Chapter 4

Results and Discussions

Part 1 Studies on callus induction from endosperm explants of Physic Nut (*J.curcas* L.)

Experiment 1 Effects of auxin types and concentrations on callus induction from endosperm explants of physic nut (J.curcas L.).

Callus induction study was carry out by culturing the sterile endosperm explants of physic nut in MS(1962) medium containing different auxin types; 2, 4-D, NAA and IBA and different concentrations; 5 and 10 µM compared to the control medium which without any addition of plant growth regulators. All the cultures were incubated at room temperature of 25±3ºC, under darkness condition. The morphologies consisted degree of callogenesis, types of callus, colors of callus and percentage of callus formation were observed and results were recorded. The results showed that the endosperm explants of physic nut could not grow in the control medium which was lacked plant growth regulators (showed in figure 6A). The same result conducted by other research, for example Kiong *et al*., 2007 who reported that callus did not formed in the control medium without plant growth regulators and Dalila *et al.* (2013) who indicated that the basal medium without plant growth regulators did not induce any callus growth of *Barringtonia racemosa*. Moreover, effects of auxin types and concentrations were shown that auxin types could induce callus formation from endosperm explants of physic nut. Our results was similar to the several researches which reported that parts of physic nut could induce callus easily (hypocotyl explant; Monacelli *et al*., 1995, leaf explants; Rajore and Batra, 2007, embryo explants; Astra *et al*., 2006). For the callogenesis from the endosperm explants of physic nut, calli were initiated from the cut margins of the explants in the presence of suitable plant growth regulators (showed in figure 6B).

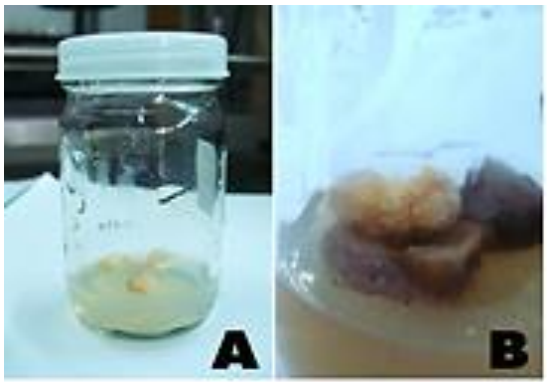

Figure 6 Callus formationfrom endosperm explant of physic nut
A. No callogenesis. B. Callogenesis at the cut margin of the endosperm explants.

In addition, it was found that the auxin concentration at 10 µM could induce callus formation while the concentration at 5 µM was not induced callus formation. The maximum percentage of callus formation was achieved on MS medium supplemented with 10 µMNAA (100%) (figure 7F and table 5) followed by 2,4-D and IBA 92% and 86% respectively(figure 7D, 7E and table 8). The results indicated that only the appropriate concentrations of auxin can be induced callus formation from endosperm explants of physic nut. Similar results was observed by Kiong *et al*. (2008) who found that initiation of callus from endosperm explants of *Cycas revolute* could be seen in suitable medium whereas in less appropriate medium, the induction was not found. Basically this could be due to the fact that plant specificity towards plant growth regulators. Although the percentage of callus induction can reach up to 100%.However, the appropriated characteristic of callus for the cell suspension culture was friable callus type. According to Trigano and Gray (2005) usually rapid growth and light color callus indicate a healthy culture and friable callus of crumbly appearance is very suitable for breaking up, either for sub-culturing or to produce a cell suspension culture.

In subsequent observation found that the presence of 10 µM 2, 4-Dand NAA gave average larger diameter callus (1.0-2.0 cm) while 10 µM IBA gave smaller callus (less than 1.0 cm).In addition, the type of callus was found that the concentration at 10 µM of 2, 4-D produced compact callus than friable callus (C/F) whereas the presence of NAA and IBA induced friable callus than compact callus (F/C)and friable callus (F) respectively (table 8). Moreover the healthiest of callus was found that the concentration of 2, 4-D

and NAA at 10 µM gave yellow (++) and pale yellow (+) callus respectively while MS medium supplemented with 10 µM IBA gave pale yellow (+) callus (figure 7A-7G). From the results, it was showed that MS medium supplemented with 10 µM NAA was suitable for induction callus from endosperm explants of physic nut. Similarly, Li *et al*. (2012) who reported that the best medium for callus induction in various tissues of physic nut were the combination of 1 mg/L NAA and 0.1 mg/L Kinetin. However, Pei *et al*. (2006) reported that among all the hormone combinations, 2.0 mg/L of 2, 4-D induced more calli of physic nutthan2.0 mg/L of NAA did whereas, Savitha and Naik (2011) reported that good callus formation was obtained on MS medium containing 0.93 µM Kinetin and 0.73 µM IBA and Dalila *et al*. (2013) found that *Barringtonia recemosa* leaf and endosperm explant in different type of basal medium, produce the highest callus induction was obtained in MS medium supplemented with1.5 mg/L 2, 4-D and 0.5 mg/L kinetin. Nonetheless, the research of the above were used plant growth regulators combination (auxin and cytokinin) while in this study used only one type of auxin. It was possible that, callus induction from endosperm of physic nut can use appropriated concentration of only one type of auxin which in this case was 10 µM of NAA.

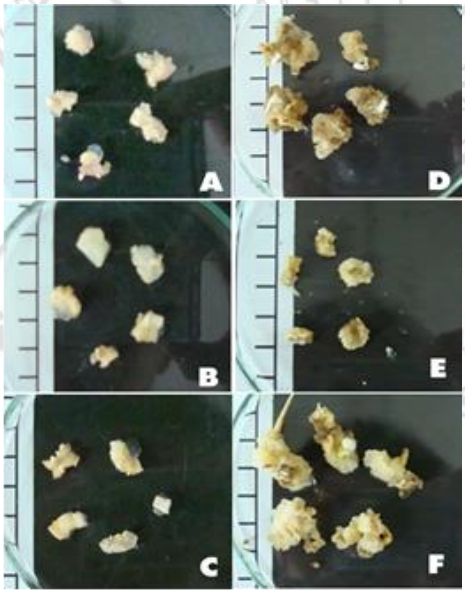

Figure 7 Callogenesis of physic nut endosperm on MS medium supplement with various auxins at different concentrations.
A. 5 µM 2, 4-D; B. 5 µM IBA; C. 5 µM NAA
D. 10 µM 2, 4-D; E. 10 µM IBA; F. 10 µM NAA

Table 8 Effects of auxin types and concentrations on callus formation from endosperm explants of *J. curcas* L.

Plant growth regulator	Concentration (µM)	Percentage of callus formation (%)[1/]	Degree of callus formation[a]	Type of callus[b]	Color of callus[c]
Control	0	0c	-	-	-
2,4 - D	5	0c	-	-	-
	10	92ab	Average	C/F	Y (++)
NAA	5	0c	-	-	-
	10	100a	Average	F/C	Y (+)
IBA	5	0c	-	-	-
	10	86b	Poor	F	Y (+)

[1/] Means within column followed by the same letters do not significant different as determined by least significant difference test at P<0.05.

[a] Degree of callus formation : absence of callus (-), less than 1.0 cm (poor), 1.0 - 2.0 cm (average), more than 2.0 (good)

[b] Type of or Callus texture : compact callus (C),friable callus (F), compact callus than friable callus (C/F), friable callus than compact callus (F/C)

[c] Color of callus : absence of callus (-), white (W), pale yellow (Y (+)), yellow (Y (++)), dark yellow (Y (+++)), brown (B)

Part 2 Study on growth and oil content from cell suspension culture of endosperm cell of Physic Nut (*J. curcas* L.)

Experiment 1 Growth and oil content from cell suspension culture of endosperm cell of J. curcas L.

The study on growth and oil contents of endosperm cell of *J. curcas* L. was conducted by use the cell suspension culture technique, in 125 mL of Erlenmeyer flasks contained 30 mL liquid MS medium supplemented with 10 µM 1-Naphthaleneacetic acid (NAA) alone. It was established by using 1 gram of 30 day-old friable callus as inoculums (figure 8A-8B).

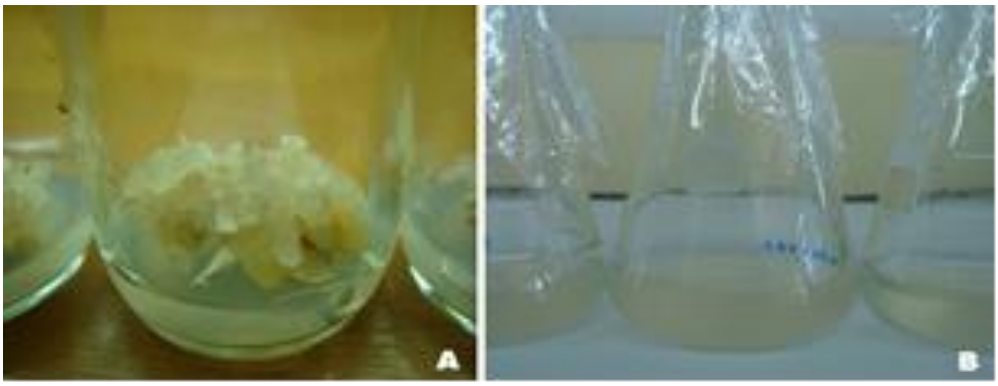

Figure 8 Cell suspension culture ofendosperm cell of *J. curcas* L.
A. Friable callus on solid MS medium.
B. Endosperm suspended cells in liquid MS medium.

