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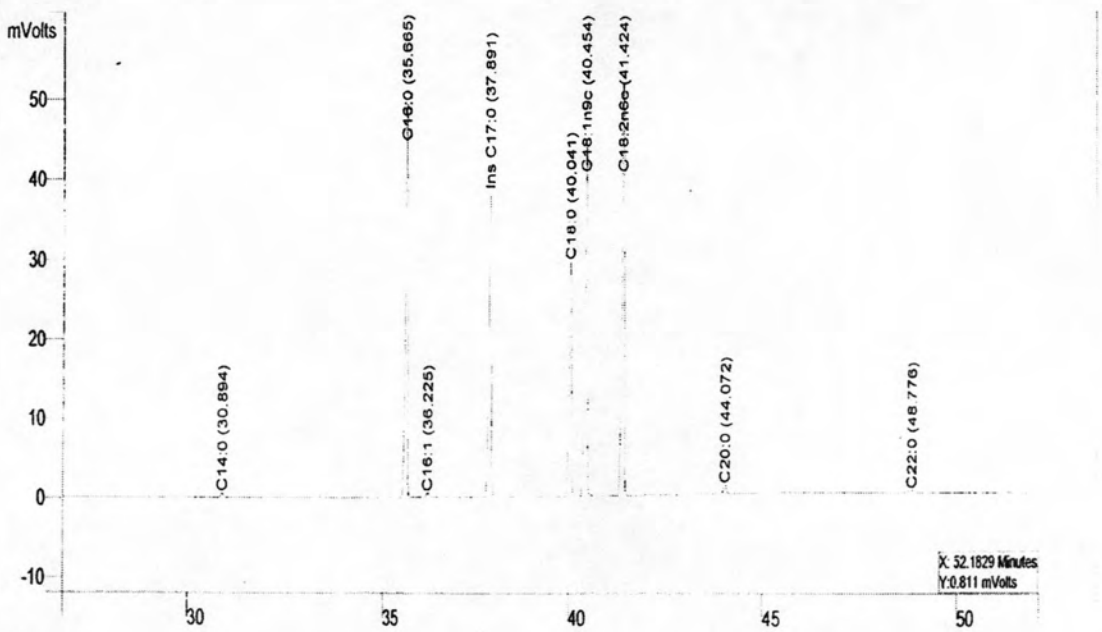
APPENDICES

APPENDIX A

CALCULATIONS

1. Molecular weight of crude oil of endophytic fungus can be calculated in following:

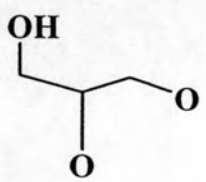


Molecular weight (approx.) of crude oil = $M_G + 3M_{Ac}$

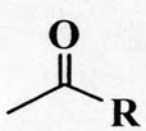
where M_G : Molecular weight of glycerol moiety = $C_3H_5O_3$ = 89.07 g/mol

M_{Ac} : Molecular weight of acyl moiety of fatty acid

M_{FFA} : Molecular weight of fatty acid



Glycerol moiety



Acyl moiety

where;

$$M_{FFA} = \frac{[(\Sigma\%\text{Area from GC} \times \text{MW of fatty acids})]}{\text{Total \%Area from GC}}$$

$$\text{So, } M_{FFA} = \frac{[(0.25\times228.38) + (22.3452\times256.43) + (0.2507\times254.41) + ...]}{100.0001}$$

$$= 274.41 \text{ g/mol}$$

and M_{Ac} = molecular weight (approx.) of fatty acid – molecular weight of (-OH)

$$= 274.41 - 17.01$$

$$= 257.398 \text{ g/mol}$$

$\therefore$ Molecular weight (approx.) of oil of endophytic fungus $= 257.398 \times 3$

$$= 772.19 \text{ g/mol}$$

The molecular weight of oil methyl ester has been calculated from the molecular weight (approx.) of acyl moiety of fatty acid, that is

