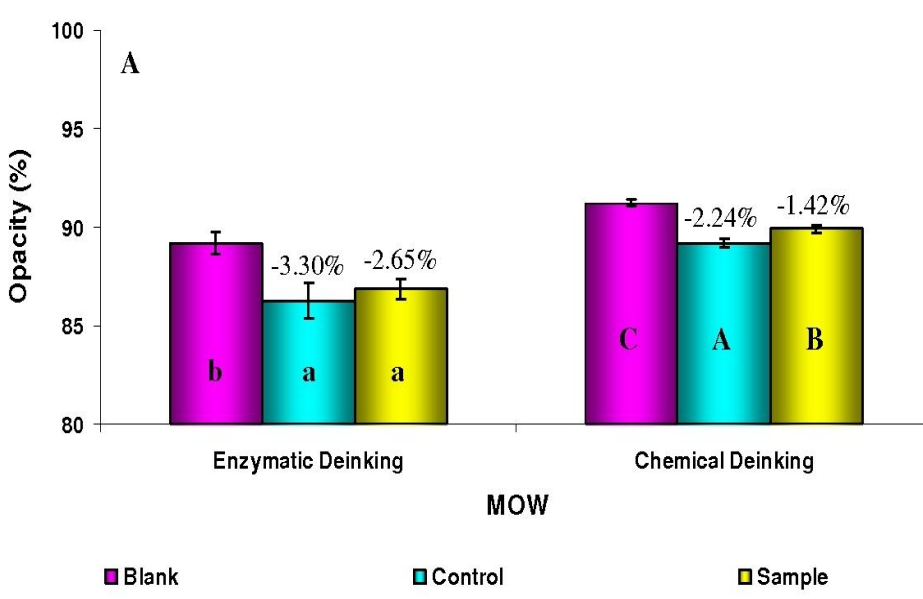
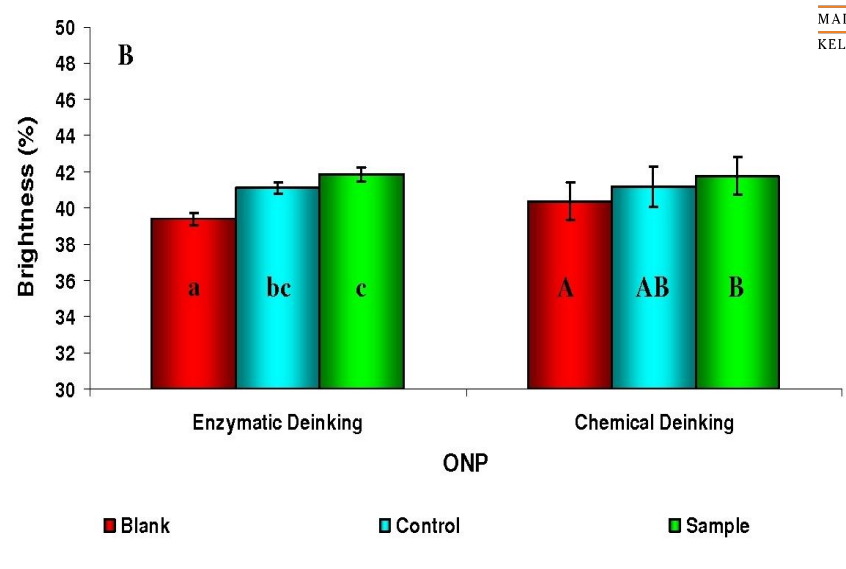
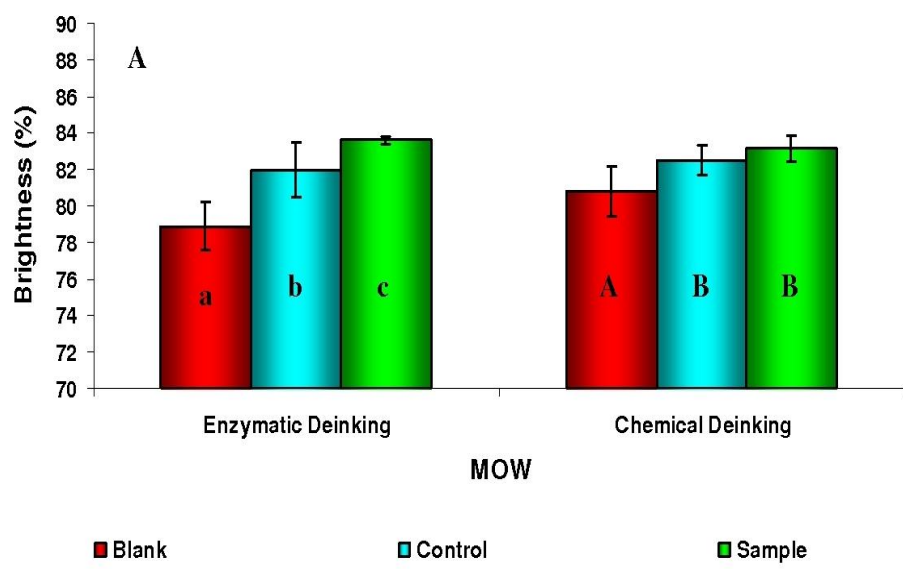


