CHAPTER IV

RESULTS AND DISCUSSION

4.1 Colony characteristics and microcharacteristics of endophytic fungi

The endophytic fungus was grown on MEA medium. Colonial morphology of endophytic fungi is shown red aerial mycelium in Figure 4.1.

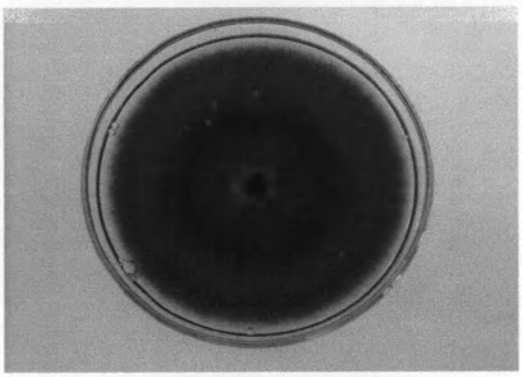


Figure 4.1 Colony of endophytic fungi.

These fungi were identified by the light microscope. From Figure 4.2, the microcharacteristics of endophytic fungus were sickle-shaped (x100) which was of *Fusarium sp.*

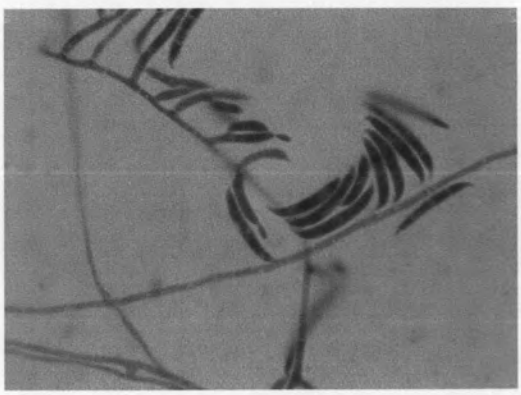


Figure 4.2 Microcharacteristics of endophytic fungi.

4.2 Effect of cultivation condition on oil production

4.2.1 Study of media selection for oil production

The endophytic fungus was grown on various 3 media including MEB, PDB and YES. The culture broths were colleted at 30 days, which MEB medium has more oil production than other media. (Table 4.1)

Table 4.1 Effect of kinds of media on oil production

Kinds of media (1 L)	C:N* Ratio	Dry weight (g)	Oil (g)	Oil (%)
Malt Extract Broth(MEB) (Malt:Glucose:Peptone(g) = 20:20:1)	109.7:1	10.62±0.03	2.92±0.01	27.50±0.02[b]
Potato Dextrose Broth(PDB) (Potato:Glucose(g) = 200:20)	8:0	10.12±0.01	2.73±0.01	26.98±0.06[a]
Yeast Extract Sucrose(YES) (Yeast extract:Sucrose(g) = 20:15)	18.9:3	10.47±0.02	2.85±0.02	27.22±0.05[ab]

* = gram of carbon to gram of nitrogen

It was found that the best results were obtained on MEB medium which dry mycelium was 10.62 g/L and amount of oil was 2.92 g/L (27.50% wt/dry weight of mycelia). Since MEB medium contains some essential nutrients such as carbon and nitrogen source (C:N ratio) more than PDB and YES, the growth and oil of endophytic fungus were enhanced. (See details in Appendix A) Therefore, the best result was obtained on MEB medium.

The difference of oil production from three media was analyzed via statistics in Duncan and F test. From the results, the calculated values of percent of oil

weight (%w/w) were shown the different value with significance levels (± 0.05). From Table 4.1, MEB[b] indicated the most quantity of oil production while PDB[a], YES[ab] have similarity values of oil production. (See details in Appendix A)

Therefore, MEB medium was selected to further study effect of media on oil production.

4.2.2 The time of extraction and extraction method on oil production

In this work MEB was selected to study on time of extraction and extraction method for the highest oil production.

Table 4.2 Effect of time and method for extract on oil production

Kind of media (1 L)	Time of extraction (days)	Dry weight (g)	Oil (g)	Oil (%w/w)
Malt Extract Broth(MEB) (Malt:Glucose:Peptone = 20:20:1)	1	10.62±0.03	2.92±0.01	27.50±0.12
Malt Extract Broth(MEB) (Malt:Glucose:Peptone = 20:20:1)	2	10.53±0.04	3.47±0.01	32.95±0.12
Malt Extract Broth(MEB) (Malt:Glucose:Peptone = 20:20:1)	3	10.22±0.02	3.73±0.02	36.50±0.19
Malt Extract Broth(MEB) (Malt:Glucose:Peptone = 20:20:1)	Soxhlet 6 h	10.20±0.02	3.75±0.03	36.76±0.20

The time for extraction on the highest oil production was extracted by soaking for 3 days and using ultrasonic bath for 30 min. So this method was selected to study effect of media on oil production.

4.2.3 Determination of growth profile of the endophytic fungus

Endophytic fungal cultivated in MEB medium. Growth of fungi was determined by mycelia dry weight and the oil of the dried mycelia was also investigated.

Table 4.3 Determination of growth profile of the endophytic fungus

Time (days)	Dry weight (g)	Amount of oil	
		(g)	%wt/wt
0	0	0	0
2	1.02±0.02	0.01±0.01	0.76±0.07
5	5.75±0.01	0.14±0.01	2.49±0.01
8	9.01±0.01	0.63±0.01	7.04±0.04
11	10.08±0.03	1.01±0.01	10.02±0.05
14	10.20±0.01	1.86±0.01	18.22±0.07
17	10.58±0.01	2.44±0.01	23.05±0.04
20	10.60±0.02	2.90±0.01	27.45 ± 0.09^{ab}
23	10.62±0.02	2.92±0.01	27.50 ± 0.09^{a}
26	10.61±0.01	2.91±0.01	27.32±0.04
29	10.59±0.01	2.86±0.01	26.87±0.01
32	10.56±0.02	2.44±0.01	23.10±0.09

From Table 4.3, it was found that the highest oil was produced at the beginning of stationary phase (20-23 days). (Figure 4.3)