MW of methyl ester = M_{Ac} + molecular weight of acyl moiety of fatty acid

$$= 772.19 + 89.07$$

$$= 861.26 \text{ g/mol}$$

So, Molecular weight of methyl ester of oil of endophytic fungus

$$= 861.26 \text{ g/mol}$$

2. %Methyl ester content from GC

%Methyl ester content from GC of crude oil of endophytic fungus can be calculated this equation

$$\left[\frac{\Sigma A - Ai}{Ai}\right] \times \frac{C \times V}{m} \times 100$$

; C = Concentration of standard
V = Volume of standard
m = Amount of sample
Ai = Peak area of Internal standard
ΣA = Total peak area

$$= \left[\frac{1008121 - 176300}{176300}\right] \times \frac{501.73}{50} \times \frac{5 \times 100}{249.77} = 94.78\%$$

3. Sugar analysis by DNS method

DNS reagent

1. Nitrosalicylic acid 1.0 g
2. Sodiumhydroxide 20.0 g
3. Mixed
4. Add Distilled water 50.0 mL
5. Add KNa tartrate 30.0 g
6. Make volume as 100.0 mL

Method

1. Sample 1 mL
2. Add DNS reagent 1 mL
3. Boiled 10 min and settle for cool at temperature
4. Add Distilled water 10 mL
5. Asorbance 540 nm

* Sugar standard curve was prepared 0 – 2.0 mg/ml of glucose.

4. Determine the %FFA (ASTM D5555-95)

Reagent

1. 2-propanol
2. Phenolphthalein
3. 0.1 M NaOH
4. 0.25 M NaOH

The process for titration is as 50 mL of 2-propanal was warm then 2 mL of Phenolphthalein was added. After that add 0.1 M NaOH was drop wise until the color of solution change to pink. Next, a mixture of the pink solution and 7.05 g oil into 250 mL flask then titrate with 0.25 M NaOH.

The calculation of %FFA was titrated from this equation.

$$\%FFA = \frac{\text{mL of NaOH} \times \text{concentration of NaOH} \times 28.2}{\text{Weight of sample}}$$

$$= \frac{12.2 \times 0.25 \times 28.2}{7.05}$$

$$= \frac{86.01}{7.05}$$

$$= 12.2\%$$

The calculation of acid value was titrated from this equation.

$$\text{Acid value} = \%FFA \times 1.99$$

$$= 12.2 \times 1.99$$

$$= 24.278$$

5. Statistics in F test and Duncan

1. The difference of oil production from three medium as MEB, PDB and YES were analyzed via statistics in Duncan and F test.

Source	Data	The difference	
MEB	27.62	28	Different
	27.45	27	
	27.42	27	
PDB	26.88	27	Not Different
	27.04	27	
	27.00	27	
YES	27.13	27	
	24.47	27	
	27.07	27	

MEB indicated the most quantity of oil production while PDB, YES have liken to values of oil production.

2. The difference of oil was extracted by soak for 3 days and using Soxhlet apparatus were analyzed via statistics in Duncan and F test.

Source	Data	The difference	
extracted by soaking for 3 days	36.50	37	Not Different
	36.51	37	
	36.50	37	
using Soxhlet apparatus	36.56	37	
	36.86	37	
	36.86	37	

The difference of oil was extracted by soak for 3 days and using Soxhlet apparatus were not different reserve 0.05 significance levels.

3. The difference of oil production from at 10 g/L and 5 g/L of malt ratio were analyzed via statistics in Duncan and F test.

Source	Data	The difference	
Malt(g):Glucose(g):Peptone(g) (1 lit) Ratio 5:20:1	33.77	34	Not Different
	34.09	34	
	34.08	34	
Malt(g):Glucose(g):Peptone(g) (1 lit) Ratio 10:20:1	33.73	34	
	33.96	34	
	34.54	34	

The difference of oil was culture in MEB ration 10 g/L and 5 g/L of malt ratio were not different reserve 0.05 significance levels.