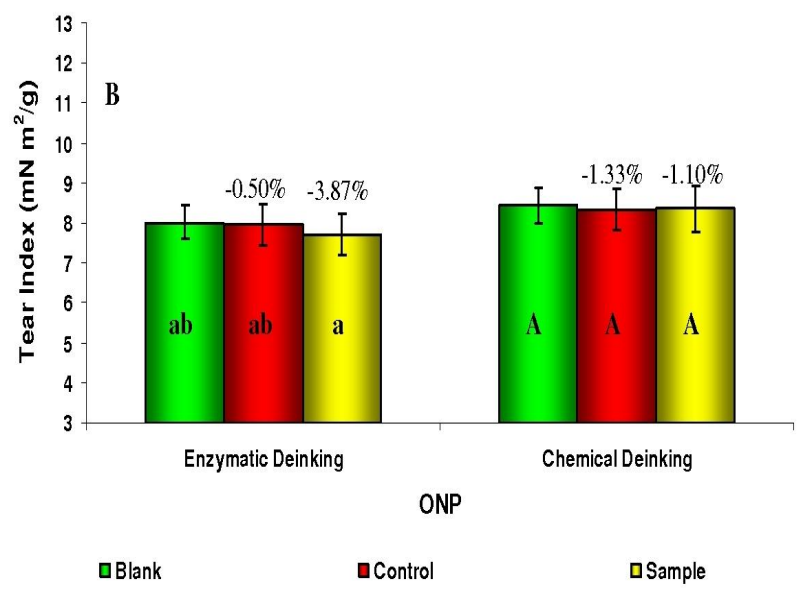
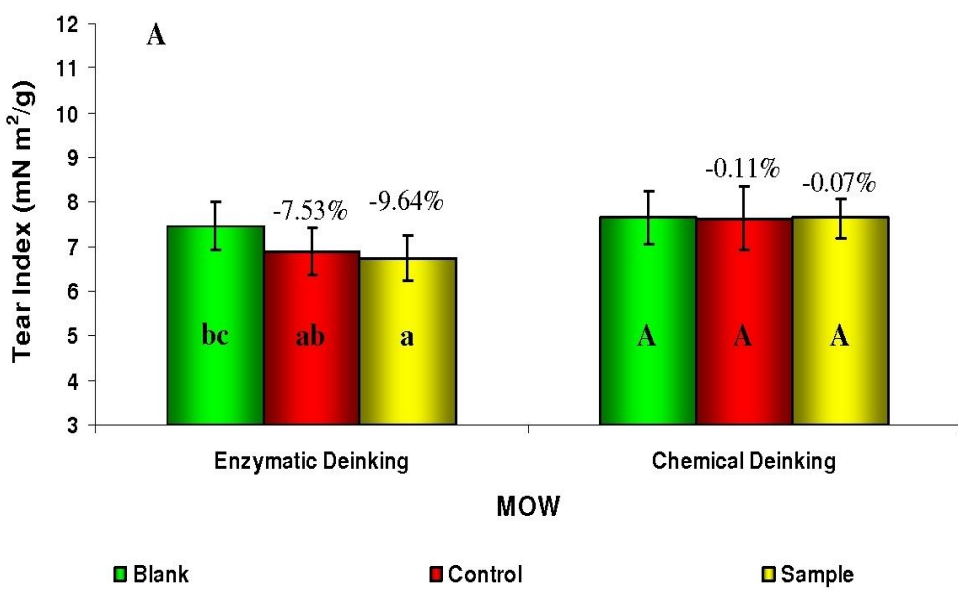
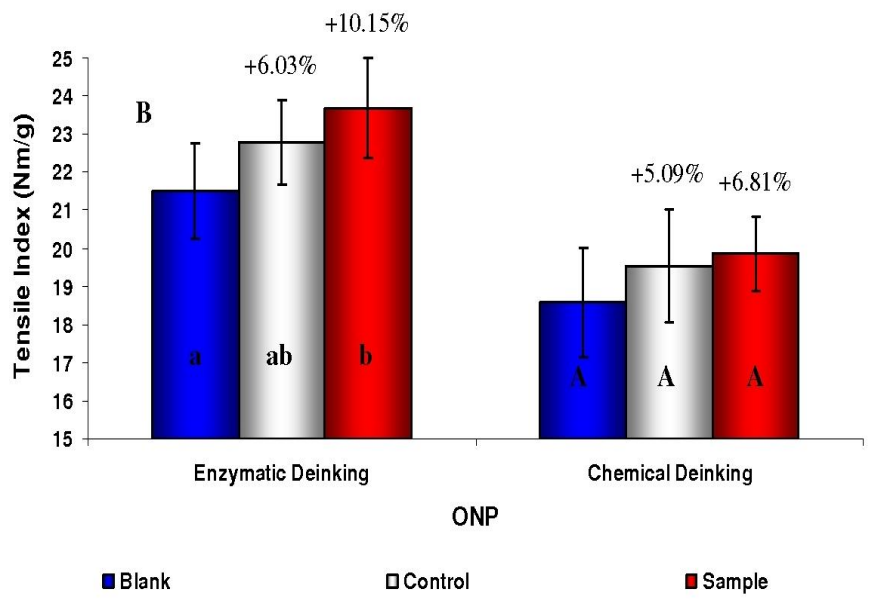
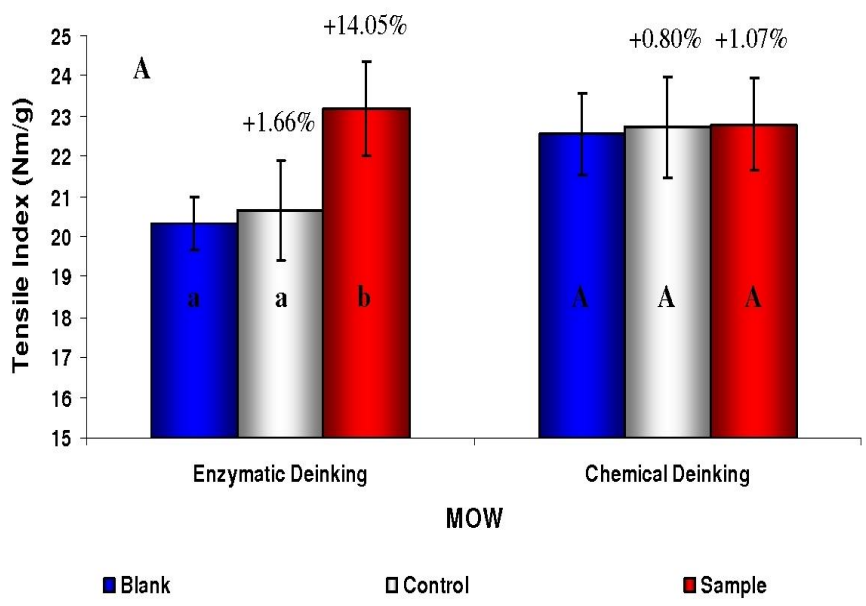
Mixed Office wastes (MOW)