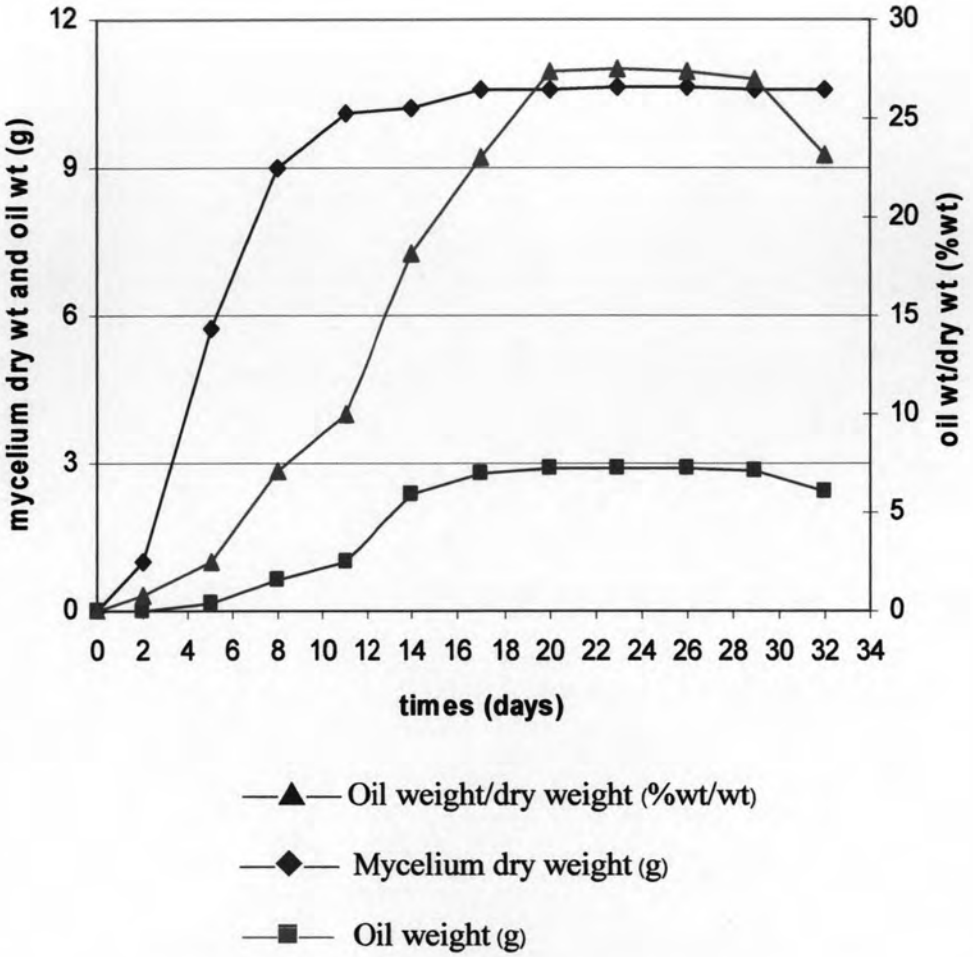


Figure 4.3 Growth curve in MEB medium.

In range of 2-20 days, the growth of fungus was increased at log phase. On 20-23 days in growth curve, it was found the fungus produced the highest oil at the beginning of stationary phase (Figure 4.3). During the first 20 days, the endophytic fungus was grown incomplete therefore the fungus produced the oil less than often 20 days. After 23 days, it was found the fungus remained stationary growth which the fungus produced the oil less than in 23 days.

In this work, the culture broth was collected at 20 days, it was selected to use for studing effect of media on oil production.

4.2.4 The influence of the amount of malt extract in MEB medium on oil production

The amount of malt extract in MEB medium was varied (0, 1, 5, 10, 20, 30 and 40 g/L). Table 4.4 presented the influence of the amount of malt extract in MEB medium on oil production.

Table 4.4 The amount of malt extract in MEB medium was varied for producing the highest oil

Malt(g):Glucose(g):Peptone(g) (1 L)	C:N* Ratio	Dry weight (g)	Oil (g)	Oil (%)
0:20:1	51.47:1	1.17±0.01	0.38±0.01	32.48±0.05
1:20:1	54.53:1	2.06±0.01	0.68±0.01	32.98±0.02
5:20:1	66.76:1	3.09±0.02	1.05±0.01	33.98±0.01[a]
10:20:1	82.05:1	5.81±0.02	1.98±0.02	34.07±0.07[ab]
20:20:1	109.65:1	10.62±0.01	2.92±0.01	27.50±0.02
30:20:1	143.20:1	13.10±0.02	2.46±0.01	18.78±0.05
40:20:1	173.77:1	16.53±0.04	3.12±0.01	18.88±0.01

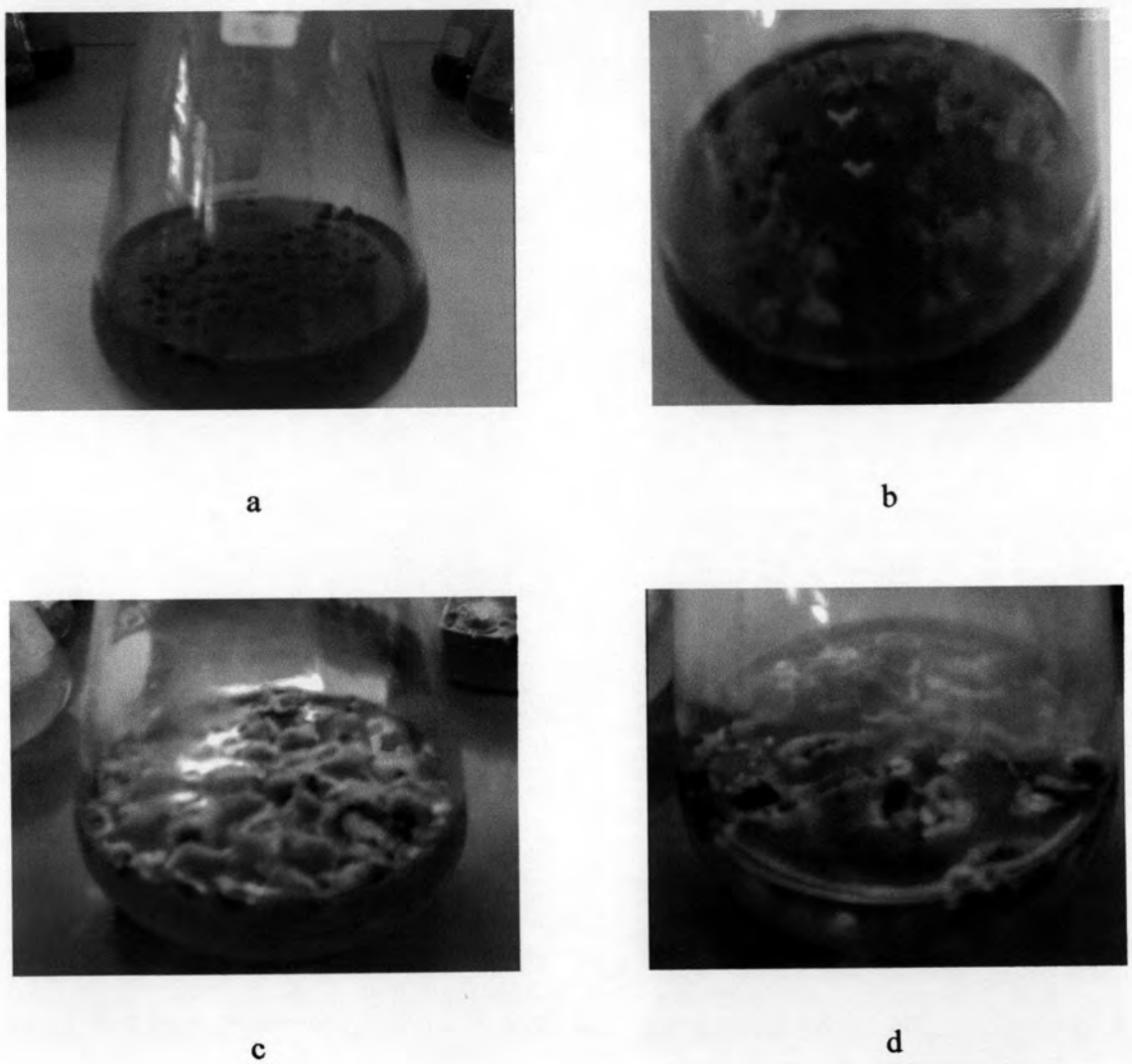
* = gram of carbon to gram of nitrogen

It was found that the best result was obtained at 10 g/L of malt ratio which dry mycelium was 5.81 g/L and amount of oil was up to 1.98 g/L (34.07% w/dry weight of mycelia). The oil production was increased in range of 0-10 g/L of malt extract because in this range C:N ratio was increased therefore the growth of endophytic fungus was increased too. On the other hand, C:N ratio was increased but the oil was decreased when above 10 g/L of malt extract was used because in this range another substance were produced from fungus so the oil was decreased.