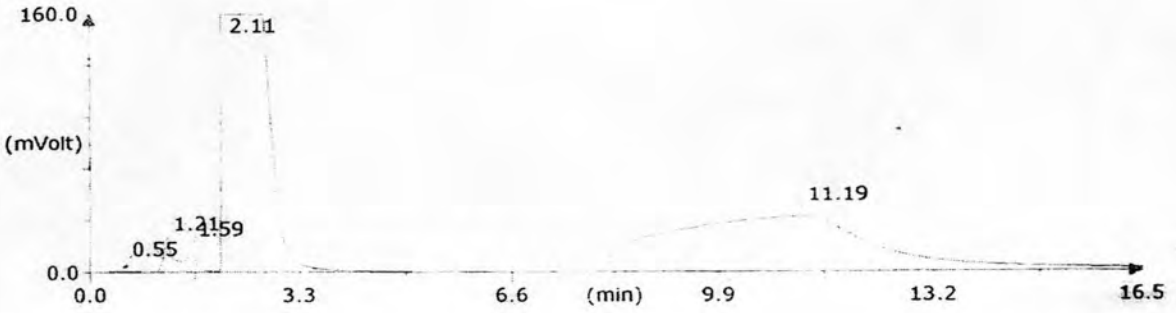
4. The difference of oil production from glucose and sucrose medium were analyzed via statistics in Duncan and F test.

Source	Data	The difference	
Glucose medium	27.62	28	Not Different
	27.45	27	
	27.42	28	
Sucrose medium	27.41	27	
	27.52	28	
	27.51	28	

The difference value with significance levels = 0.05, glucose and sucrose have similarity values of oil production.

6. The analysis of C:N ratio by elemental analyzer method

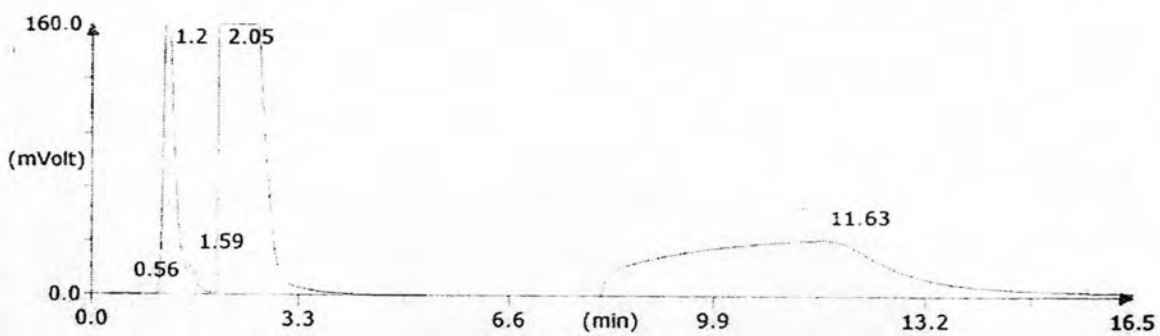
Malt extract



Retention Time (Min)	Component Name	Area (.1*uV*sec)	Area % (%)	Element %	Peak Height (uV)
0.55		157048	0.061	0.0000	511
1.21	Nitrogen	2069893	0.804	-1.9061	16322
1.59		1489508	0.579	0.0000	12736
2.11	Carbon	180077500	69.986	50.6019	647276
11.19		73509960	28.569	0.0000	32689
		257303900		48.6958	3553909

Malt extract composition as carbon 50.6% and nitrogen 0%.