Afterday 5th of the culture, the growth and the oil contents of Jatropha endosperm cells were measured. The friable calli of endosperm of *J. curcas* L. were easily broken apart and dispersed into single cells or aggregated cells (figure 9A - 9B) in liquid MS medium. Similar results were observed by Trigano and Gray (2005) who reported that the friable calli usually rapid growth and light color calli indicate a healthy culture. Furthermore, friable calli of crumbly appearance is very suitable for breaking up, either for sub-culturing or to produce a cell suspension culture. In this study, the cells showed different shapes under the light microscopy(figure 9C) and some cells were elongated and highly vacuolated with sparse cytoplasm (figure 9D).

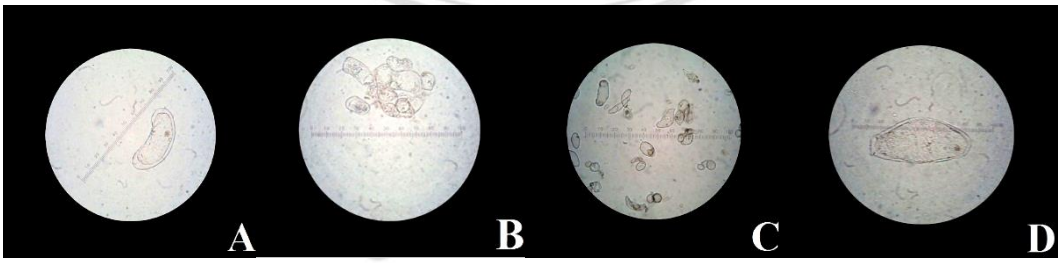

Figure 9 Endosperm cells of physic nut in liquid MS medium
Supplemented with 10 µM NAA.

A. Single cell, B. Aggregated cells, C. Different of cell shapes in suspension culture, D. Highly vacuolated in some cells. (Magnification 40X, Olympus CX31)

A. Growth of cell suspension culture

A1. Fresh weight (FW) and Dry weight (DW)

At day 5th of the culture, fresh weight (FW) and dry weight (DW) had slowly increased(1.1030±0.0381 g/30ml and 0.0790±0.0022 g/30ml respectively) until day 15thof the culture (1.7606±0.0244 g/30ml and 0.1040±0.0010 g/30ml respectively).The maximum FW and DW were received on day20th of the culture(2.3538±0.0066 g/30mland 0.1827±0.0058 g/30ml respectively) then, had continuously decreased during day 25thto day 40thof the culture time (showed in figure 10 and figure 11). Similar result was reported by Soomro and Memon (2007) in cell suspension culture of physic nut derived from hypocotyl callus which reported that the growth rate were initially slow during first 3 days (lag phase) and significantly accumulated great amount of fresh weight over a period 15 days (log phase) and maximum increase of fresh weight was reached on day 21thwhich was about 5-6 fold over initial fresh weight, similarly the cell dry weight gradually increased which almost corresponded to fresh weight.

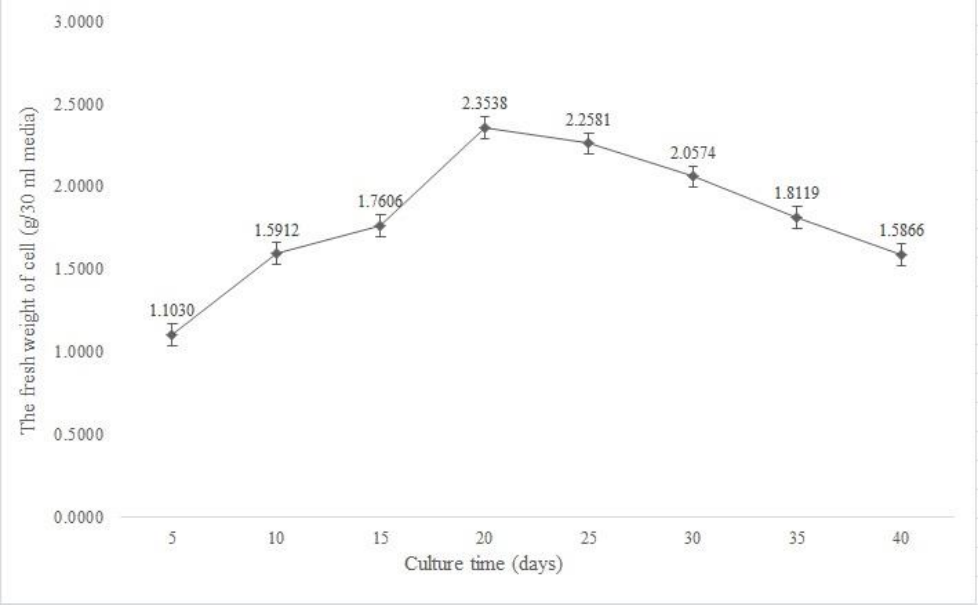

Figure 10 The fresh weight of endosperm suspended cell of physic nut every 5 days of culture.

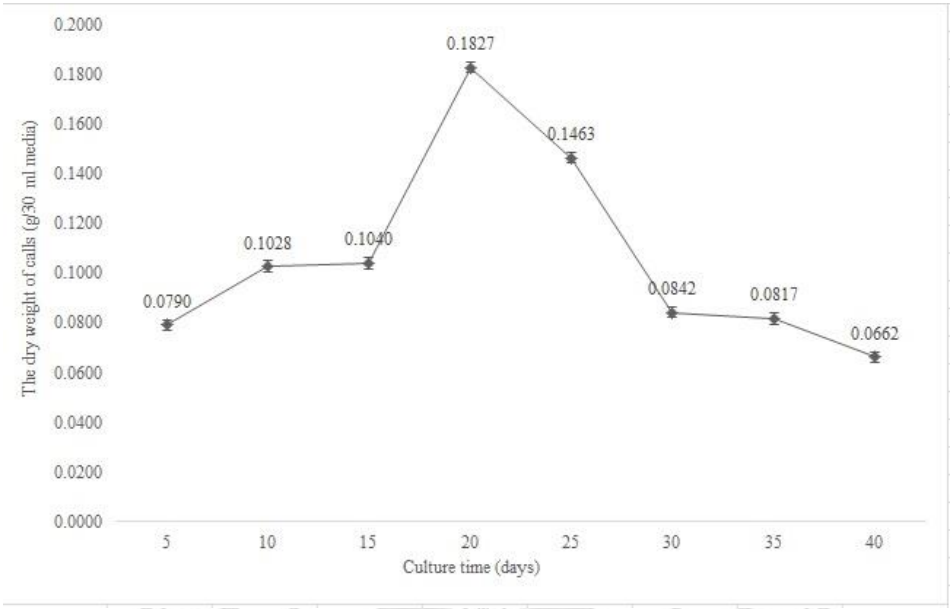

Figure 11 The dry weight of endosperm suspended cell of physic nut every 5 days of culture.

A2. The cell number

At day 5th of the culture, the cell number of endosperm suspended cell of *J. curcas* L. has continuously increased during day 5th to day 15th of the culture same with the result of fresh weight and dry weight. Maximum increased was reached on day 20th of the culture time (7.7933±0.0233cell x 10^4) then, the cell number has declined during day 25th (7.0533±0.0267 cellx10^4) until day 40th (5.5633±0.1014 cellx10^4) (figure 12). The result was shown that endosperm suspended cell could grow well and multiplied in liquid MS medium supplemented with 10 µM NAA. The result was agree with Dixon and Gonzales (1994) who found that generally concentration around 10 µM of auxin can be help maintain dedifferentiated cell and promote cell division. Demissie and Lele (2013) who also reported that maximum growth was found from jatropha cells cultivated in MS medium.