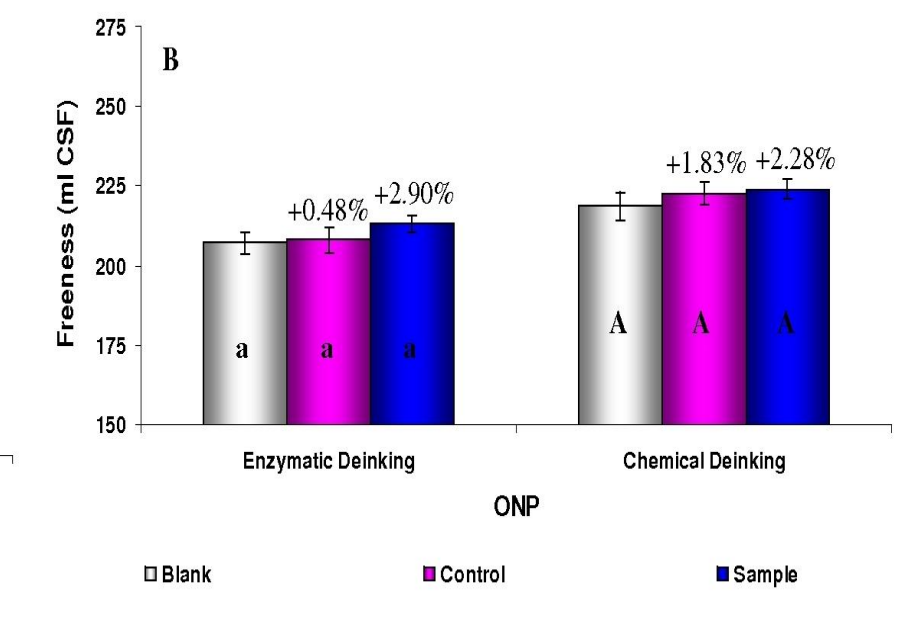
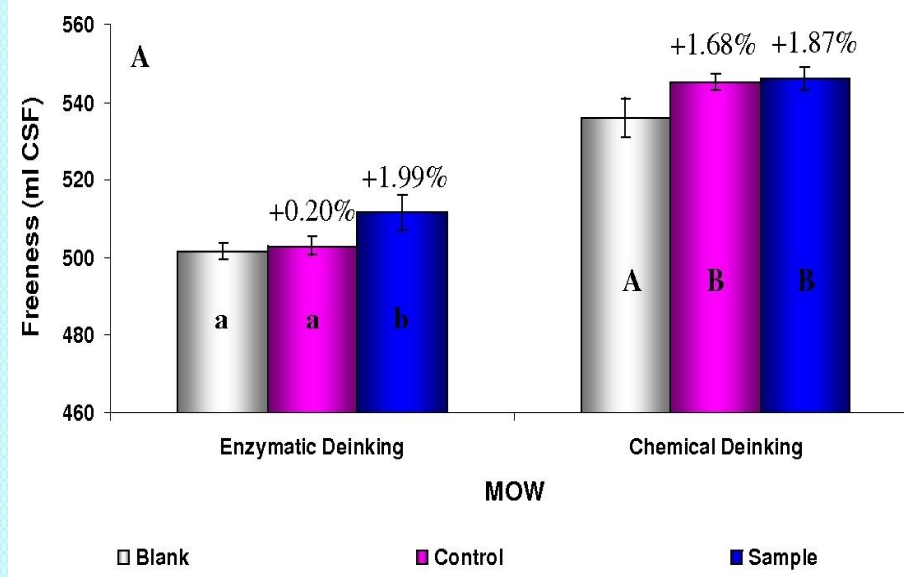
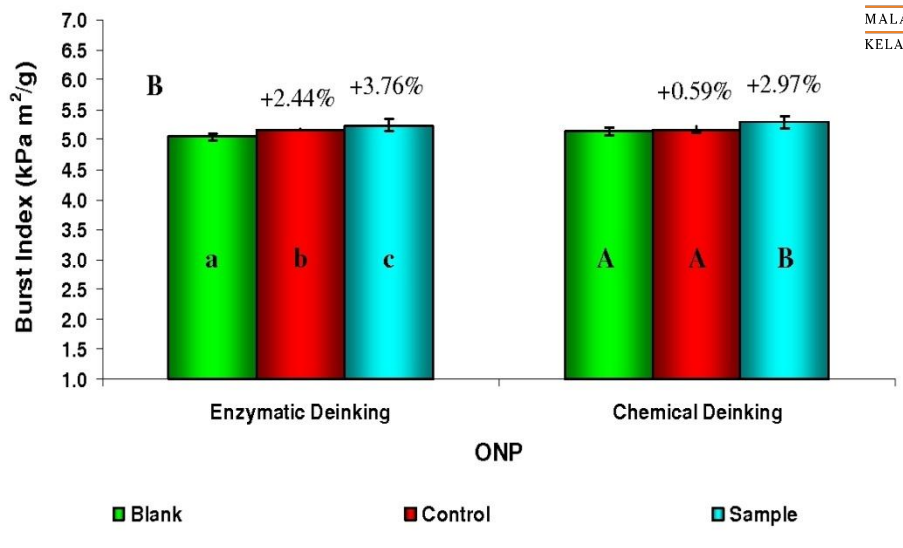
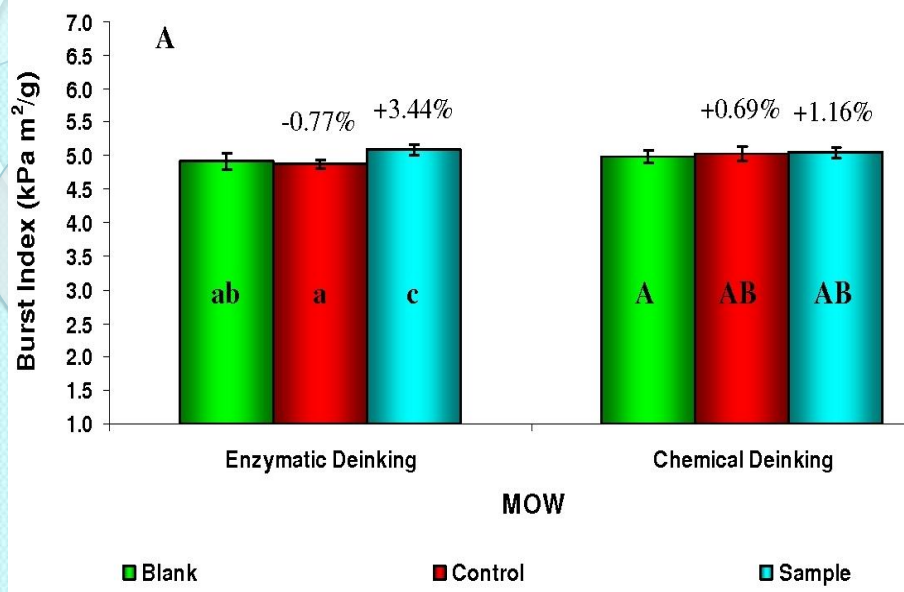