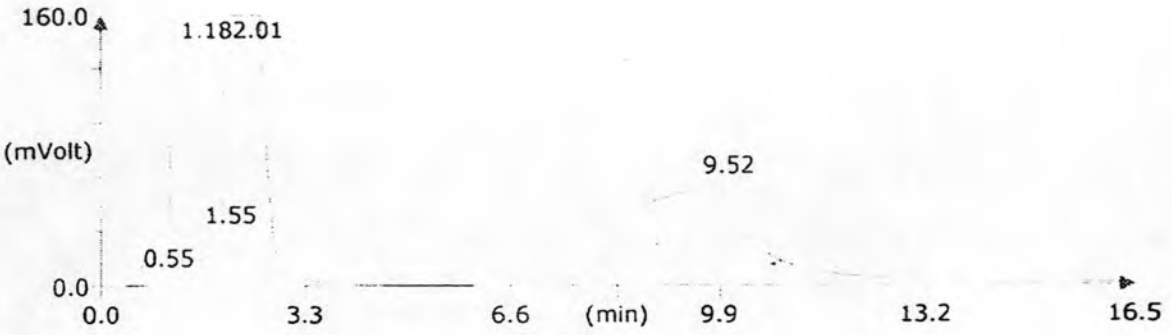
Peptone



Retention Time (Min)	Component Name	Area (.1*uV*sec)	Area % (%)	Element %	Peak Height (uV)
0.56		159896	0.053	0.0000	575
1.20	Nitrogen	24634970	8.098	16.5536	221886
1.59		347525	0.114	0.0000	7787
2.05	Carbon	200182100	65.806	51.8929	786924
11.63		78877760	25.929	0.0000	32186
		304202200		68.4465	5304261

Peptone extract composition as carbon 51.89% and nitrogen 16.55%.

Yeast extract



Retention Time (Min)	Component Name	Area (.1*uV*sec)	Area % (%)	Element %	Peak Heigh (uV)
0.55		135863	0.045	0.0000	552
1.18	Nitrogen	20337360	6.802	12.4658	198596
1.55		2355753	0.788	0.0000	25697
2.01	Carbon	189479000	63.377	46.8345	794097
9.52	Hydrogen	86662580	28.987	6.8696	53799
		298970600		66.1700	5369226

Yeast extracts composition as carbon 46.83% and nitrogen 12.47%.

In MEB medium; Malt extract 20 g: Glucose 20 g: Peptone 1 g / 1 lit

- Malt extracts 100 g Carbon 50.60 g

  Malt extract 20 g Carbon $\frac{50.60}{100} \times 20 = 10.12$ g

- Peptone 100 g Carbon 51.89 g

  Peptone 1 g Carbon $\frac{51.89}{100} \times 1 = 0.52$ g

  Peptone 100 g Nitrogen 16.55 g

  Peptone 1 g Nitrogen $\frac{16.55}{100} \times 1 = 0.17$ g

- Glucose 180 g Carbon 72.00 g

  Glucose 20 g Carbon $\frac{72.00}{180} \times 20 = 8.00$ g

Total; C = 10.12 + 0.52 + 8 = 18.64

N = 0.17

Therefore C: N = 18.64:0.17 = 109.65:1

In PDB medium; Potato 200 g: Glucose 20 g / 1 lit

- Glucose 100 g Carbon 72.00 g

Glucose 20 g Carbon $\frac{72.00}{100} \times 20 = 8.00$ g

Total; C = 8

N = 0

Therefore C: N = 8:0

In YES medium; Yeast extracts 20 g: Sucrose 15 g / 1 lit

- Yeast extracts 100 g Carbon 46.83 g

Yeast extracts 20 g Carbon $\frac{46.83}{100} \times 20 = 9.37$ g

Yeast extracts 100 g Nitrogen 12.47 g

Yeast extracts 20 g Nitrogen $\frac{12.47}{100} \times 20 = 2.49$ g

- Sucrose 342 g Carbon 144 g

Sucrose 15 g Carbon $\frac{144}{342} \times 15 = 6.32$ g

Total; C = 6.32 + 9.34 = 15.69

N = 2.49

Therefore C: N = 15.69:2.49= 18.9:3

In Malt extract 20 g: Sucrose 20 g: Peptone 1 g / 1 lit

- Malt has carbon 10.12 g
- Peptone has carbon 0.52 g

Peptone has nitrogen 0.17 g

- Sucrose has carbon 6.32 g

Total; C = 10.12 + 0.52 + 6.32 = 16.96

N = 0.17

Therefore C: N = 16.69:0.17= 99.76:1

In Malt extract 20 g: Glucose 20 g: Sodium nitrate 1.03 g / 1 lit

- Malt extracts has carbon 10.12 g
- Glucose has carbon 8.00 g
- Sodium nitrate 1.03 g from