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. From the results, the calculated

values of percent of oil weight (%w/w) were shown the different value with significance levels (±0.05). From Table 4.4, at both conditions have similarity values of oil production. (See details in Appendix A)

Therefore, 10 g/L of malt extract was selected to culture endophytic fungus in large scale.



a b

c d

Figure 4.4 Sample of the amount of malt extract in MEB medium was varied.

a. 0:20:1 b. 10:20:1

c. 20:20:1 d. 30:20:1

4.2.5 The effect of various carbon sources in MEB on oil production

From the effect of cultivated condition that carbon source was essential for growth and on oil production therefore the effect of various carbon sources including glucose, sucrose, cassava and molasses on the oil production were investigated. Table 4.5 showed the effect of various carbon sources in MEB on oil production.

Table 4.5 Carbon source of MEB media were varied for producing the highest oil

Carbon source	Carbon source (g)/L	C:N* ratio	Dry weight (g)	Oil (g)	Oil (%)
glucose	20	109.70:1	12.15±0.03	3.34±0.01	27.50±0.02[a]
sucrose	20	99.76:1	10.62±0.02	2.92±0.01	27.48±0.05[ab]
starch	20	115.52:1	4.30±0.03	0.77±0.02	17.91±0.03
molasses	40	58.31:1	3.08±0.02	0.31±0.01	10.06±0.03

* = gram of carbon to gram of nitrogen

From Table 4.5, the best result was obtain at glucose and sucrose for carbon source which dry mycelia 10.62 g/L and amount of oil up to 2.92 g/L (27.50%w/w dry weight) were obtained. Glucose has important for growth of fungus and then it found that the fungi produced the highest oil.

The different of oil production from each medium were analyzed via statistics in Duncan and F test. From the results, the calculated values of percent of oil weight (%w/w) were shown the different value with significance levels (±0.05). From Table 4.5, glucose[a] and sucrose[ab] have similarity values of oil production. (See details in Appendix A)

Therefore, glucose was selected to culture endophytic fungus in large scale.

Cassava

Molasses

Sucrose

Glucose

Figure 4.5 The endophytic fungus was growth in MEB various carbon sources.

4.2.6 The effect of various nitrogen sources in MEB on oil production

From the effect of cultivated condition, carbon source was essential for growth and oil production. The effects of various nitrogen sources including sodium nitrate, ammonium chloride, urea and yeast extract on the oil production were investigated.

Table 4.6 Nitrogen source of MEB media were varied for producing the highest oil

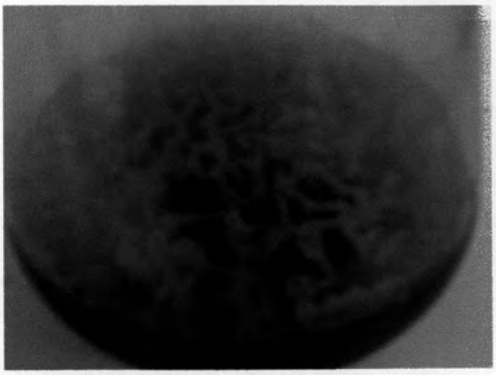
Nitrogen source	Nitrogen source (g)	C:N* ratio	Dry weight (g)	Oil (g)	Oil (%)
No extra nitrogen	0	18.12:0	13.12±0.04	3.65±0.01	27.79±0.02
Sodium Nitrate	0.85	18.12:0.14	15.21±0.02	3.93±0.01	25.84±0.08
Ammonium Chloride	0.5	18.12:0.13	12.31±0.02	0.64±0.01	5.20±0.08
Urea	0.6	18.24:0.28	22.73±0.01	2.16±0.01	9.50±0.05
Yeast Extract	1.1	18.64:0.14	16.91±0.01	5.73±0.02	33.89±0.09
Peptone	1.0	18.64:0.17	10.62±0.03	2.92±0.01	27.50±0.02

* = gram of carbon to gram of nitrogen

From Table 4.6, the variation of nitrogen sources showed that yeast extract gave the best result. Dry mycelium 16.91 g/L and amount of oil up to 5.73 g/L (33.89%w/w dry weight) were obtained. Since yeast extract contains some necessary nutrition such as vitamins or minerals, the growth and oil of endophytic fungus were enhanced.

Therefore, yeast extract was selected to culture endophytic fungus in large scale.

Sodium Nitrate



Ammonium Chloride

Urea

Yeast Extract

Peptone

no nitrogen

Figure 4.6 The endophytic fungus was growth in MEB various nitrogen sources.

4.3 The analysis of free fatty acid (FFA) and unsaturated fatty acids in the oils

In addition, free fatty acid (FFA) and unsaturated fatty acids in the oils produced from all culture conditions were analyzed by ASTM D-5555. The FFA of about 12.2% was contained in the oil cultured in all culture condition (See details in Appendix A). The unsaturated fatty acids present in the oil were determined by ^{1}H-nuclear magnetic resonance spectroscopy. The result showed that the unsaturated fatty acids of the oil were varied and up to 53.01% of the unsaturated fatty acids were obtained from the fungus cultured in MEB. [37]