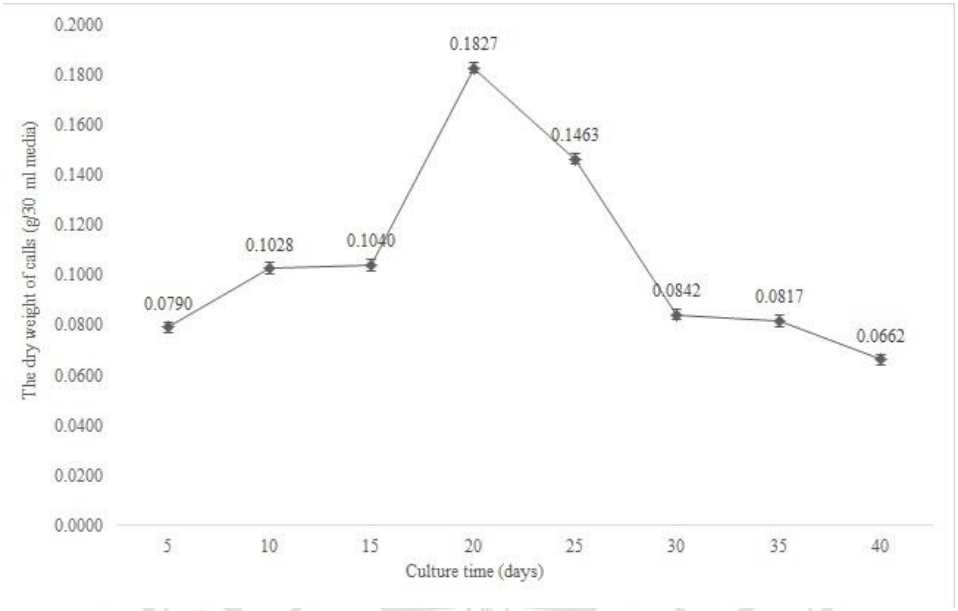

Figure 12 The cell number of jatropha endosperm suspended cell every 5 days ofculture.

A3. Packed cell volume (PCV)

At day 5th of the culture, the packed cell volume (PCV) has continuously increased during day 5th to day 15th of the culture. Maximum increased was reached on day 20th of the culture (0.6 cm^3/cm^3). Then, PCV has declined during day 25th of the culture (0.5 cm^3/cm^3) until day 40th of the culture (0.4 cm^3/cm^3) (figure 13). The result was shown that at day 20thof the culture time, the endosperm suspended cells could grow best. Similarly results observed by Collin and Edward (1998) who showed that a vigorously growing cell suspension will show a 40-50% PCV while our experiment was about 60%.

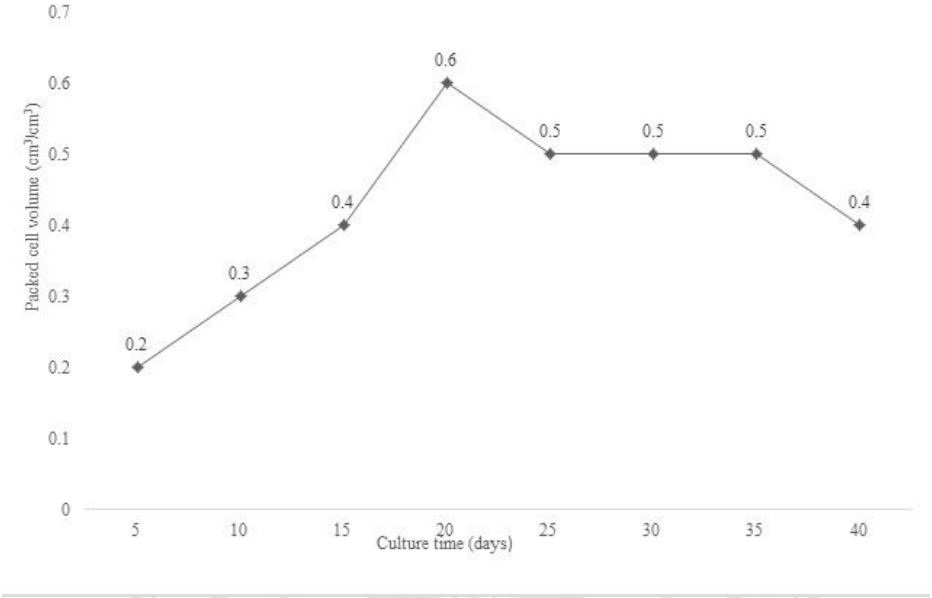

Figure 13 Packed cell volume (data were taken every 5 days)

From the results, the growth of jatropha endosperm cells had similar pattern. The initial lag phase was about day 5th to day 10th of the culture, then the exponential phase initiated around day 10thtoday 20th and the maximum growth occurred at day 20th of the culture. Although, the experiment was not clearly showed phase of stationary however, it expected was around day 20th to day 25th of the cell culture. Then, jatropha endosperm cell was entered to dead phase after day 25th of the culture time. Similar resulted was reported by Soomro and Memon (2007) in cell suspension culture of physic nut derived from hypocotyl callus which reported that the growth rate were initially slow during first 3 days (lag phase) and significantly accumulated great amount of fresh weight over a period 15 days (log phase) and maximum increase of fresh weight was reached on days 21which was about 5-6 fold over initial fresh weight. Similarly the cell dry weight gradually increased which almost corresponded to fresh weight.

B. Oil contents of jatropha endosperm suspended cell

Percentage of total lipid extract, %TLE (w/w)

At day 5th of culture, the oil contents in the form of percentage of total lipid extract (%TLE) has slowly increased (5.8238±0.0507% (w/w)) to 10th day (7.3423± 0.0263% (w/w)). In addition, between days 10th to day 20th, the oil contents has rapidly

increased. Maximum increased were reached on day 20th (27.8324±0.3954 % (w/w)). Then, the oil contents decreased on day 25th until day 40th of culture. At day 40th of the culture, the oil content was about (5.5866±0.0213% (w/w)).From these results, it was indicated that the endosperm suspended cells could grow and produced oil in the liquid MS medium supplemented with 10 µM NAA alone. The growth and oil contents of jatropha endosperm suspended cells tend to be change in the same pattern. The result was agreed with Hapsari *et al*. (2011) who reported that calli from hypocotyl explants from physic nut had the oil contents (%TLE) only 15.2% (w/w) and oil contents increased along with maturation of culture stage. In this study, the oil contents has decreased rapidly after culture for 20 days until 40 days. Moreover, it was possible that the improvement in the growth of suspended cells might affect oil content. Similarly, Kharenko *et al*. (2010) who reported that the exogenous factors i.e., temperature, osmotic potential and externally applied plant hormones have an effects on the storage lipids including; triacylglycerol (TAG) content and fatty acid composition in various plant species.

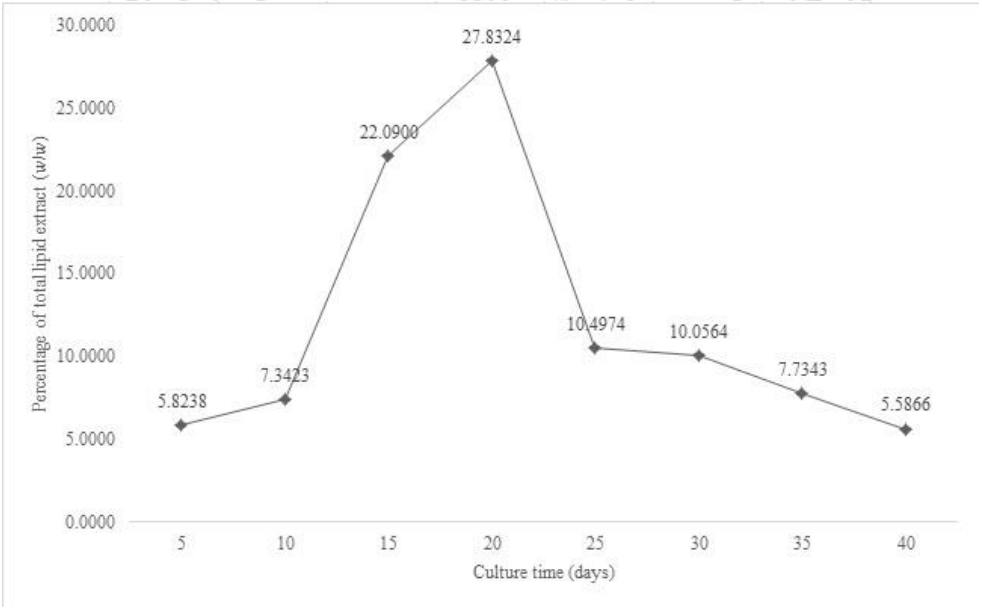

Figure 14 The percentage of oil content (%TLE)(data were taken every 5 days)

Experiment 2 Effects of callus amount on growth and oil contents of endosperm cell of J. curcas L.

The objective of this study was to find out the effects of callus amount on growth and oil contents of endosperm cell of *J. curcas* L. The experiment consisted of 3 treatments; 1 gram, 2 grams and 3grams of callus amount and used a completely randomized design (CRD) for experimental design. Friable calli of *J. curcas* L. were cultured in liquid MS medium supplemented 10µM1-Naphthaleneacetic (NAA) and with 30g/L sucrose. The cultures were placed on 120rpm rotary shaker and kept under darkness at 25±2°C for 20days.The growth consist of fresh weight (FW), dry weight (DW), the number of cells, packed cell volume (PCV) and oil content(%w/w)of each treatments were measured. And the results were as follows

The friable calli of endosperm cells were easily broken apart and dispersed into single cells or aggregated cells in liquid MS medium supplemented with 10µM NAA(figure 16).Similarly Demissie and Lele (2013) reported that the highest biomass productivity was obtained in liquid MS medium at the day 16th of the culture.