Old newspapers (ONP)









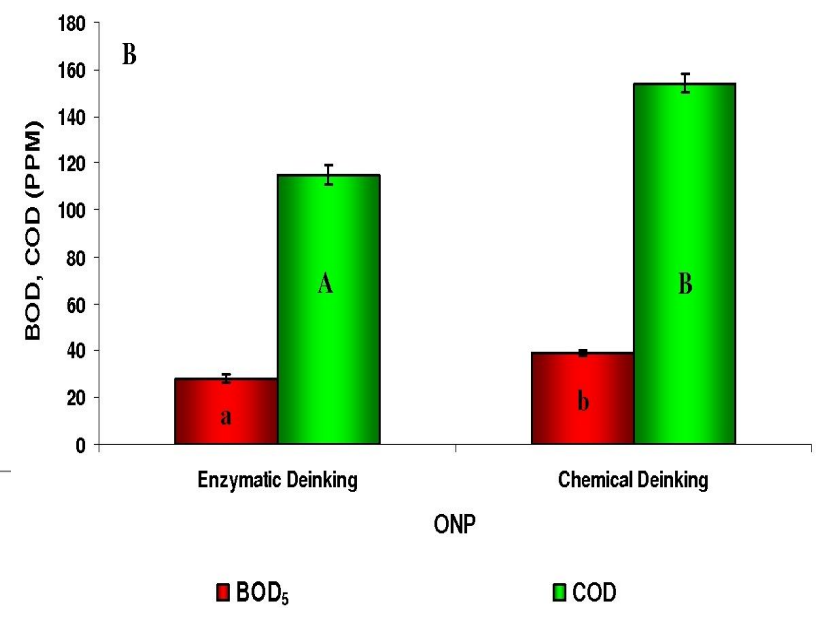
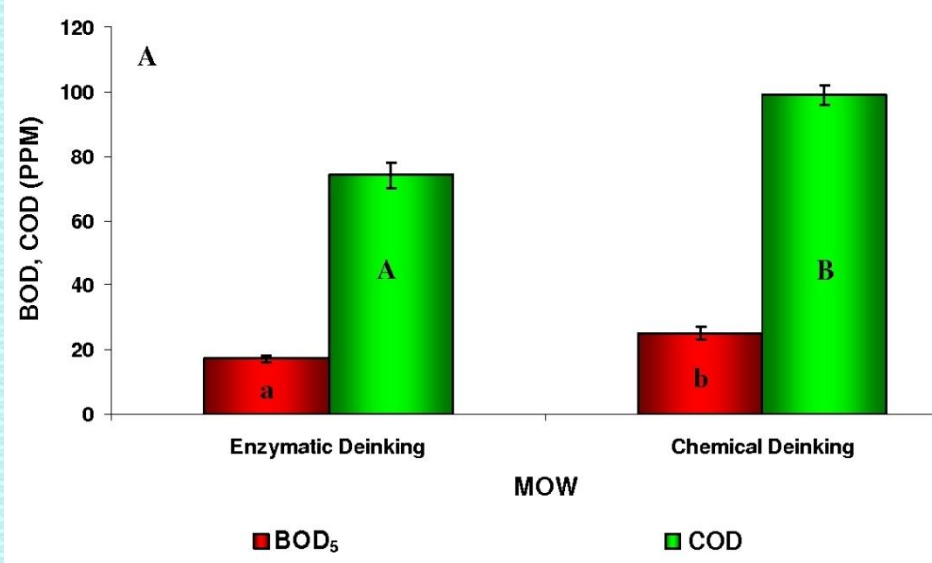
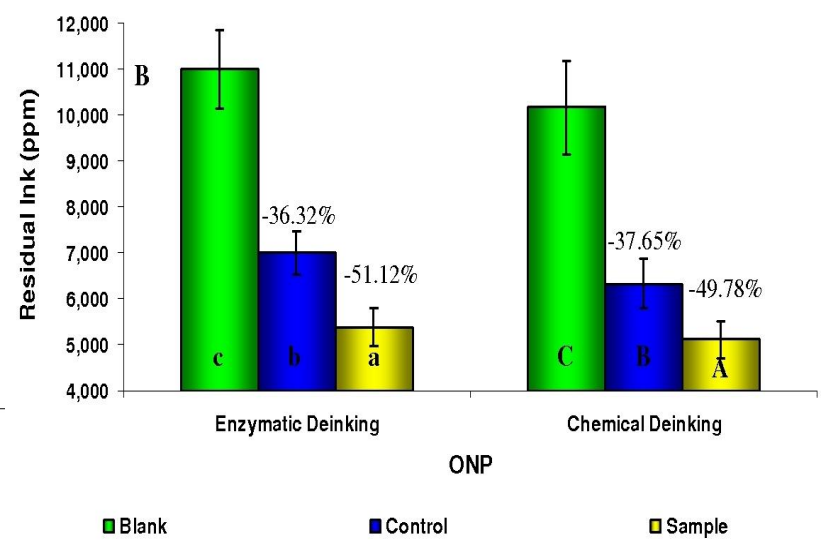
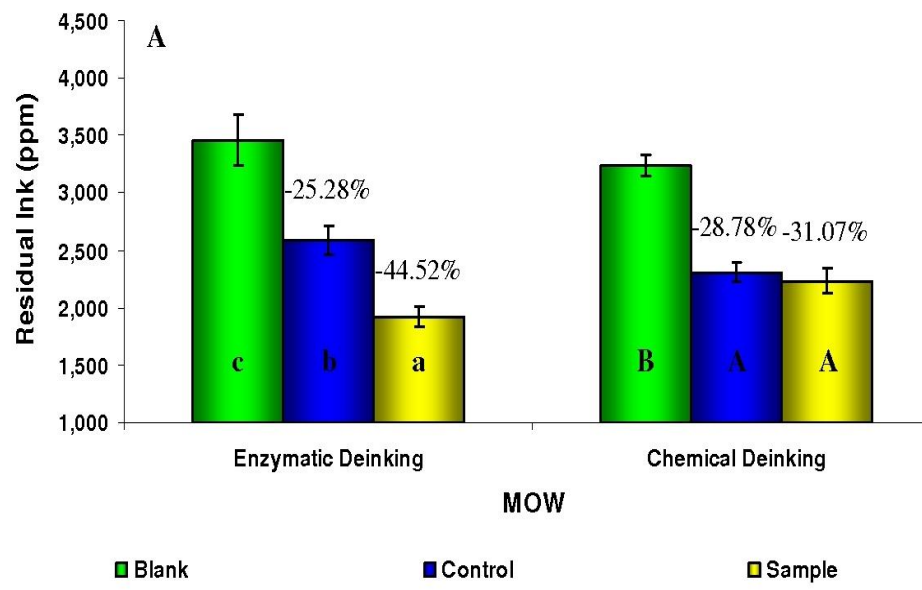


Table 6.1: Summary of pulp and paper properties after deinking process.