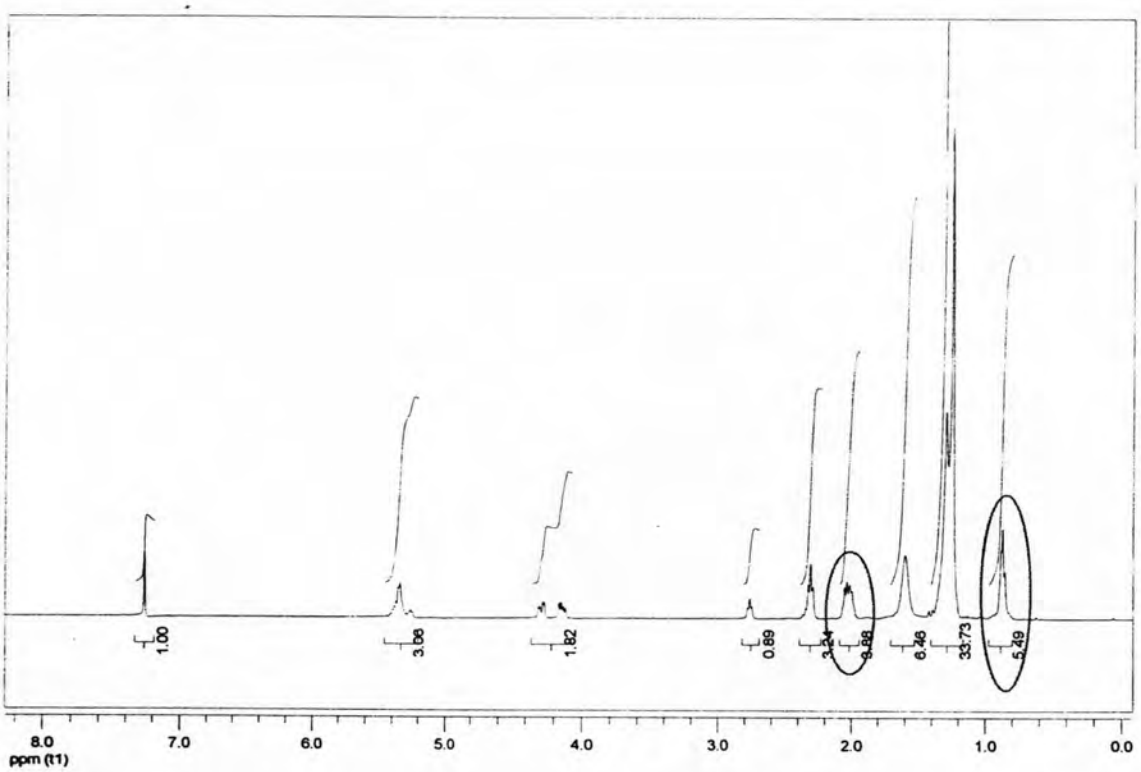


Figure 4.7 ^{1}H NMR spectrum of oil of endophytic fungus.

$$\text{A unsaturated} = \frac{I\,exper, allylic}{4} = \frac{3.88}{4} = 0.97$$

$$\text{Terminal methyl group} = \frac{5.49}{3} = 1.83$$

(I exper,allylic at 2.0-2.1 ppm and terminal methyl group at 0.8-0.9 ppm)

$$\text{Therefore, \%unsaturated} = \frac{0.97}{1.83} \times 100 = 53.01\%$$

4.4 Hydrolysis cellulose

From the effect of various carbon sources, glucose was the best carbon source. In this work, bagasse was hydrolyzed to glucose in medium for oil production.

The reducing sugar from hydrolysis cellulose (Bagasse) was determined by dinitrosalicylic acid method (DNS method).

1. Standard curve

Standard curve of absorbance versus glucose concentration was determined by microplate reader (Figure 4.8 and Figure 4.9), record the absorbance at 540 nm (See details in Appendix A).

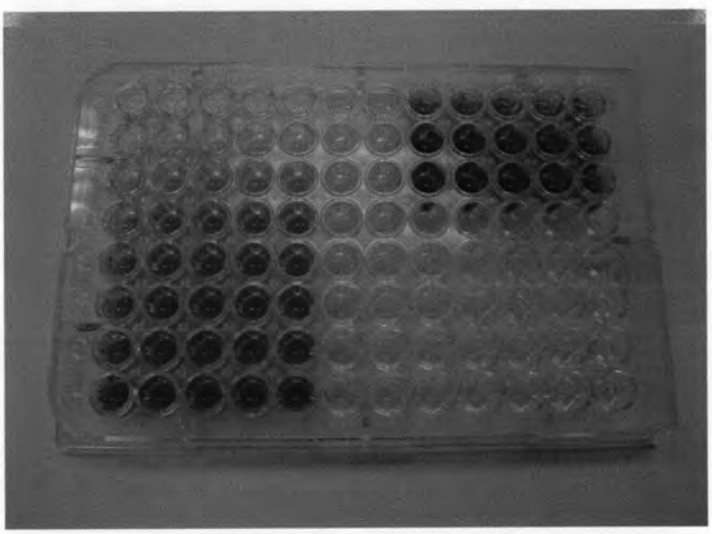


Figure 4.8 Glucose concentration was determined by microplate reader.

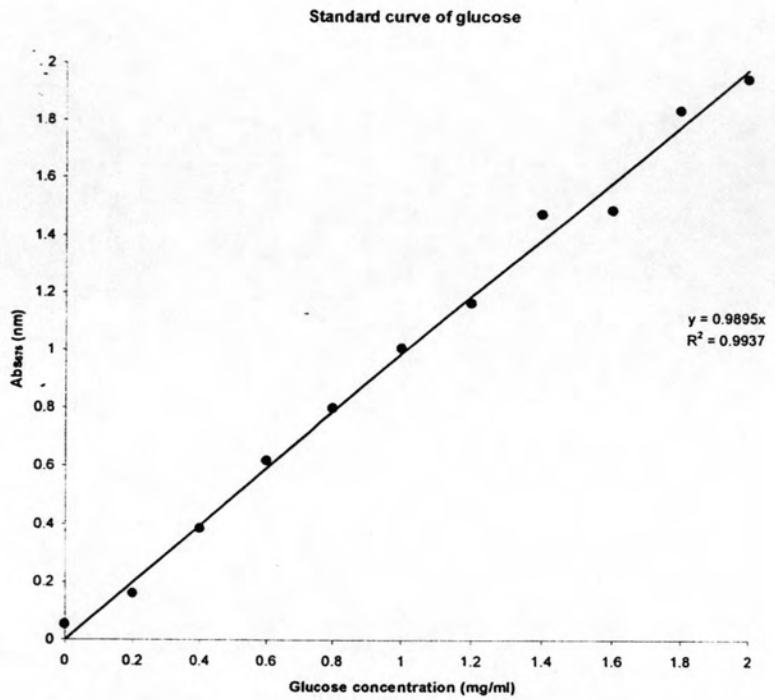


Figure 4.9 Standard curve of absorbance versus glucose concentration.

In the Figure 4.9, it shows the equation of standard curve as $y = 0.9895x$. The amount of sugar was calculated from this equation.

2. Reducing sugar

Reducing sugar of hydrolysis cellulose was determined by microplate reader (Figure 4.10 and Table 4.7), recorded the absorbance at 540 nm.

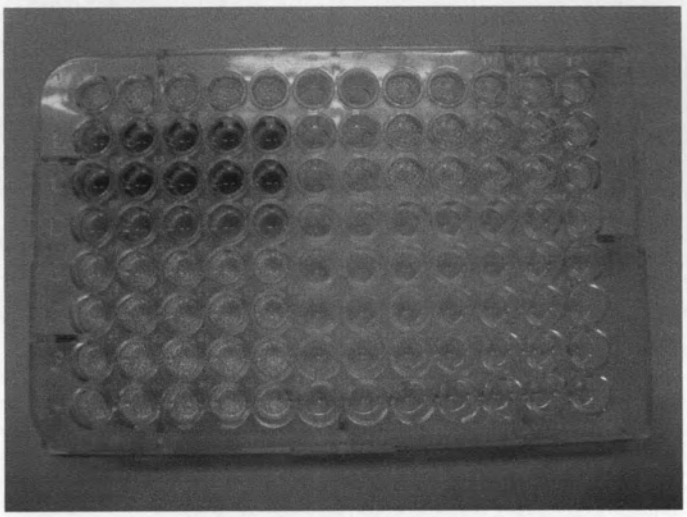


Figure 4.10 Hydrolysis cellulose was determined by microplate reader.