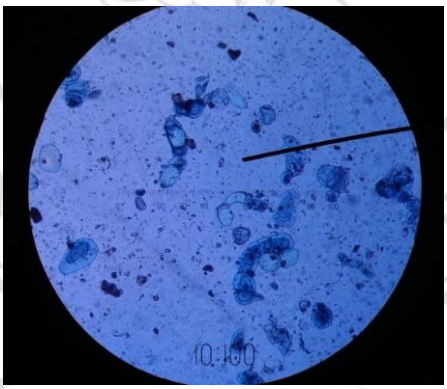

Figure 15 Cell suspension culture of jatropha endosperm cells in liquid MS medium supplemented with 10 µM NAA.(Magnification 40X, Olympus CX31)

A. Growth of cell suspension culture

A1. Fresh weight and dry weight

The results were shown that the callus contents had significantly affected to fresh weight and dry weight of jatropha endosperm suspended cells at statistical confidence level of 95 %.In addition, an increase of callus amount resulted in increasing of fresh weight and dry weight. At day 20th of the culture, the amount of callus at 3 grams gave maximum of fresh weight and dry weight (9.0561±0.003 g/30ml and

1.0670±0.017 g/30ml) respectively. Followed by the amount of callus at 2 grams (6.0722±0.059 g/30ml and 0.3151 ± 0.001 g/30ml) and1 gram (3.7708±0.057 g/30ml and 0.1698±0.010 g/30mlrespectively) (figure 16 and figure 17).

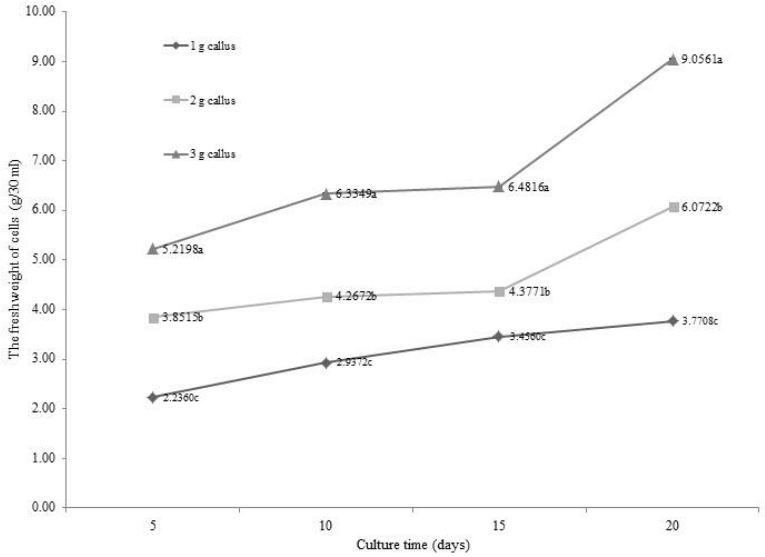

Figure 16 The increased of fresh weight during the cell suspension culture of *J. curcas* L. in different amounts of callus contents. (Different letters mean averages are different significant statistical percent confidence level 95($p \leq 0.05$)).

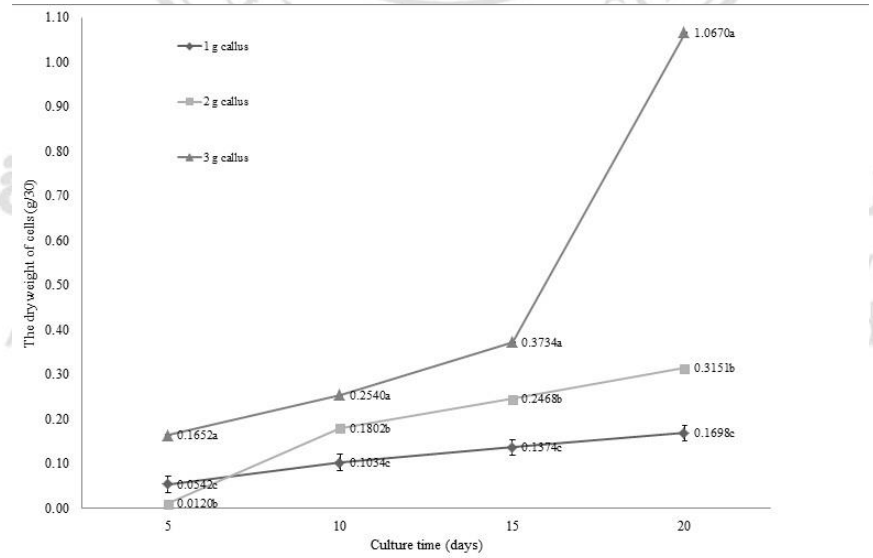

Figure 17 The increased of dry weight during the cell suspension culture of *J. curcas* L. in different amounts of callus contents.

(Different letters mean averages are different significant statistical percent confidence level 95 ($p \leq 0.05$)).

A2. The number of cells

The result showed that the callus amount as significantly affected to the number of cells of jatropha endosperm suspended cells at statistical confidence level of 95 % (figure 18). An increasing of callus contents had resulted to the increasing of the number of cells. At day 20th of the culture, the results showed that the amount of callus at 3 grams gave the highest the number of cells (19.917±0.795x10^4 cell/cm^3) followed by the amount of callus at 2 grams (14.7805±0.518x10^4 cell/cm^3) and 1 gram (8.527±0.055x 10^4 cell/cm^3).

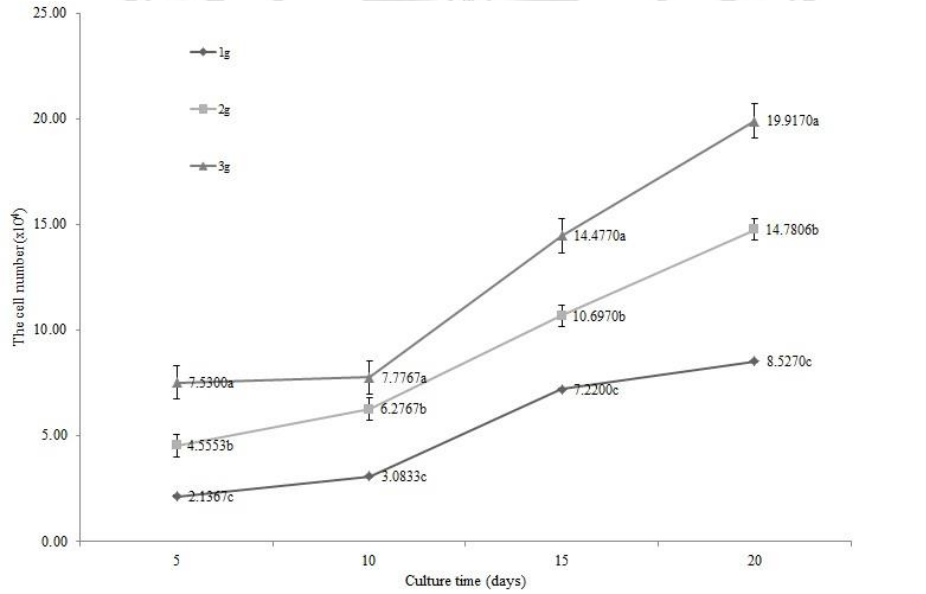

Figure 18 The increased of the number of cells of the cell suspension culture of *J. curcas* L. in different amounts of callus contents.

(Different letters mean averages are different significant statistical percent confidence level 95($p \leq 0.05$)).

A3. Packed cell volume, PCV

The result was shown that the callus amount had significantly affected to the packed cell volume (PCV) of jatropha endosperm suspended cell at statistical confidence level of 95 %. In addition an increasing of callus contents has resulted to the increasing of PCV. At 20th of the culture time, the amount of callus at 3 grams gave the

highest PCV(1.75±0.02 cm^3/cm^3) followed by the amount of callus 2 grams (1.23±0.03 cm^3/cm^3) and1 gram of callus (0.63±0.03 cm^3/cm^3), respectively (figure 19).