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)

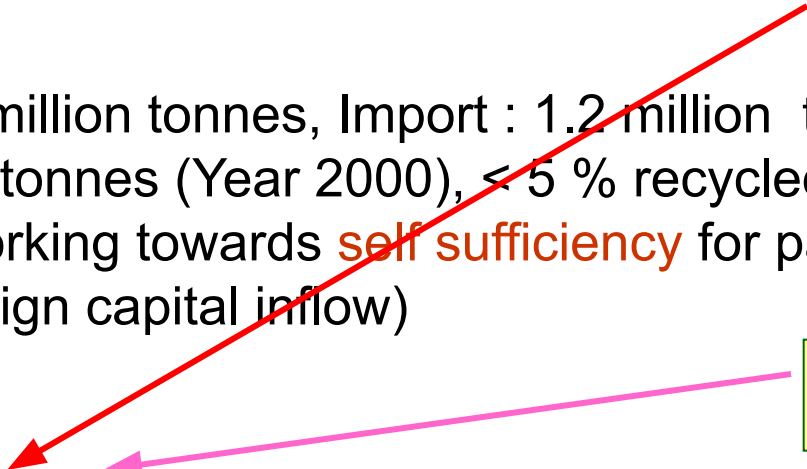
~ RM 4.5 billion

2.2 million tonnes of papers available for enzymatic deinking per year

Environmental issues and sustainable development

Enzymatic processes cover large market potential

Advancement of Industrial/Env Biotechnology and bioengineering



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) effluent

PRODUCTION OF BIOPRODUCTS FROM WASTES

- Bioplastics
- Fermentable sugars (Ethanol)

Inoculum development for hydrocarbon bioremediation



(a)



(b)

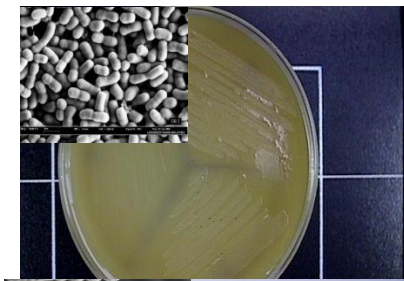
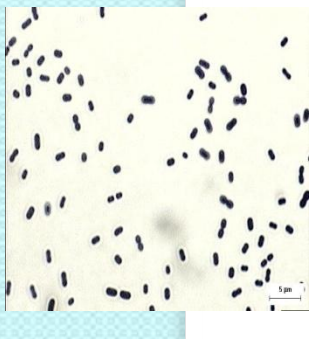
Isolation of hydrocarbon degrading microorganisms:
Oil contaminated soil/water (Penang, Kedah)
Soils at oil refineries (Melaka and Kerteh, Terengganu)



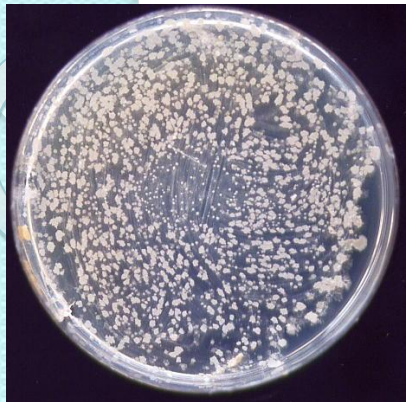
Potential isolates for consortia development