The amount of sugar was calculated from y = 0.9895x, the result was shown in table 4.7

Table 4.7 The absorbance and amount of sugar of hydrolysis cellulose

Days	Absorbance	Amount of sugar (mg/100mL)
0	0.0564	56.99
3	0.7954	803.84
6	0.8102	818.80
9	0.6592	666.20
12	0.2188	221.12
15	0.3170	320.36
18	0.0884	89.34
21	0.0666	67.31
24	0.0588	59.42
27	0.0624	63.06
30	0.0752	75.99

Calculation ; From Equation $y = 0.9895x$

$$0.0564 = 0.9895x$$

$$X = \frac{0.0564}{0.9895} = 0.057$$

Dilute 5 twice $\longrightarrow$ $0.057 * 5 = 0.285$

Hydrolysis cellulose 500 μL Sugar 0.285 mg

Hydrolysis cellulose 100,000 μL Sugar $\frac{0.285 * 100{,}000}{500}$ mg

$= 56.99$ mg/100 mL

Bagasse was hydrolyzed to glucose for medium on oil production that glucose was product up to 56.99 mg/100 mL of medium.

3. Cultivation fungus in glucose from hydrolysis cellulose

The growth of endophytic fungus was cultivated in MEB medium that containing malt extract 5 g, yeast extract 1.1 g, hydrolyzed solution 500 mL and distilled water 500 mL, to produce oil.

Table 4.8 Determination of growth profile of the endophytic fungus

Time (days)	**Dry weight** (g)	**Amount of oil**	
		(g)	%wt/wt
0	0	0	0
2	0	0	0
5	0.02±0.01	0.30±0.01	0.87±0.03
8	0.09±0.03	1.02±0.01	2.96±0.05
11	2.01±0.01	0.13±0.01	6.47±0.05
14	2.32±0.01	0.33±0.01	14.22±0.07
17	2.46±0.01	0.49±0.01	19.92±0.04
20	2.47±0.02	0.52±0.01	21.05±0.09
23	2.48±0.02	0.51±0.01	20.65±0.09
26	2.47±0.01	0.50±0.01	20.24±0.04
29	2.43±0.01	0.47±0.01	19.34±0.01
32	2.36±0.02	0.37±0.01	15.68±0.09

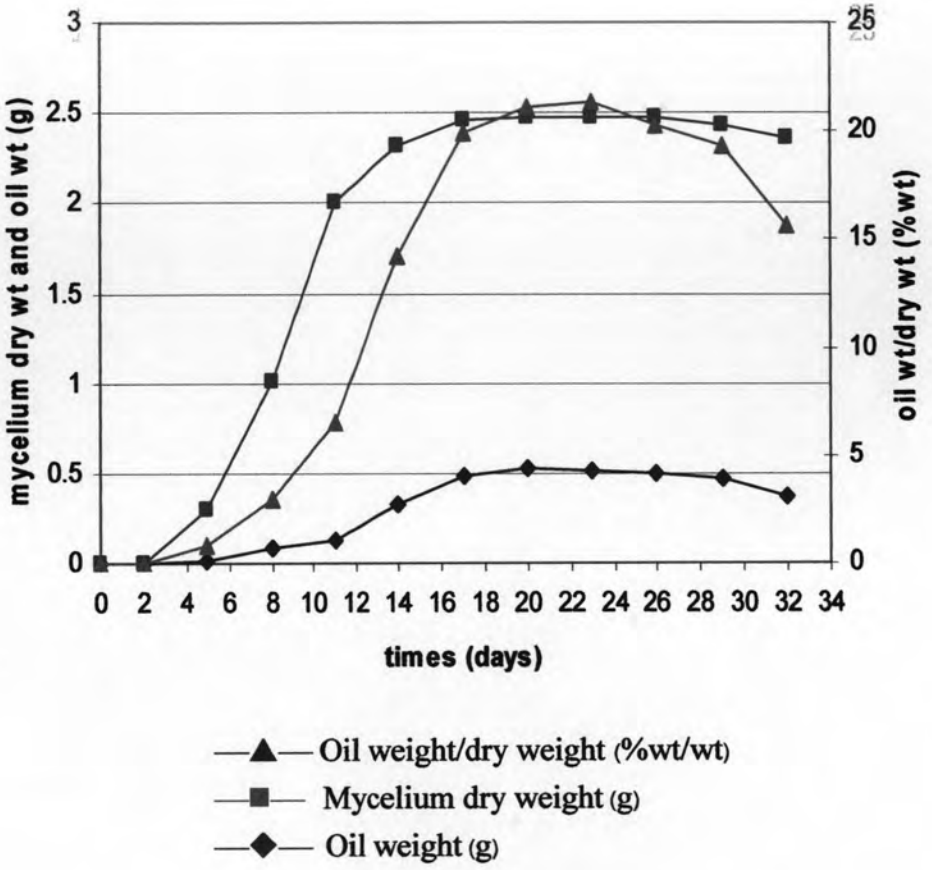


Figure 4.11 Growth curve in hydrolysis glucose medium.

It was found that the endophytic fungus produced the highest oil at the beginning of stationary phase (20 days) which dry mycelium was 2.47 g/L and amount of oil was up to 0.52 g/L (21.05% w/dry weight of mycelia). The oil production was increased in range of 2-20 days. On the other hand, the oil was decreased when above 20 days that corresponding to used glucose. (Figure 4.11)

This medium can be used for produced the same amount of oil as glucose. However, the cost of hydrolyzed cellulose from bagasse is much less than glucose.

4.5 Biodiesel production

The compositions of fatty acids of crude oil of endophytic fungus are also analyzed using AOAC method (AOAC 969.33). The results are shown in Table 4.9.

Table 4.9 Compositions of fatty acids in crude oil of endophytic fungus (See details in Appendix A)

Fatty acid composition	% Total fatty acids
Myristic acid, C14:0	0.24
Pentadecanoic acid, C15:0	0.07
Palmitic acid, C16:0	24.42
Palmitoleic acid, C16:1	0.28
Stearic acid, C18:0	17.81
Oleic acid, C18:1	25.02
Linoleic acid, C18:2	30.14
α-Linolenic acid, C18:3	0.08
Arachidic acid, C20:0	0.75
Beheric acid, C22:0	0.36
Lignoceric acid, C24:0	0.19

It was found that the oil of endophytic fungus mainly consisted of linoleic acid and oleic acid. The component is saturated and unsaturated fatty acid of the oil of endophytic fungus similarly to palm oil. [38]

4.5.1 Synthesis of biodiesel from crude oil of endophytic fungus

1. One-step alkali base catalyzed transesterification

The transesterification process was carried out at four different oil: methanol ratios as shown in Table 4.10 It found that the transesterification at 65°C for 1 h gave 30.20% conversion when the ratio of oil : methanol was 1:40.