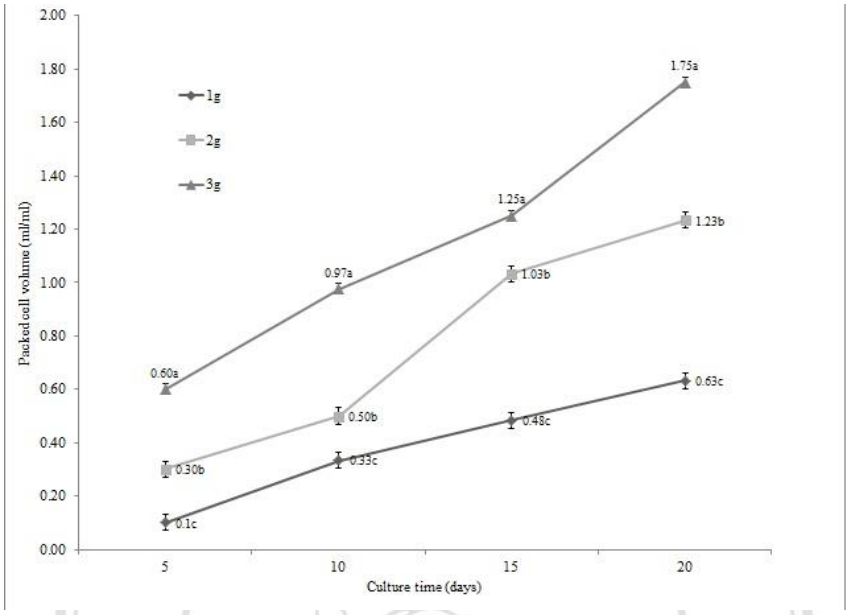

Figure 19 The increased of the packed cell volume of the cell suspension culture of *J. curcas* L. in different amounts of callus contents.
(Different letters mean averages are different significant statistical percent confidence level 95($p \leq 0.05$)).

B. Oil contents of jatropha endosperm suspended cell

Percentage of Total Lipid Extract, %TLE (w/w)

The effects of callus contents on oil content (%TLE**)** of jatropha endosperm suspended cell was shown in figure 20. The result was shown that the callus contents had significantly affected oil content of jatropha endosperm suspended cell at statistical confidence level of 95%. In addition, an increasing of callus amount resulted in increasing of %TLE of jatropha endosperm suspended cell. At 20thof culture, the results found that the amount of callus at 3 grams gave the highest % TLE equaled (45.79±0.56% (w/w)) followed by the amount of callus at 2 grams (35.85±0.96 (w/w)) and1 gram(26.41±0.86 (w/w)). However, an increasing of initial callus content from 1 to 2 grams trended to promote higher oil content (35.75 %) than that increasing callus content from 2 to 3 grams (22.72 %).Therefore, we recommended that the use 2 grams of initial callus were optimal for growth and oil content production in Jatropha endosperm suspended cell.

Biosynthetic process and changing biosynthetic product of lipids in plant cell suspension culture were continuously studied (Staba *et al*., 1971; James, 1985, and Mangold, 1986). Plant tissue culture studies of *J. curcas* L. and closely related genus such as *Rhicinas communis* have been reported in previous study (Brown *et al*., 1970 and Alam *et al*., 2010). In plant tissue culture of *J. curcas* L. found that it has been continuously reported. Especially, the micropropagation of *J. curcas* L. in vitro (Attaya *et al*., 2012). In addition the callus induction and cell suspension culture of *Jatropha curcas* L. were studies (Soomro and Memon, 2007). However the affecting factors the lipid biosynthesis of *J. curcas* L. have only few communicated (Hapsari *et al*., 2011).

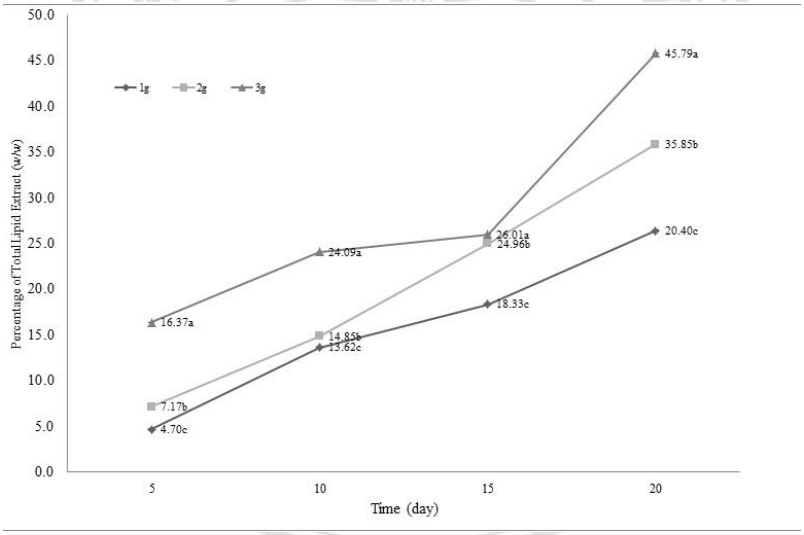

Figure 20 The increased of the oil contents of the cell suspension culture of *J.curcas* L. in different amounts of callus contents.
(Different letters mean averages are different significant statistical percent confidence level 95 ($p \leq 0.05$)).

The culturing system in this study was the cell suspension culture. The oil production of jatropha endosperm cells by the used different initial callus; 1 gram, 2 grams and 3 grams and cultured in the liquid MS medium supplemented with 10 µM NAA and 30 g/L of sucrose, under shaking at 120 rpm and darkness condition at 25±2ºC were evaluated. The results were indicated that the callus amount significantly affected growth and oil contents of jatropha endosperm suspended cells. In addition the

increasing of callus contents could improve growth and oil contents as well. Consistent with the previous study which were reported that the cell suspension of jatropha endosperm cells could grow and produce oil in liquid MS medium under the same condition. This results was supported with Demissie and Lele (2013) who reported that the the maximum growth and fatty acid contents obtained from jatropha cells which were cultivated in liquid MS medium. Moreover they study only the part of media formulation which effected growth and fatty acid yield. Different from our study that aimed to study the effect of callus contents to growth and oil contents. In addition, the results indicated that the improving biomass of endosperm cell of jatropha could increase the oil contents of jatropha endosperm suspended cells. Consistent with Somporn (2009) who reported that the callus contents was an important factor for the growth and the chemical biosynthesis of plant cell in vitro. In this study the results showed that the increasing of initial callus content from 1 to 2 grams trended to promote higher oil content (35.75%) than that increasing callus content from 2 to 3 grams (22.72%). The results was agree with Demissie and Lele (2013) who reported that unlike the microbial cells, the cell density, the inoculum size and heterogeneous cellular displays of the plant cell are the major impediment for such strategies. Thus, from this results, we recommended that the use 2 gram of initial callus was optimal for growth and oil content production in *J. curcas* L. endosperm suspended cell.

Experiment 3 Effects of temperature and sucrose concentrations on growth and oil contents of endosperm cell of J. curcas L.

This experiment was carried out to find effects of temperature and sucrose concentrations and their interaction on growth and oil contents of jatropha endosperm suspended cell. The experiment has two main factors consisted of temperature at 3 levels (15, 20, 25 degree Celsius) and 5 concentrations of sucrose (20, 25, 30, 35 and 40 g/L). It was conducted in a 3x5 factorials in completely randomized design (CRD) with three replications. The growth consist of fresh weight (FW), dry weight (DW), the number of cells, packed cell volume (PCV) and oil content (%w/w) of each treatments were measured. The results were as follows

A. Growth of cell suspension culture

A1. Fresh weight

A1.1 Effect of temperature on fresh weight

The use of different temperature gave significantly affected on fresh weight of jatropha endosperm suspended cell at confidence level of 95 percent. Increasing of temperature resulted decrease on fresh weight in in all during culturing (table 9). At day 20th of the culture, using of 15°C gave maximum of fresh weight (3.6859±0.0384g/30ml) followed by 20°C (3.4609±0.0731g/30ml) and 25°C (2.9119±0.0501g/30m) (table 9).

A1.2 Effect of sucrose concentration on fresh weight

Using of different sucrose concentrations has significantly affected on fresh weight of jatropha endosperm suspended cell at confidence level of 95 %. In contrast with temperature, increasing of sucrose concentrations resulted in increasing on fresh weight in all during culturing (table 10). In addition, at day 20th of the culture, adding of sucrose at 35 g/L gave maximum of fresh weight (3.5830±0.1110g/30ml) but it had no significantly different when compared to40g/L(3.5747±0.1170g/30ml). The minimal fresh weight was obtained when using of sucrose 20 g/L of sucrose was add to the medium (3.0971±0.1106g/30ml).

Table 9 Effect of temperature on fresh weight (g/30ml) of jatropha endosperm suspended cell during culturing for 20 days.

Temperature (°C)	Time after culturing (days)			
	5 days	10days	15days	20days
15	3.0492b	3.2546a	3.5455a	3.6859a
20	3.0718a	3.0268b	3.2508a	3.4609b
25	2.2871c	2.5142c	2.6430b	2.9119c
Turkey$_{.05}$	0.0000	0.0000	0.0000	0.0000

Means within the same column followed by different characters showed significant different by Turkey HSD all-pairwise comparison test at $P \leq 0.05$.

Table 10 Effect of sucrose concentration on fresh weight(g/30ml)of jatropha endosperm suspended cell during culturing for 20 days.