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



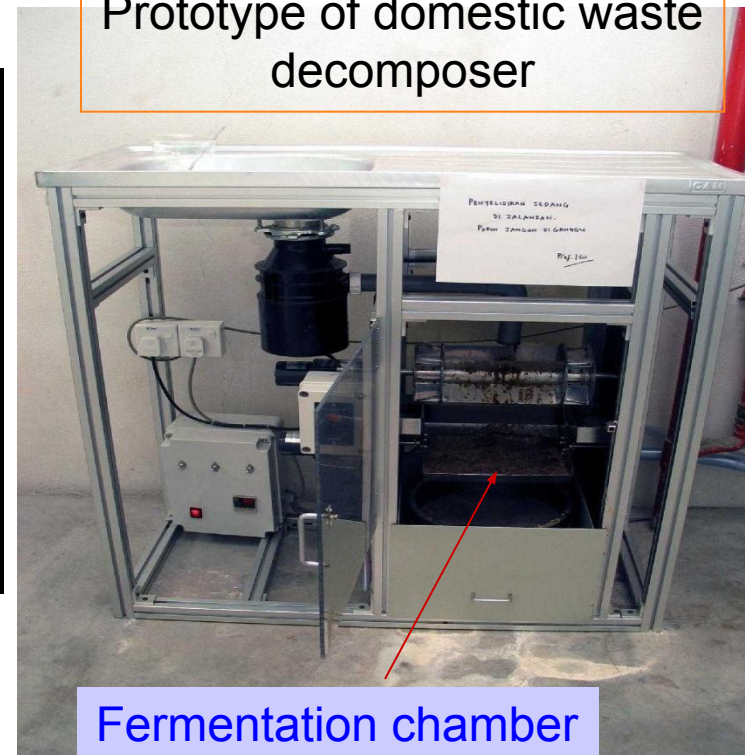
Inoculum in rice husk as binder



Prototype of domestic waste decomposer

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

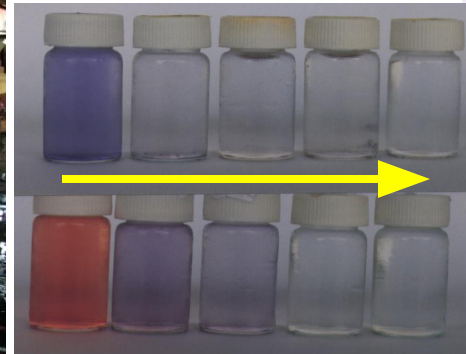


Fermentation chamber

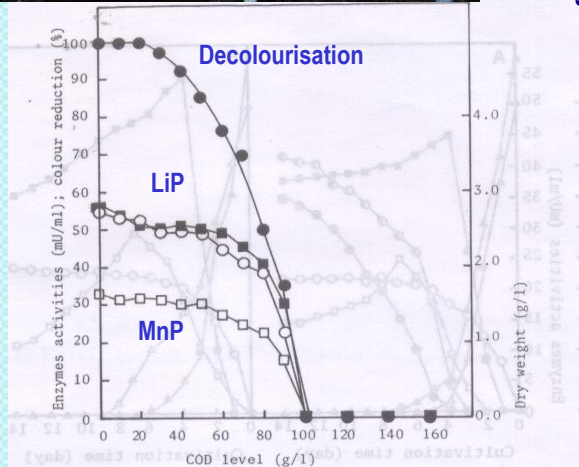
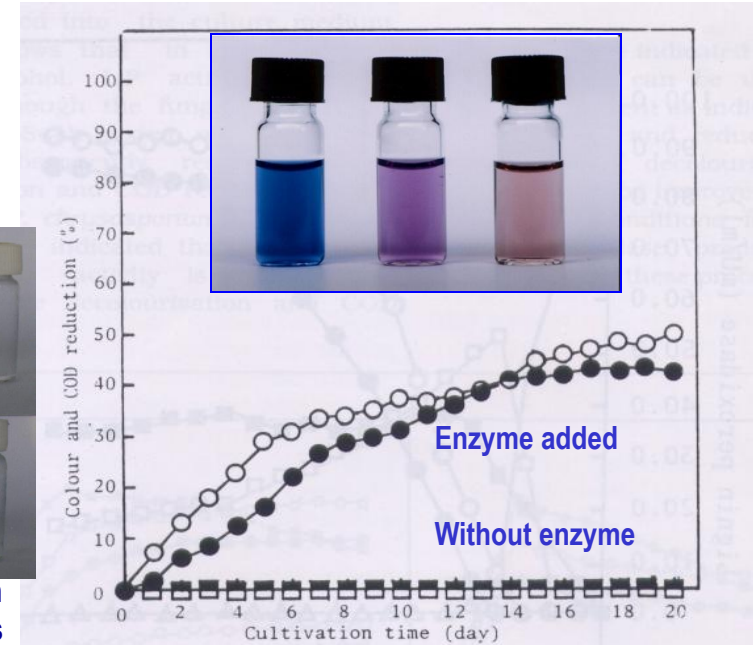
Decolourisation of dyes from effluent of batik industries

Enzymes involved: Lignin peroxidase (LiP)
manganese peroxidase (MnP)

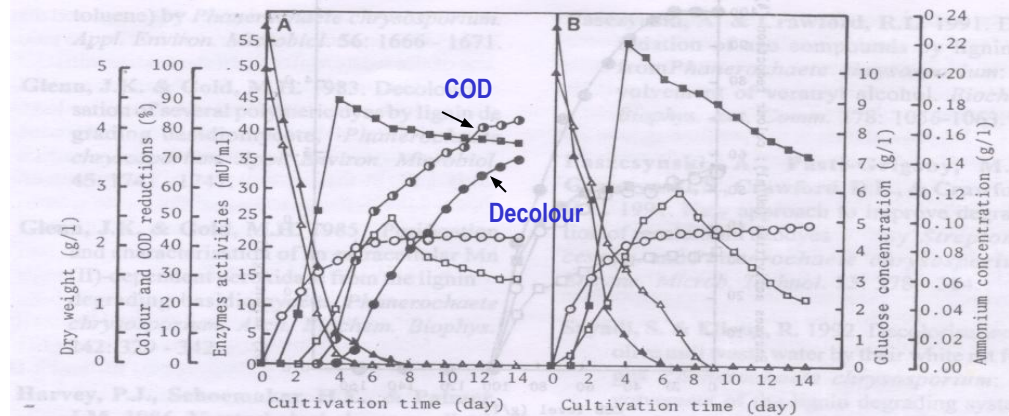
Source: *Phanerochaete chrysosporium*.
Waste water: batik effluent



Time profiles of decolorisation using lignin degrading enzymes



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



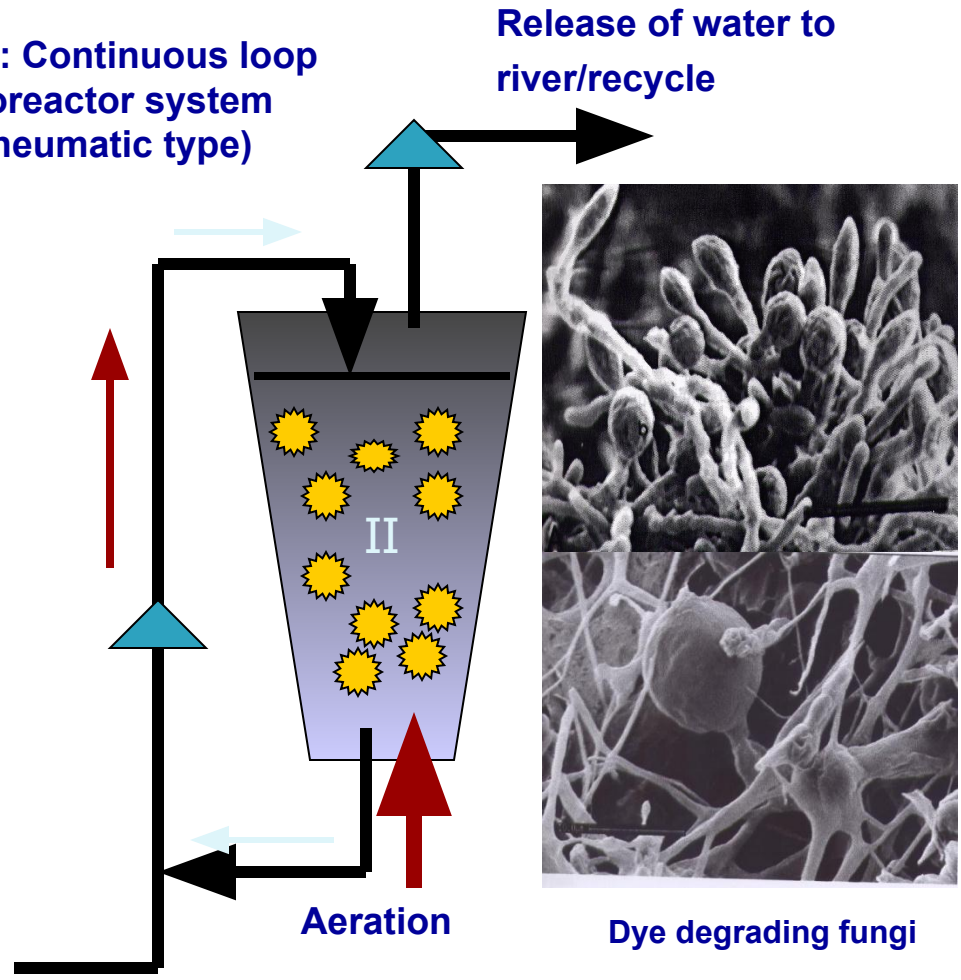
Direct cultivation of *Phanerochaete chrysosporium* in the medium containing waste water
A. In the presence of waste water, B: absence of waste water.