- Effect of molar ratio of methanol/oil

The molar ratio of methanol to oil of endophytic fungus is one of the important factors that affect the conversion to methyl esters. Table 4.10 shows the effect of methanol/oil molar ratio on %product and %conversion.

Table 4.10 Molar ratio converts crude oil as oil methyl ester via base-catalyzed process (1% NaOH).

Molar ratio (Oil : CH_3OH)	**%Product**	**% Conversion**
1:10	-	-
1:20	-	-
1:30	-	-
1:40	26.21	30.20

Remark ; - = not detected product

From the Table 4.10, The high conversion was obtained with 1:40 of oil:methanol as 20.21 %product and 30.20 %conversion.

- Effect of catalyst amount

Effect of catalyst amount was investigated. The results are shown in Table 4.11.

Table 4.11 The amount of NaOH converts crude oil as oil methyl ester via base-catalyzed process (Oil : MeOH ratio 1:40).

Amount of NaOH (%w/w oil)	**%Product**	**% Conversion**
0.5	28.87	30.11
1.0	26.21	30.20
1.5	25.09	30.21

From Table 4.11, the high product and the high conversion was obtained with 1% wt oil of NaOH as 20.21 %product and 30.20 %conversion.

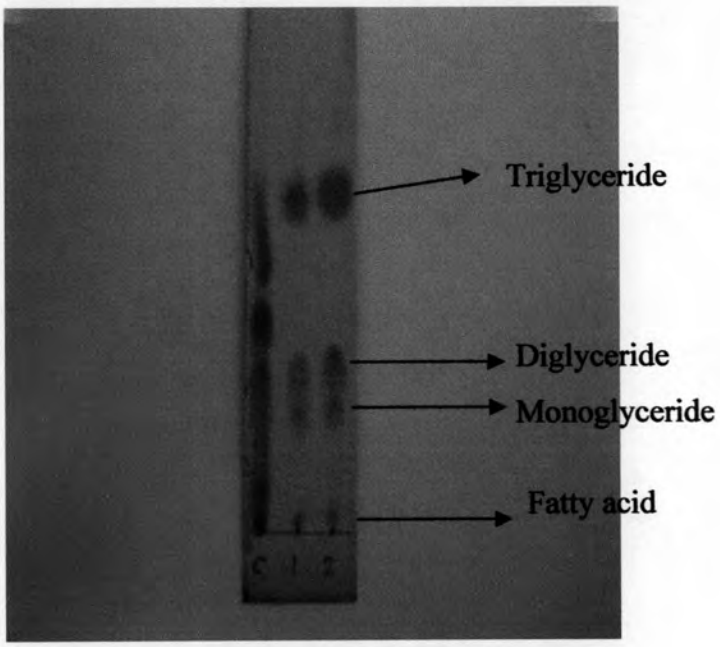
The results of one-step base catalyzed process (1%NaOH) were shown in Table 4.10 and 4.11 indicated that the optimum condition of alkali base catalyzed transesterification for oil of endophytic fungus required more catalyst and methanol. The optimum amount of NaOH and the optimum methanol to oil ratio were 1.0% wt and 1:40, respectively. The reason of higher consumption catalyst NaOH and methanol in the transesterification process of oil of endophytic fungus is considered to be FFA.

2. One-step acid catalyzed transesterification

2.1 1.0% H_2SO_4 catalyst

The oil of endophytic fungus was used to produce biodiesel using 1.0% (% oil weight) H_2SO_4 catalyst and Oil : MeOH ratio 1:10 at 65°C. The results are shown in figure 4.13 and table 4.12.

The reproductive TLC of FFA was converted into FAME in this Figure 4.12.



C : Crude oil of endophytic fungus

1 : Adsorption time of methyl ester via acid-catalyzed process 1h.

2 : Adsorption time of methyl ester via acid-catalyzed process 2 h.

Mobile phase: a mixture of hexane, ethyl acetate (90:10 v/v)

Stationary phase: TLC aluminium sheet, silica gel 60 F_{254}

Figure 4.12 Reproductive TLC of FFA was converted into FAME (H_2SO_4 catalyst).

The analysis was remained of FFA after acid pretreatment with 1.0% (% w/w) H_2SO_4 by titration method. The results are shown in Table 4.12.

Table 4.12 Acid pretreatment with titration method

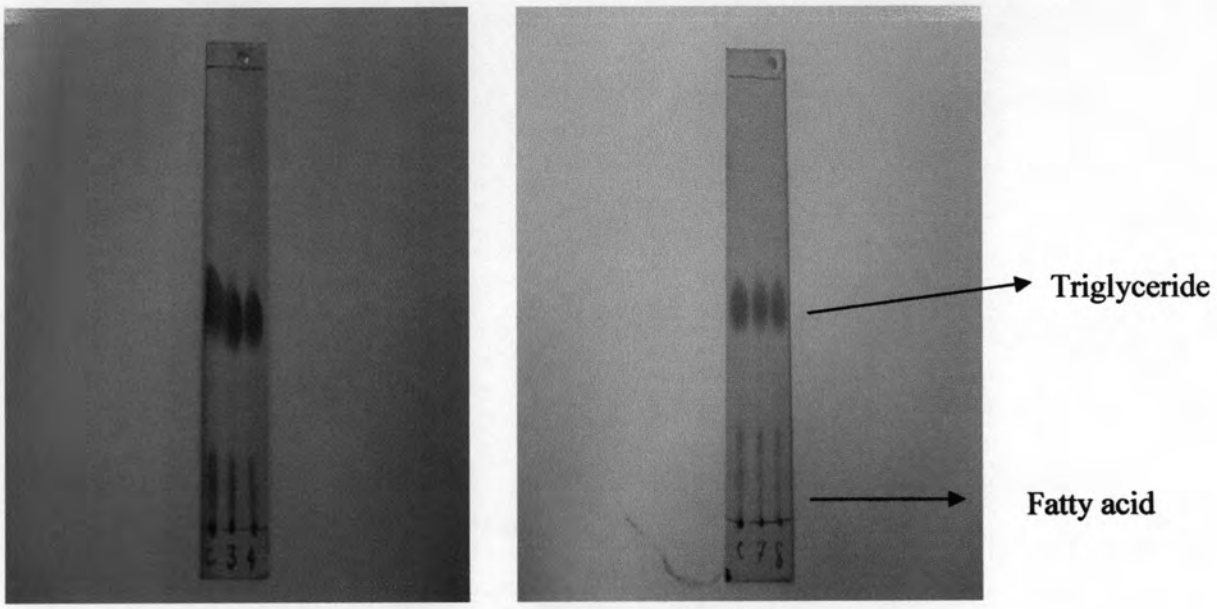
Reaction Time (hour)	**%FFA**
0	12.2
1	1.4
2	0.9

The results indicated that esterification production of oil of endophytic fungus using 1% H_2SO_4 (%w/w) as catalyst produced. However, oil of endophytic fungus can be produced when decreasing FFA to 0.9% and then it was converted to transesterification in basic condition at 90°C to 2 hours up to 76.38%.