Peptone 100 g has Nitrogen 16.55 g

Peptone 1 g has Nitrogen $\frac{16.55}{100} \times 1 = 0.17$ g

Nitrogen 14.00 g in $NaNO_3$ 84.995 g

Nitrogen 0.17 g in $NaNO_3$ $\frac{84.995}{14} \times 0.17 = 1.03$ g

Total; C = 10.12 + 8.00 = 18.12

N = 1.03

Therefore C: N = 18.12:1.03= 17.59:1

In Malt extract 20 g: Glucose 20 g: Ammonium chloride 0.65 g / 1 lit

- Malt extracts has carbon 10.12 g
- Glucose has carbon 8.00 g
- Ammonium chloride 0.65 g from

Peptone 100 g has Nitrogen 16.55 g

Peptone 1 g has Nitrogen $\frac{16.55}{100} \times 1 = 0.17$ g

Nitrogen 14.00 g in NH_4Cl 53.5 g

Nitrogen 0.17 g in $NaNO_3$ $\frac{53.5}{14} \times 0.17 = 0.65$ g

Total; C = 10.12 + 8.00 = 18.12

N = 0.65

Therefore C: N = 18.12:0.65 = 27.88:1

In Malt extract 20 g: Glucose 20 g: Urea 0.36 g / 1 lit

- Malt has carbon 10.12 g
- Glucose has carbon 8.00 g
- Urea 0.36 g from

Peptone 100 g has Nitrogen 16.55 g

Peptone 1 g has Nitrogen $\frac{16.55}{100} \times 1 = 0.17$ g

Nitrogen 28 g in $CO(NH_2)_2$ 60 g

Nitrogen 0.17 g in $CO(NH_2)_2$ $\frac{60}{28} \times 0.17 = 0.36$ g

Carbon 12 g in $CO(NH_2)_2$ 60 g

Carbon 0.36 g in $CO(NH_2)_2$ $\frac{60}{12} \times 0.36 = 1.8$ g

Total; C = 10.12 + 8.00 + 1.8 = 19.92

N = 0.85

Therefore C: N = 19.92:0.85 = 23.44:1

In Malt extract 20 g: Glucose 20 g: Yeast extract 1.36 g / 1 lit

- Malt extracts has carbon 10.12 g
- Glucose has carbon 8.00 g
- Yeast extracts 1.36 g from

Peptone 100 g has Nitrogen 16.55 g

Peptone 1 g has Nitrogen $\frac{16.55}{100} \times 1 = 0.17$ g

Nitrogen 12.47 g in Yeast extracts 100 g

Nitrogen 0.17 g in Yeast extracts $\frac{100}{12.47} \times 0.17 = 1.36$ g

Carbons 46.83 g in Yeast extracts 100 g

Carbons 1.36 g in Yeast extracts $\frac{100}{46.83} \times 0.36 = 0.77$ g

Total; C = 10.12 + 8.00 + 0.77 = 18.89

N = 1.36

Therefore C: N = 18.89:1.36 = 13.89:1

7. %Product

$$\%\text{Product} = \frac{\text{Amount of oil before reaction (g)}}{\text{Amount of product (g)}} \times 100$$