Sucrose (g/L)	Time after culturing (days)			
	5 days	10days	15days	20days
20	2.5815e	2.7613d	2.9049c	3.0971d
25	2.6419d	2.7517d	2.9698c	3.1853c
30	2.7422c	2.9098c	3.1107b	3.3245b
35	3.1437b	3.0963b	3.3708a	3.5830a
40	2.9141a	3.1403a	3.3758a	3.5747a
$Turkey_{.05}$	0.0000	0.0000	0.0000	0.0000

Means within the same column followed by different characters showed significant different by Turkey HSD all-pairwise comparison test at $P \leq 0.05$.

A1.3 *Combinatory effect of temperature and sucrose concentrations on fresh weight*

The treatments combination of different temperature and sucrose concentrations gave significantly different results in fresh weight at confidence level of 95 % (table 11). In addition, at 20^{th} of the culture, using temperature at 15°C and 35 g/L sucrose gave maximum fresh weight (3.8489±0.0132 g/30ml), but not different with using temperature at 15°C and 40g/L which gave fresh weight (3.5802±0.0132g/30ml) followed by using temperature at 20°C and 40g/L sucrose and using using temperature at 20°C and 35 g/L sucrose which gave fresh weight 3.7580±0.0132g/30ml and 3.7647±0.0132g/30ml respectively. The minimal fresh weight was obtained from using of temperature at 25°C and 25g/L sucrose (2.6900±0.0132g/30ml).

Table 11 Combinatory effect of temperature and sucrose concentration on fresh weight (g/30ml)of jatropha endosperm suspended cell during culturing for 20 days.

Treatment No.	Treatment Temperature (ºC) *Sucrose (g/L)	Time after culturing (days)			
		5 days	10days	15days	20days
1	15*20	3.0225c	3.1452d	3.3740de	3.4954d
2	15*25	2.9858c	3.1452d	3.4430cde	3.5859c
3	15*30	2.9877c	3.1636cd	3.4839bcd	3.6475c
4	15*35	2.9794c	3.2428bc	3.6648ab	3.8489a
5	15*40	3.2703b	3.5763a	3.7620a	3.8502a
6	20*20	2.6136e	2.7675e	2.8686f	3.0644g
7	20*25	2.8042d	2.7675e	2.9475f	3.2760e
8	20*30	2.9799c	3.1868cd	3.2754e	3.4413d
9	20*35	3.9780a	3.2760b	3.6060abc	3.7580b
10	20*40	2.9835c	3.1363d	3.5563bcd	3.7647b
11	25*20	2.1085h	2.3711f	2.4721g	2.7316i
12	25*25	2.1358h	2.3424f	2.5189g	2.6900i
13	25*30	2.2589g	2.3789f	2.5729g	2.8845h
14	25*35	2.4736f	2.7700e	2.8416f	3.1442f
15	25*40	2.4585f	2.7085e	2.8092f	3.1092fg
Turkey.05		0.0000	0.0000	0.0000	0.0000
CV (%)		0.81	0.98	2.11	0.68

Means within the same column followed by different characters showed significant different by Turkey HSD all-pairwise comparison test at $P \leq 0.05$.

A2. Dry weight

A2.1 Effect of temperature on dry weight

Similar to the results of fresh weight, the use of different temperature gave significantly affected on dry weight at confidence level of 95%. Increasing of temperature resulted decrease on dry weight in all during culturing (table 12). At day 20^{th} of the culture, using 15°C gave maximum of dry weight (0.3003 ±0.0023g/30ml)

followed by 20°C (0.2977 ±0.0030g/30ml). The minimal fresh weight was obtained when using temperature at 25°C (0.2773±0.0013g/30ml).

Table 12 Effect of temperature on dry weight(g/30ml)of jatropha endosperm suspended cell during culturing for 20 days.

Temperature (°C)	Time after culturing (days)			
	5 days	10days	15days	20days
15	0.1252a	0.1928a	0.2412a	0.3003a
20	0.1164b	0.1899b	0.2410a	0.2977b
25	0.0866c	0.1705c	0.2132b	0.2773c
Turkey.05	0.0000	0.0000	0.0000	0.0000

Means within the same column followed by different characters showed significant different by Turkey HSD all-pairwise comparison test at $P \leq 0.05$.

A2.2 Effect of sucrose concentration on dry weight

Using different sucrose concentrations was significantly affected on dry weight of jatropha endosperm suspended cell at confidence level of 95 %. In contrast to temperature, increasing of sucrose concentrations has resulted in increasing dry weight in all during culturing (table 13). In addition, at day 20^{th} of the culture, using sucrose concentration at 35 and 40 g/L gave same maximum of dry weight (0.3017±0.0047g/30ml) followed by using 30 g/L(0.2.880±0.0028g/30ml). The minimal dry weight was obtained when using sucrose 20 and 25 g/L (0.2838±0.0031g/30ml).

Table 13 Effect of sucrose concentration on dry weight(g/30ml)of jatropha endosperm suspended cell during culturing for 20 days.

Sucrose (g/l)	Time after culturing (days)			
	5 days	10days	15days	20days
20	0.1034e	0.1795e	0.2295c	0.2838c
25	0.1057d	0.1805d	0.2279d	0.2838c
30	0.1063c	0.1822c	0.2292c	0.2880b
35	0.1149b	0.1887b	0.2349b	0.3017a
40	0.1167a	0.1913a	0.2376a	0.3017a
$Turkey_{.05}$	0.0000	0.0000	0.0000	0.0000

Means within the same column followed by different characters showed significant different by Turkey HSD all-pairwise comparison test at $P \leq 0.05$.

A2.3 Combinatory effect of temperature and sucrose concentrations on dry weight

The treatments combination of different temperature and sucrose concentrations gave significantly different results in dry weight at confidence level of 95 % (table 14). In addition, at 20^{th} of the culture, using temperature at 20°C and 35 g/L sucrose gave maximum dry weight (0.3124±0.0020 g/30ml) but not different when using temperature at 15°C with10 g/L and 20°C and 40 g/L(0.3111±0.0020 g/30ml and 0.3112±0.0020 g/30ml), respectively. The minimal fresh weight was obtained from using of temperature at 25°C and 20 gram/litter of sucrose (0.2714±0.0020 g/L).

Table 14 Combinatory effect of temperature and sucrose concentration on dry weight (g/30ml) of jatropha endosperm suspended cell during culturing for 20 days.

Treatment No.	Treatment Temperature (ºC) *Sucrose (g/L)	Time after culturing (days) 5 days	10days	15days	20days
1	15*20	0.1241	0.1880	0.2393	0.2911
2	15*25	0.1238	0.1890	0.2404	0.2918
3	15*30	0.1244	0.1894	0.2412	0.2974
4	15*35	0.1268	0.1986a	0.2422	0.3102
5	15*40	0.1268b	0.1991a	0.2426b	0.3111ab
6	20*20	0.1030	0.1810	0.2374	0.2888
7	20*25	0.1106	0.1834	0.2315	0.2878
8	20*30	0.1124	0.1898	0.2323	0.2885
9	20*35	0.1279ab	0.1967	0.2484b	0.3124a
10	20*40	0.1282a	0.1987a	0.2555a	0.3112ab
11	25*20	0.0830	0.1694	0.2118	0.2714h
12	25*25	0.0828	0.1691	0.2117j	0.2719
13	25*30	0.0821i	0.1673k	0.2141	0.2781
14	25*35	0.0901	0.1709	0.2139	0.2826
15	25*40	0.0952	0.1759	0.2147	0.2827
Turkey.05		0.0000	0.0000	0.0000	0.0000
CV (%)		0.37	0.10	0.20	0.16

Means within the same column followed by different characters showed significant different by Turkey HSD all-pairwise comparison test at $P \leq 0.05$.

A2. The ratio of fresh weight to dry weight (FW/DW)

A2.1 Effect of temperature on the ratio of fresh weight to dry weight (FW/DW)

The use of different temperature gave significantly affected on FW/DW ratio at confidence level of 95%. At day 20th of the culture, using of temperature at 20°C

gave maximum FW/DW ratio (23.807±0.0447) followed by using of temperature at 15°C (22.896±0.2187) and 25°C (22.458±2871 g/30ml) (table 15).

Table 15 Main effect of temperature on the ratio of fresh weight to dry weight (FW/DW) of jatropha endosperm suspended cell during culturing for 20 days.

Temperature (°C)	Time after culturing (days)			
	5 days	10days	15days	20days
15	24.450a	24.159c	23.007a	22.869c
20	25.659a	26.053a	24.383a	23.807a
25	25.735b	23.250b	22.754b	22.458b
$Turkey_{.05}$	0.0000	0.0000	0.0000	0.0000

Means within the same column followed by different characters showed significant different by Turkey HSD all-pairwise comparison test at $P \leq 0.05$.