Thus, this condition was selected to reduce free fatty acid in oil of endophytic fungus to further synthesis biodiesel with base catalyzed.

2.2 1.0% Amberlyst 15 catalyst

The oil of endophytic fungus was used to produce biodiesel using 1.0% Amberlyst 15 catalyst (% w/w) and oil : MeOH ratio 1:10 at 65°C. The results are shown in Figure 4.15.



C : Crude oil of endophytic fungus

1-11 : Adsorption time of methyl ester via acid-catalyzed process 1-11 h.

Mobile phase: a mixture of hexane, ethyl acetate (90:10 v/v)

Stationary phase: TLC aluminium sheet, silica gel 60 F_{254}

Figure 4.14 Reproductive TLC of FFA was converted into FAME.

The analysis was remained of FFA after acid pretreatment with 1.0% Amberlyst 15 (% w/w) catalyst. The results are shown in table 4.13.

Table 4.13 Acid pretreatment by Amberlyst 15 catalyst

Time (hour)	%FFA
0	12.2
1	11.8
2	11.7
3	11.1
4	10.2
5	9.9
6	9.3
7	8.1
8	6.3
9	5.5
10	5.2
11	5.2

The results are shown in Figure 4.15.

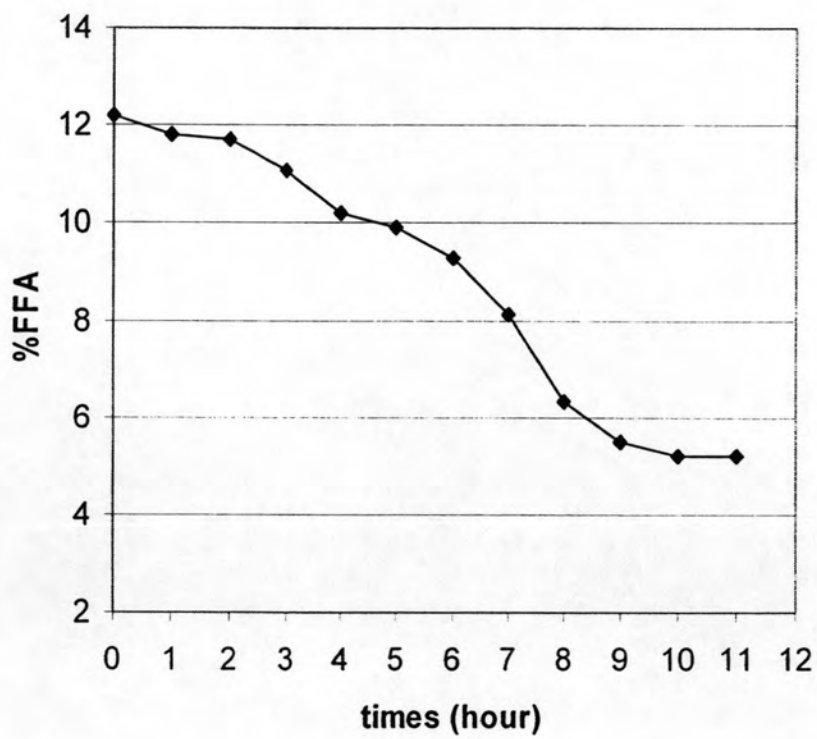


Figure 4.15 Acid pretreatment with titration method of Amberlyst 15 catalyst.

From the results in Table 4.13 and Figure 4.15, when considering at FFA content in oil of endophytic fungus, FFA content in oil of endophytic fungus was decreased from 12.2% to 5.2% in the reaction time up to 11 hours and FFA content in oil of endophytic fungus up to 5.2% then 1.0% Amberlyst 15 (% w/w) catalyst was not suitable for synthesis biodiesel. Therefore, 1% H_2SO_4 (% w/w) was selected to reduced FFA in acid pretreatment.

3. Enzymatic catalyzed transesterification

The oil of endophytic fungus was used to produce biodiesel using 10% (%w/w) enzyme catalyst (Noyozyme 435) in oil : MeOH ratio (1:2). The results are shown in Table 4.14 and Figure 4.17.

Table 4.14 Conversion (%) oil of endophytic fungus by enzyme catalyst in oil : MeOH ratio 1:2.

Time (hour)	% Conversion	Time (hour)	% Conversion
1	11.56	11	12.58
2	11.68	12	14.60
3	12.67	13	34.33
4	12.06	14	55.07
5	10.27	15	18.06
6	9.15	16	12.42
7	1.24	17	11.33
8	0.98	18	11.09
9	0.29	19	10.59
10	12.41		

The reproductive TLC of FFA was converted into FAME as shown in this Figure 4.16.

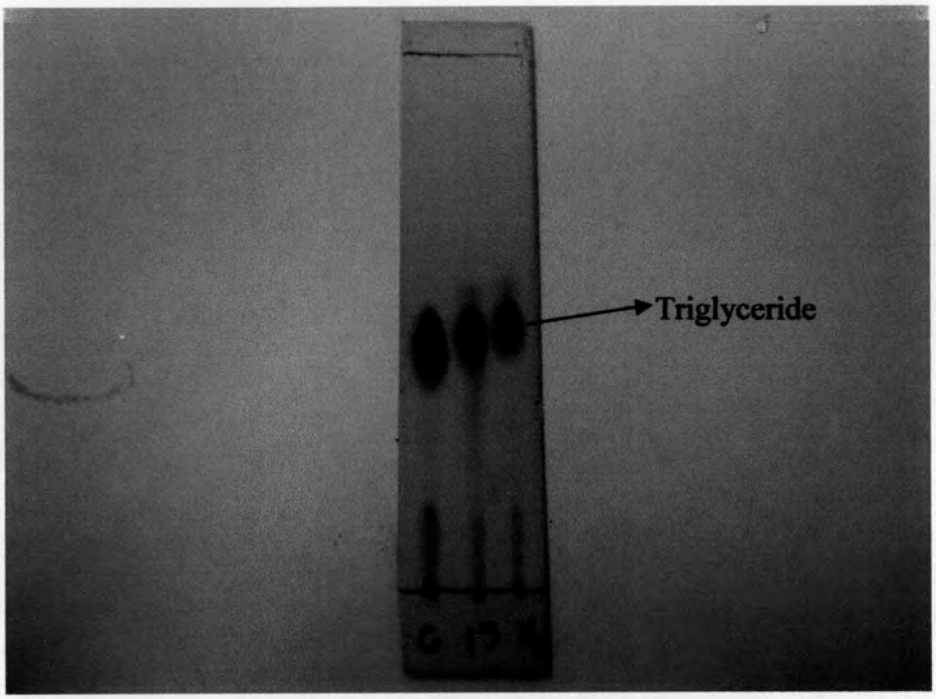


Figure 4.16 Reproductive TLC of FFA was converted into FAME.