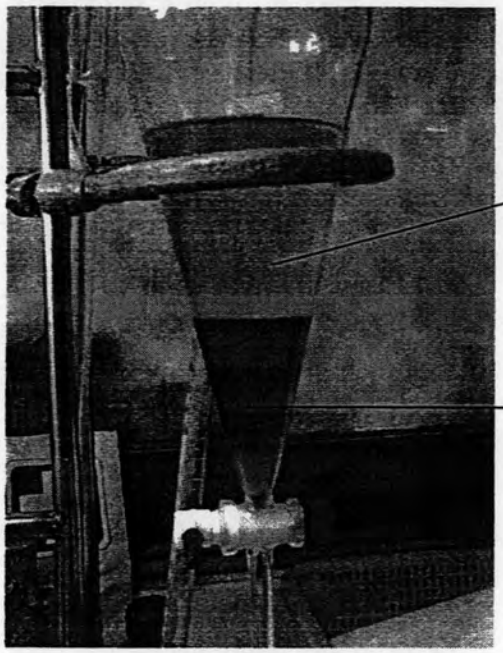
8. Molasses

Component (% by weight);

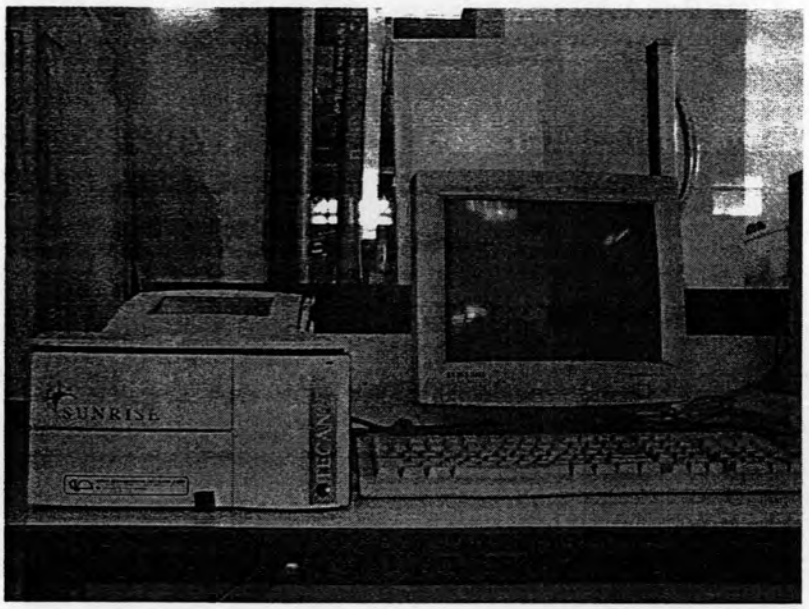
1. H_2O 20.65
2. Reducing sugar 13.00
3. Sugar 50.50
4. Sulfur 15.00
5. Rubber 3.01
6. Starch 0.42
7. Wax 0.38
8. Nitrogen 0.95
9. SO_2 0.46
10. P_2O_5 0.12
11. K_20 4.19
12. CaO 1.35
13. MgO 1.12