A2.2 Effect of sucrose concentration on the ratio of fresh weight to dry weight (FW/DW)

Using of different sucrose concentrations significantly affected on FW/DW ratio of jatropha endosperm suspended cell at confidence level of 95%. Similarly with temperature, increasing of sucrose concentrations has resulted in increasing on FW/DW ratio in all during culturing (table 16). In addition, at day 20^{th} of the culture using of sucrose concentration at 35 g/L gave maximum of FW/DCW ratio (23.294±0.2205) but not significantly different of FW/DW ratio when using 25, 30 and 45 g/L. The minimal dry weight was obtained when using of sucrose 25 g/L (22.415±0.3940).

Table 16 Main effect of sucrose concentration on the ratio of fresh weight to dry weight (FW/DW) of jatropha endosperm suspended cell during culturing for 20 days.

Sucrose (g/l)	Time after culturing (days)			
	5 days	10days	15days	20days
20	23.879	23.602	23.088	23.088a
25	25.094	23.432	22.415	22.415b
30	26.399	25.152	23.138	23.138a
35	25.575	24.979	22.289	23.289a
40	25.460	23.411	23.294	23.294a
$Turkey_{.05}$	0.0000	0.0000	0.0000	0.0000

Means within the same column followed by different characters showed significant different by Turkey HSD all-pairwise comparison test at $P \leq 0.05$.

A2.3 Combinatory effect of temperature and sucrose concentrations on the ratio of fresh weight to dry cell weight (FW/DW)

The combination treatments of different temperature and sucrose concentrations gave significantly different results in FW/DW ratio at confidence level of 95% (table 17). In addition, at 20^{th} of the culture time, using temperature at 15°C and 20 g/L of sucrose gave maximum FW/DW ratio (24.075±0.2134), but not significantly different with 20°C and 30 g/L, 20°C and 40 g/L, 20°C and 25 g/L, 25°C and 35 g/L, 20°C and 20 g/L and 20°C and 35 g/L (23.927±0.2134, 23.867±0.2134, 23.831±0.2134, 23.790±0.2134, 23.786±0.2134 and 23.625±0.2134) respectively. The minimal FW/DW ratio was obtained when using of temperature at 25°C and 20 g/L of sucrose (21.131±0.2134).

Table 17 Combinatory effect of temperature and sucrose concentration on the ratio of fresh weight to dry weight (FW/DW) of jatropha endosperm suspended cell during culturing for 20 days.

Treatment No.	Treatment Temperature (ºC) *Sucrose (g/L)	Time after culturing (days) 5 days	10days	15days	20days
1	15*20	24.362	24.572	24.262	24.075a
2	15*25	25.335	24.545	24.456	23.786
3	15*30	21.941	21.688	21.147	21.403
4	15*35	24.124	24.401	22.826	22.282
5	15*40	25.362	24.404	24.353	23.831
6	20*20	25.796	21.490	22.285	21.131f
7	20*25	25.054	24.432	23.450	23.018
8	20*30	26.504	26.609	26.321	23.927a
9	20*35	27.638	24.416	22.609	22.471
10	20*40	23.955	23.774	22.335	22.453
11	25*20	25.304	25.830	23.589	23.625ab
12	25*25	27.465	25.333	25.284	23.790
13	25*30	24.757	23.616	22.164	22.520
14	25*35	25.790	28.876	22.198	23.867a
15	25*40	25.834	23.322	22.444	23.495
Turkey.05		0.0000	0.0000	0.0000	0.0000
CV (%)		0.85	6.17	0.86	1.60

Means within the same column followed by different characters showed significant different by Turkey HSD all-pairwise comparison test at $P \leq 0.05$.

A3. The cell number

A3.1 Effect of temperature on the cell number

The use of different temperature gave significantly affected on the cell number of jatropha endosperm suspended cell at confidence level of 95 %. Increasing of temperature resulted to decrease in the cell number in all treatments during culturing

(table 18). At day 20th of the culture, using of temperature at 15°C gave maximum of the cell number (25.933±1.0931x10^4 cell) followed by 20°C (24.000±1.1912x10^4 cell). The minimal fresh weight was obtained when using of temperature at 25°C (19.667±1.1899x10^4 cell).

Table 18 Effect of temperature on the cell number of jatropha endosperm suspended cell during culturing for 20 days.

Temperature (°C)	Time after culturing (days)			
	5 days	10days	15days	20days
15	9.333a	11.000a	16.667a	25.933a
20	9.333a	11.000a	17.333b	24.000b
25	7.600b	9.000b	14.600c	19.667c
$Turkey_{.05}$	0.0000	0.0000	0.0000	0.0000

Means within the same column followed by different characters showed significant different by Turkey HSD all-pairwise comparison test at $P \leq 0.05$.

A2.2 Effect of sucrose concentration on the cell number

Using of different sucrose concentrations has significantly affected on the cell number of jatropha endosperm suspended cell at confidence level of 95%. In contrast to the temperature, increasing of sucrose concentrations resulted in increasing on the cell number during culturing time (table 19). In addition, at day 20th of the culture, using 40 g/Lof sucrose gave maximum of the cell number (29.222±00.9246x10^4 cell) followed by 35 g/L (27.444±0.8184x10^4 cell). The minimal of the cell number was obtained when using of sucrose 20 g/L (18.778±0.9095x10^4 cell).

Table 19 Effect of sucrose concentration on the cell number of jatropha endosperm suspended cell during culturing for 20 days.

Sucrose (g/l)	Time after culturing (days)			
	5 days	10days	15days	20days
20	7.333d	8.222c	12.444e	18.778d
25	7.667d	8.667c	13.667d	19.444d
30	8.444c	9.111c	15.222c	21.111c
35	9.778b	12.000b	19.444b	27.444b
40	10.556a	13.667a	20.222a	29.222a
$Turkey_{.05}$	0.0000	0.0000	0.0000	0.0000

Means within the same column followed by different characters showed significantly different by Turkey HSD all-pairwise comparison test at $P \leq 0.05$.

A2.3 Combinatory effect of temperature and sucrose concentrations on the cell number

The combination treatment of different temperature and sucrose concentrations has significantly different results in the cell number at confidence level of 95% (table 20). In addition, at 20^{th} of the culture, using temperature at 15°C and 40 g/Lof sucrose gave maximum the cell number ($31.667\pm0.7638x10^4$ cell), but not different with using 20°C and 40 g/L($30.333\pm0.7638x10^4$ cell) followed by 15°C and 35 g/L of sucrose and 20°C and 35 g/L of sucrose gave the cell number ($29.667\pm0.7638 \times 10^4$ cell and $28.333\pm0.7638x10^4$ cell), respectively. The minimal fresh weight was obtained when using of temperature at 15°C and 20 g/L of sucrose ($15.333\pm0.7638x10^4$ cell).

Table 20 Combinatory effect of temperature and sucrose concentration on the cell number of jatropha endosperm suspended cell during culturing for 20 days.

Treatment No.	Treatment Temperature (ºC) *Sucrose (g/L)	Time after culturing (days)			
		5 days	10days	15days	20days
1	15*20	8.000	9.000	13.667	21.333
2	15*25	8.333	9.667	14.333	22.333
3	15*30	9.333	10.000	15.000	24.667
4	15*35	10.000	13.000	19.667	29.667bc
5	15*40	11.000a	13.333a	20.667ab	31.667a
6	20*20	8.000	9.333	13.333	19.667
7	20*25	8.667	9.333	14.333	20.333
8	20*30	9.000	9.667	16.000	21.333
9	20*35	10.333ab	13.333a	21.333a	28.333c
10	20*40	10.667a	14.333	21.667a	30.333ab
11	25*20	6.000	6.333d	10.333j	15.333h
12	25*25	6.000g	7.000	12.333	15.667
13	25*30	7.000	7.667	14.667	17.333
14	25*35	9.000	9.667	17.333	24.333
15	25*40	10.000	14.333a	18.333	25.667
Turkey.05		0.0040	0.0040	0.0004	0.0718
CV (%)		3.18	8.89	3.32	2.49

Means within the same column followed by different characters showed significant different by Turkey HSD all-pairwise comparison test at $P \leq 0.05$.

A4. Packed Cell Volume, PCV

A4.1 Effect of temperature on packed cell volume, PCV

The use of different temperature gave significantly affected on PCV of jatropha endosperm suspended cell at confidence level of 95%. Increasing of temperature resulted in decreasing PCV in all during culturing (table 21). At day 20th of the culture, using of temperature at 15°C gave maximum of PCV (1.3207±

0.0163cm^3/cm^3) followed 20°C (1.3033±0.0188 cm^3/cm^3). The minimal PCV was obtained when using of temperature at 25°C (1.2660±0.0185cm^3/cm^3).

Table 21 Effect of temperature on packed cell volume (cm^3/cm^3)of jatropha endosperm suspended cell during culturing for 20 days.