The conversion was increased in range of 1-4 days. On the other hand the conversion was decreased in range of 5-9 days because methanol was losing in the reaction. After 9 hours, methanol was added to reaction so the conversion was increased up to 55.07% for 4 hours. After 14 hours, the conversion was reduced because the reaction was reversed.

The percent conversion was 55.07 %conversion for 14 hours. The enzymatic catalyst was used to produce biodiesel in the reaction time up to 14 hours that this method was not suitable for synthesis biodiesel from oil of endophytic fungus.

4. Synthesis of methyl ester via 2-step catalyzed process

The first step was acid pretreatment with 1%H_2SO_4 (% w/w) catalyst then in the second step the oil (from first step) was synthesized methyl ester by base catalyst (1%NaOH and K_2CO_3 (% w/w)) at 65°C to produce biodiesel.

- Effect of molar ratio of methanol to oil

Table 4.15 shows the effect of molar ratio of methanol to oil on %conversion and %product.

Table 4.15 Product and conversion (%) of the synthetic methyl ester from oil of endophytic fungus by two-step process (1% H_2SO_4 and 1% NaOH (% w/w) catalyst)

Molar ratio (Oil : CH_3OH)	**Product (%)**	**Conversion (%)**
1:10	95.7	97.34
1:8	91.3	93.40
1:6	88.5	90.20

When the methanol amount was decreased, it was found that %conversion and %product were decreased considerably. The best ratio of oil : MeOH was 1:10.

Additionally the methyl ester was confirmed by analysis of ^{1}H NMR was 100%conversion as showed in Figures 4.18.

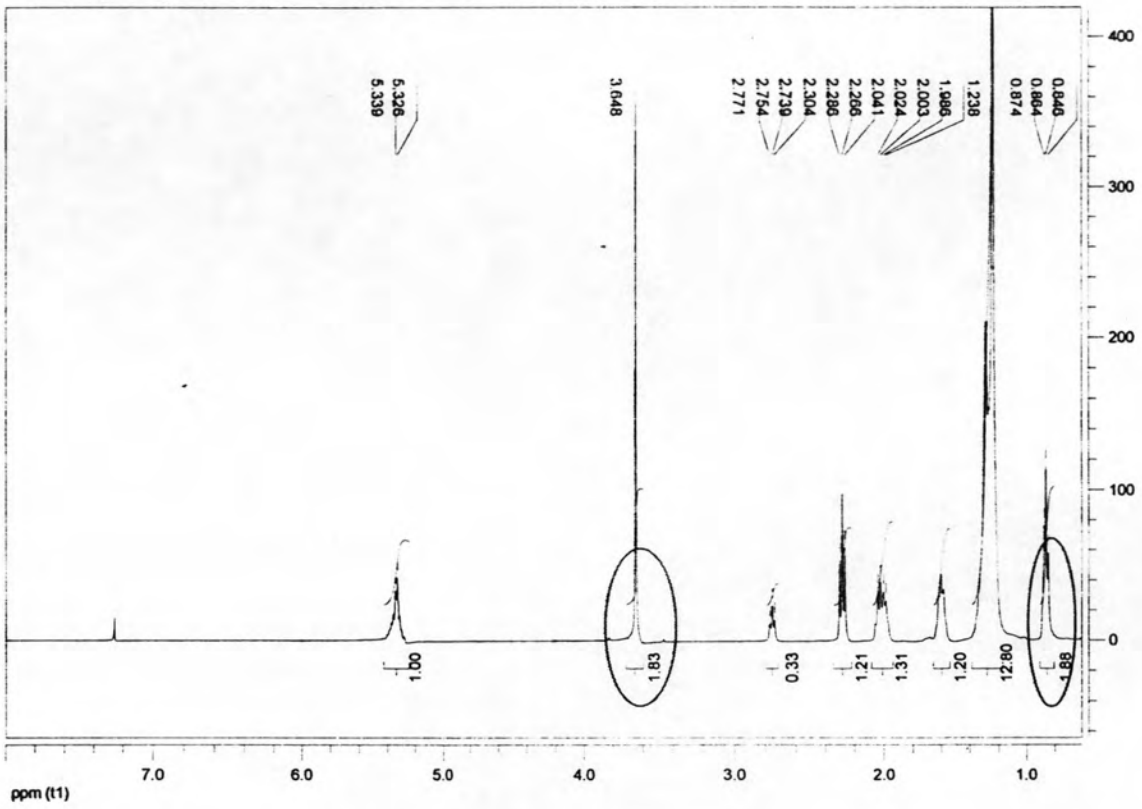


Figure 4.18 ^{1}H NMR spectrum of biodiesel.

$$\%\text{conversion} = \frac{1.83}{1.88} = 97.34\%$$

Table 4.16 Product and conversion (%) of the synthetic methyl ester from oil of endophytic fungus by two-step process (1% H_2SO_4 and 1% K_2CO_3 (% w/w) catalyst)

Molar ratio (Oil : CH_3OH)	**Product (%)**	**Conversion (%)**
1:10	18.2	21.2
1:20	26.2	38.7
1:30	39.7	53.9

Table 4.16 , The best ratio of oil : methanol was 1:30 that the conversion was 53.9%. NaOH and K_2CO_3 were compared, the result indicated that at reaction condition; 1%catalyst, 1:10 molar ratio of oil : methanol, at 65°C, NaOH gave higher conversion than K_2CO_3 because NaOH has higher basicity than K_2CO_3. Thus, NaOH catalyst was selected to catalyze transesterification of oil of endophytic fungus.

The suitable method for biodiesl production was produced biodiesel by a two-stage transesterification process. The first stage was acid pretreatment process by 1%H_2SO_4 (% w/w) at 65°C, which could reduce the FFA level of oil of endophytic fungus to less than 1%. The second stage, alkali base catalyzed transesterification process 1%NaOH (% w/w) at 65°C gave 94.78% methyl ester yield and 97.34% conversion.

4.5.2 Properties of biodiesel

The physical properties of biodiesel were determined as shown in Table 4.17.

Table 4.17 Properties of biodiesel

Fuel Property	Standard Biodiesel	Biodiesel from oil of endophytic fungus
Fuel Standard	ASTM PS 121	ASTM PS 121
Fuel composition	C12-C22 FAME	C14-C24 FAME
Methyl ester, %wt.	>96.5	98.92
Viscosity, @ 40° C	1.9-5.0	4.83
Specific Gravity kg/l @ 15° C	0.87 – 0.90	0.8746
Density, lb/gal @ 60° C	7.328	7.018
Flash Point (°C)	100-170	150
Pour point (°C)	-15 - 10	6
Cloud point (°C)	-3 - 12	9

* The analysis from Department of Energy

The fuel properties of the oil with respect to the viscosity, specific gravity, density, flash point, pour point and cloud point of biodiesel are close to standard. Viscosity (40°C) is 4.83 cSt/s. Specific gravity is 0.8746 kg/l. Density is 7.018 lb/gal. Flash Point is 150°C. Pour point is 6°C. Cloud point is 9°C.