APPENDIX B

MATERIAL PICTURE



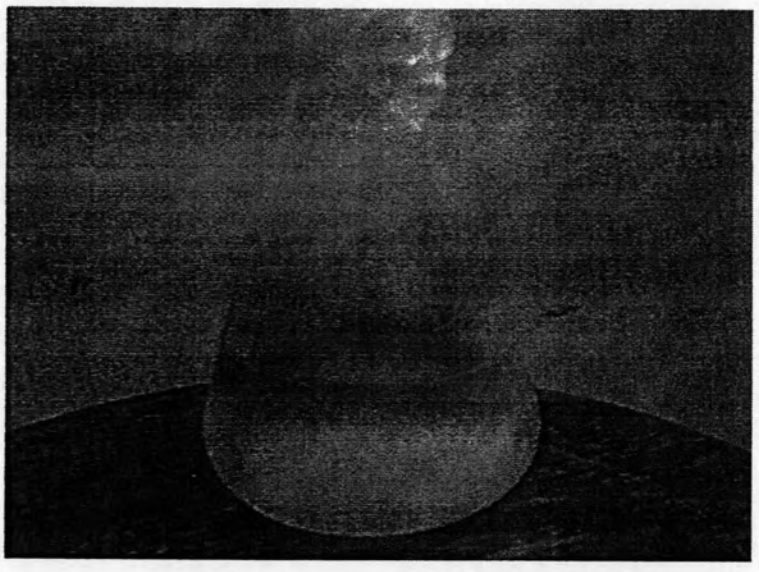
Biodiesel separation



Microplate reader

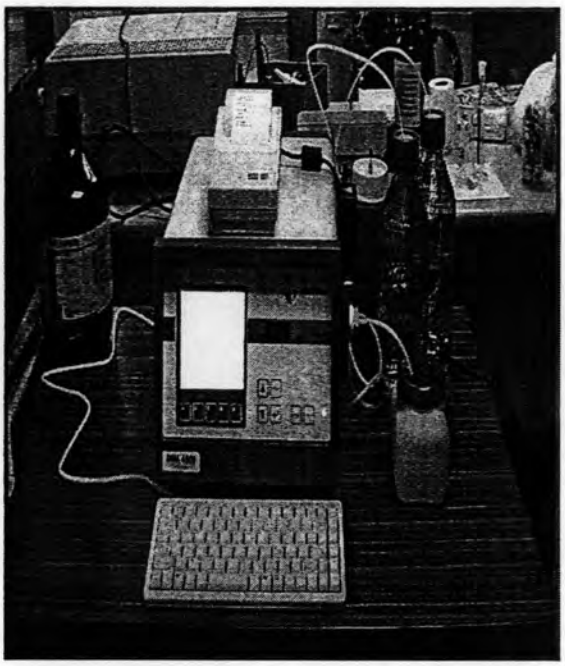
Crude oil of endophytic fungus

Biodiesel

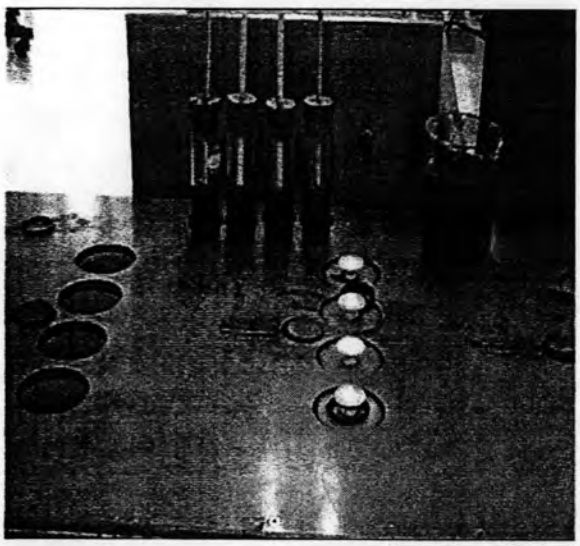


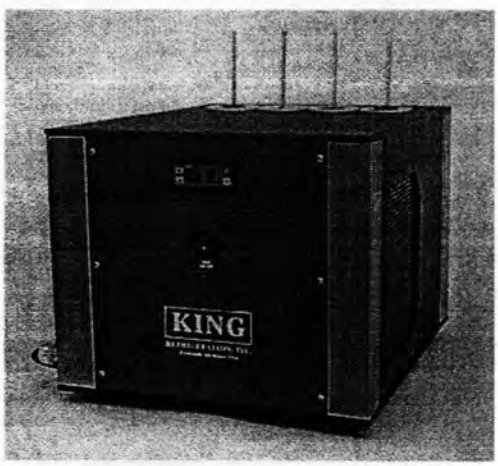
Hydrolysis cellulose solution

Flash point



Density and Specific gravity





Cloud point and Pour point

VITA

Miss Natta Rattanapanya was born on January 27, 1983 in Suphanburi, Thailand. She graduated at Satitkaset Kamphangsean School in 2000. She received the Bachelor Degree of Science in General Science, Kasetsart University in 2004. She continued her Master study in Program of Petrochemistry and Polymer Science, Faculty of Science, Chulalongkorn University in 2005 and completed the program in 2007.

Her present address is 50 Soi 3, Srisamran 2 Road, Songphinong, Suphanburi, Thailand 72110, Tel 0818417833, 035531655.