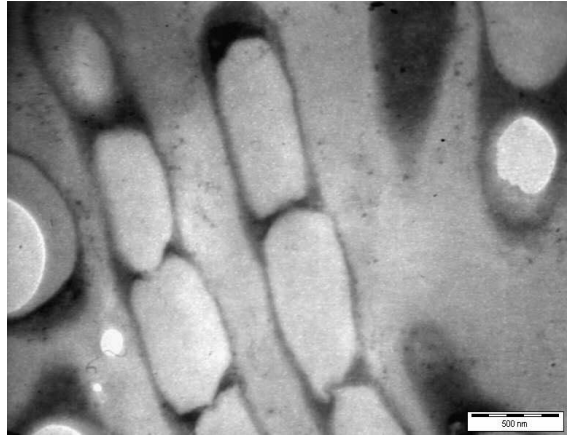
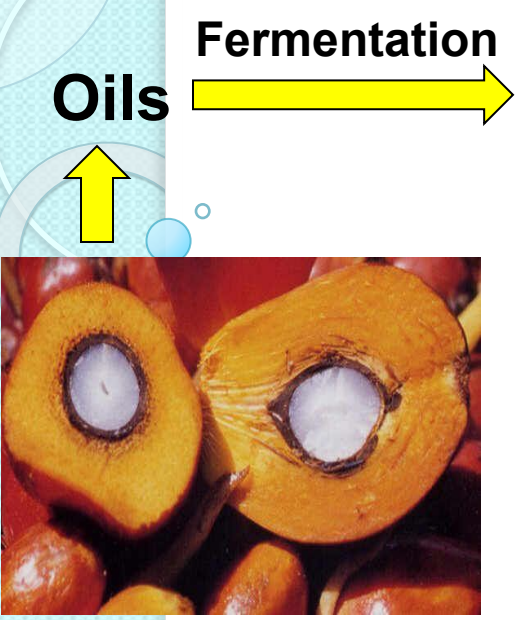
Physical Method



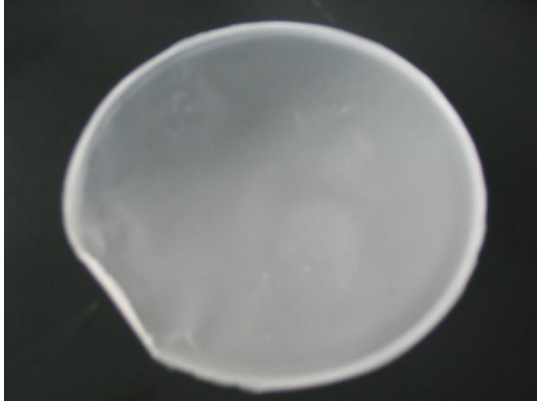
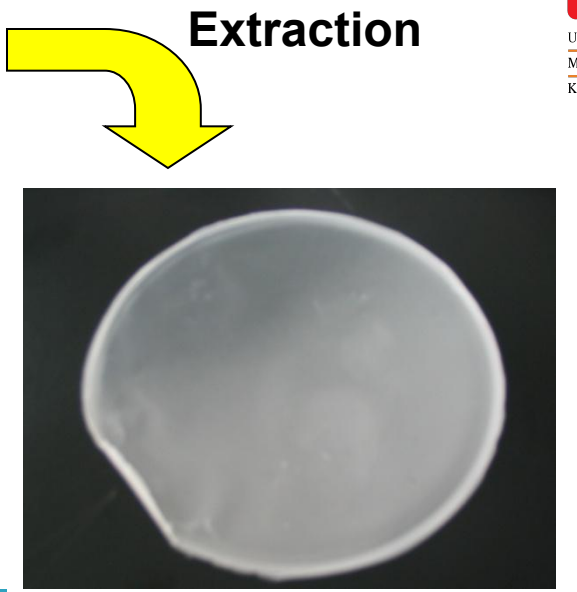
Decolourisation process by biological method

II : Continuous loop bioreactor system (Pneumatic type)





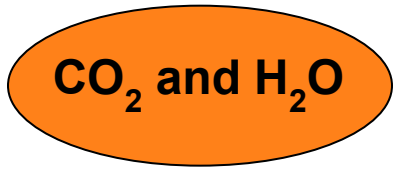
PHA in bacterial cells



Ecologically sustainable 'green' processes



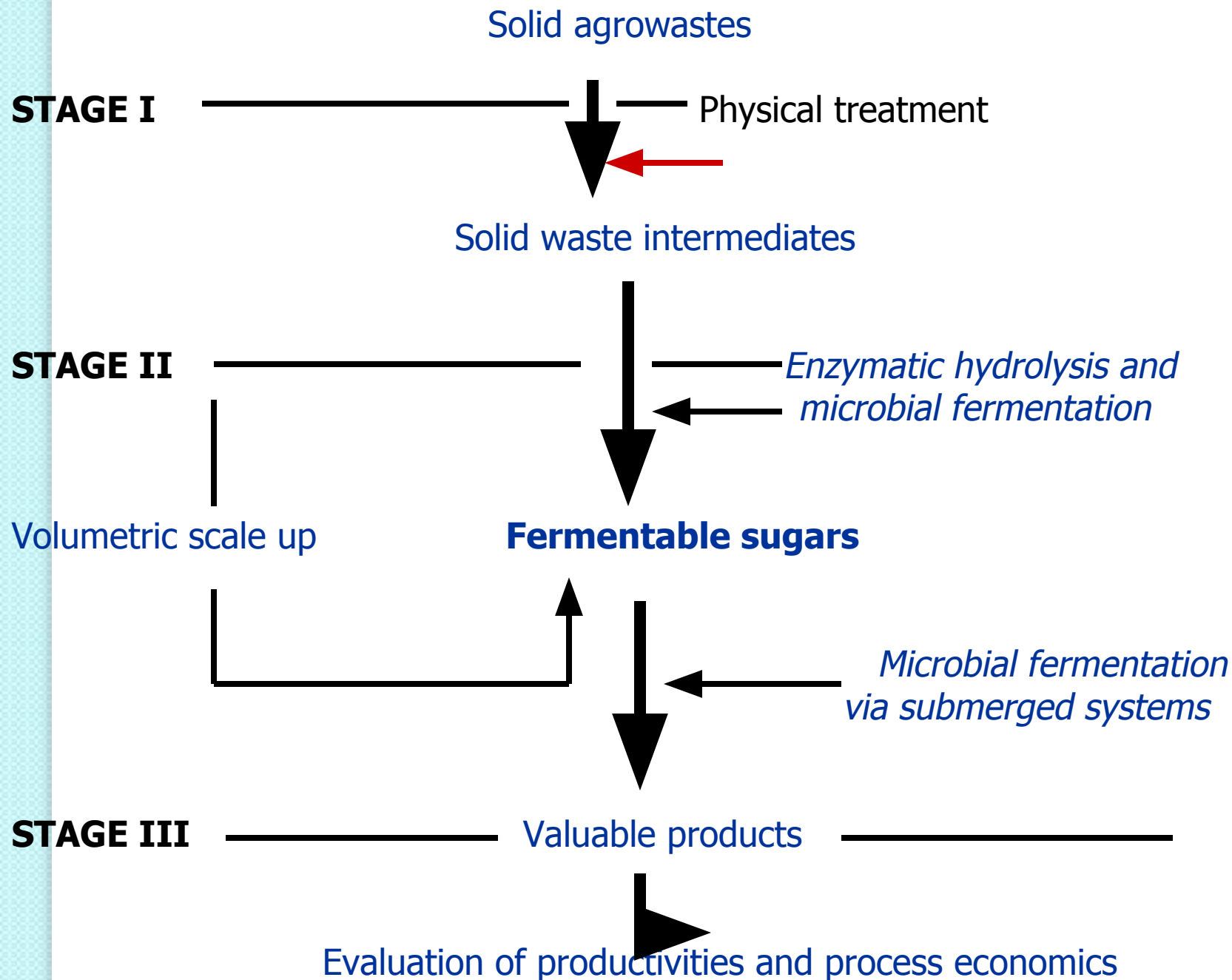
Renewable plant resources (palm oil)



Biodegradable bottles

Biodegradation Test (30 days)





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

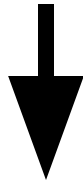
- *Candida utilis*
- *Saccharomyces cerevisiae*
- *Xanthophyllomyces dendrorhous*
- 30°C, aerobic
- Applications in aquaculture industries as feeds

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

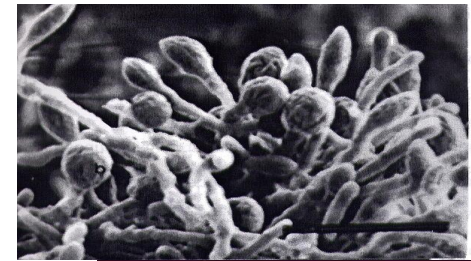
Ethanol production as fuel

Agrowastes



Delignification by fungi
Solid state fermentation (SSF)

Phanerochaete chrysosporium
Aspergillus niger
Aspergillus sp.

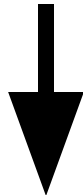


Fermentable sugars



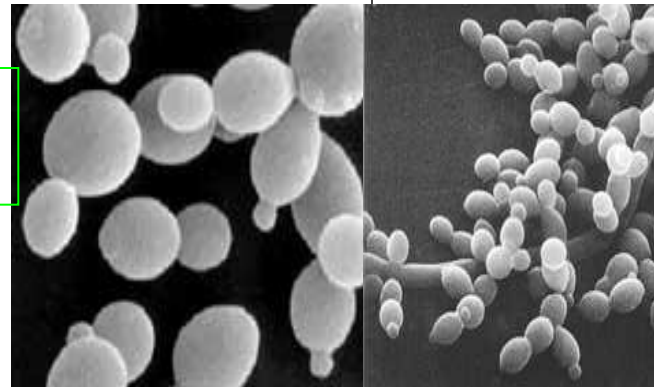
Optimization of SSF

Yeast fermentation

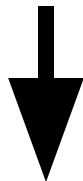


Optimization of ethanol fermentation

Saccharomyces cerevisiae

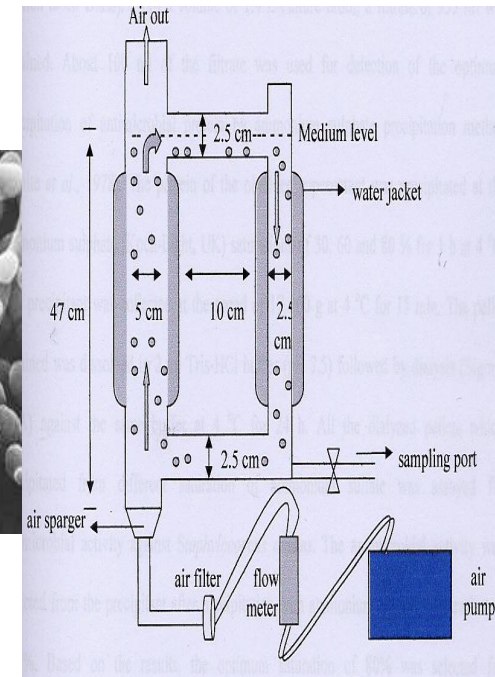
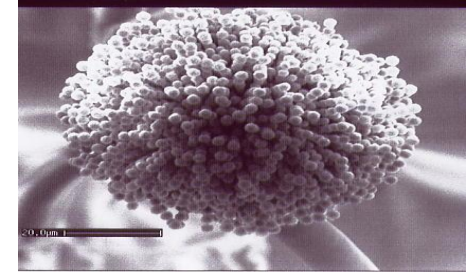
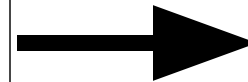


Ethanol fermentation



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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