Temperature (°C)	Time after culturing (days)			
	5 days	10days	15days	20days
15	0.3467a	0.5667a	1.2200a	1.3207a
20	0.3333ab	0.5533a	1.1593b	1.3033b
25	0.3000b	0.4467b	1.1187c	1.2660c
$Turkey_{.05}$	0.0147	0.0000	0.0000	0.0000

Means within the same column followed by different characters showed significant different by Turkey HSD all-pairwise comparison test at $P \leq 0.05$.

A2.2 Effect of sucrose concentration on packed cell volume, PCV

Using of different sucrose concentrations significantly affected on PCV of jatropha endosperm suspended cell at confidence level of 95 %. In contrast to the temperature, increasing of sucrose concentrations also resulted in increasing on PCV in all during culturing (table 22). In addition, at day 20^{th} of the culture time using of sucrose concentration at 40 g/L gave maximum of PCV (1.3878±0.0068cm^3/cm^3) followed by 35 g/L of sucrose (1.3644±0.0093cm^3/cm^3). The minimal PCV was obtained from 20 g/L of sucrose (1.2267±0.0072 cm^3/cm^3).

Table 22 Effect of sucrose concentration on packed cell volume (cm^3/cm^3) of jatropha endosperm suspended cell during culturing for 20 days.

Sucrose (g/l)	Time after culturing (days)			
	5 days	10days	15days	20days
20	0.2889b	0.5000b	1.0889d	1.2267d
25	0.2778b	0.4444c	1.1000cd	1.2467c
30	0.3000b	0.4778bc	1.1322c	1.2578c
35	0.3667a	0.6111a	1.2322b	1.3644b
40	0.4000a	0.5778a	1.2767a	1.3878a
$Turkey_{.05}$	0.0000	0.0000	0.0000	0.0000

Means within the same column followed by different characters showed significant different by Turkey HSD all-pairwise comparison test at $P \leq 0.05$.

A2.3 Combinatory effect of temperature and sucrose concentrations on packed cell volume, PCV

The combination treatments of different temperature and sucrose concentrations gave significantly different results in PCV at confidence level of 95% (table 23). In addition, at 20^{th} of the culture, using temperature at 15°C and 40 g/L of sucrose (1.4067±0.00258 cm^3/cm^3), but not different with using temperature at 20°C and 40 g/L and using temperature at 20°C and 35 g/L (1.3933±0.00258 $cm^3/cm^3$and 1.3833±0.00258 cm^3/cm^3), respectively. The minimal PCV was obtained from using of temperature at 25°C and 20 g/L of sucrose (1.2000±0.00258 cm^3/cm^3).

Table 23 Combinatory effect of temperature and sucrose concentration on packed cell volume (cm^3/cm^3) of jatropha endosperm suspended cell during culturing for 20 days.

Treatment No.	Treatment Temperature (ºC) *Sucrose (g/L)	Time after culturing (days)			
		5 days	10days	15days	20days
1	15*20	0.200	0.500	1.167	1.2500
2	15*25	0.300	0.500	1.167	1.2700
3	15*30	0.333	0.500	1.233	1.3000
4	15*35	0.300a	0.567a	1.233	1.3767bc
5	15*40	0.367ab	0.567a	1.300ab	1.4067a
6	20*20	0.233	0.500	1.000f	1.2300
7	20*25	0.300	0.433	1.033	1.2533
8	20*30	0.300	0.533	1.100	1.2167
9	20*35	0.367a	0.667a	1.300ab	1.3833abc
10	20*40	0.400	0.633	1.367a	1.3933ab
11	25*20	0.200	0.500	1.100	1.2000i
12	25*25	0.233c	0.400	1.100	1.2167
13	25*30	0.300	0.400d	1.067	1.2167
14	25*35	0.333	0.500	1.167	1.3333
15	25*40	0.333	0.533	1.167	1.3633
Turkey.05		0.4784	0.0003	0.0000	0.0024
CV (%)		12.91	7.55	2.24	0.70

Means within the same column followed by different characters showed significant different by Turkey HSD all-pairwise comparison test at $P \leq 0.05$.

B. Oil contents of jatropha endosperm suspended cell

B1. Percentage of Total Lipid Extract, %TLE (w/w)

B1.1 Main effect of temperature on Total Lipid Extract, %TLE (w/w)

The use of different temperature gave significantly affected on% TLE of jatropha endosperm suspended cell at confidence level of 95%. At day 20th of the

culture, using of temperature at 20°C gave maximum of %TLE(33.078±2.2580% (w/w)) followed by using temperature at 15°C (29.927±1.4088% (w/w)) and 25°C (19.163±0.5890% (w/w)), respectively (table 24).

Table 24 Effect of temperature on total lipid extract (TLE) (w/w) of jatropha endosperm suspended cell during culturing for 20 days.

Temperature (°C)	Time after culturing (days)			
	5 days	10days	15days	20days
15	17.863a	20.379b	26.243b	29.927a
20	17.667a	20.755a	28.473a	33.078b
25	13.647b	15.985c	18.267c	19.163c
$Turkey_{.05}$	0.0000	0.0000	0.0000	0.0000

Means within the same column followed by different characters showed significant different by Turkey HSD all-pairwise comparison test at $P \leq 0.05$.

A2.2 Effect of sucrose concentration on Total Lipid Extract, %TLE (w/w)

Using of different sucrose concentrations significantly affected on % TLE of jatropha endosperm suspended cell at confidence level of 95%. Increasing of sucrose concentrations also resulted to increasing on %TLE in all during culturing (table 25). In addition, at day 20^{th} of the culture time using of sucrose concentration at 40 g/L gave maximum of %TLE (32.524±3.4211% (w/w)), but it had no significantly different when compared with the use of sucrose concentration at 35 g/L (32.524±3.3837% (w/w)). The minimal TLE was obtained when using of sucrose 20 g/L (20.848±1.6889% (w/w)).

Table 25 Main effect of sucrose concentration on total lipid extract (TLE) (w/w) of jatropha endosperm suspended cell during culturing for 20 days.

Sucrose (g/l)	Time after culturing (days)			
	5 days	10days	15days	20days
20	14.882c	16.680d	19.061c	20.848c
25	14.909c	17.144c	20.499c	24.693b
30	15.943bc	18.239b	23.738b	25.576b
35	19.482a	21.643a	28.668a	32.307a
40	16.746b	21.492a	29.672a	33.524a
$Turkey_{.05}$	0.0000	0.0000	0.0000	0.0000

Means within the same column followed by different characters showed significant different by Turkey HSD all-pairwise comparison test at $P \leq 0.05$.

A2.3 Combinatory effect of temperature and sucrose concentrations on Total Lipid Extract, %TLE (w/w)

The treatments combination of different temperature and sucrose concentrations gave significantly different results in %TLE at confidence level of 95 percent (table 26). In addition, at 20^{th} of the culture time, using temperature at 20°C and 35 gram/litter of sucrose gave maximum %TLE 45.530±1.2628% (w/w), but not different with using temperature at 20°C and 40 gram/litter which gave %TLE 42.057±1.2628% (w/w). The minimal fresh weight was obtained from using of temperature at 25°C and 30 gram/litter of sucrose (18.077±1.2628% (w/w)).

Table 26 Combinatory effect of temperature and sucrose concentration on total lipid extract (TLE)(w/w)of jatropha endosperm suspended cell during culturing for 20 days.

Treatment No.	Treatment Temperature (ºC) *Sucrose (g/L)	Time after culturing (days) 5 days	10days	15days	20days
1	15*20	16.510	17.550	17.827	21.997
2	15*25	16.727	16.727	23.050	28.073
3	15*30	19.287ab	19.520	29.047	28.067
4	15*35	20.253a	24.753	32.087	36.380b
5	15*40	16.540	23.347a	32.730b	35.120
6	20*20	16.287	17.023	21.753	21.913
7	20*25	16.120	18.223	21.030	27.307
8	20*30	16.170	19.477	29.047	30.583
9	20*35	20.913a	23.613b	34.083ab	43.530a
10	20*40	18.847	25.440a	36.453a	42.057a
11	25*20	11.850	15.467	17.603	18.633
12	25*25	11.880	16.483h	17.417	18.700
13	25*30	12.373e	15.720	16.647h	18.077e
14	25*35	17.280	16.563	19.833	20.663
15	25*40	14.850	15.690	19.833	19.743
Turkey.05		0.0000	0.0000	0.0000	0.0000
CV (%)		5.02	1.62	4.99	5.59

Means within the same column followed by different characters showed significant different by Turkey HSD all-pairwise comparison test at $P \leq 0.05$.

From the overall results, we found that growth and oil content could be increase by increasing sucrose concentration and reducing cultured temperature. From previous studies, jatropha oil yield from jatropha endosperm suspended cell could be improved by increased appropriated initial callus. In these studies, use of appropriate initial callus amount join with appropriated sucrose concentrations and temperature were studied.

The results indicated that sucrose concentration significantly affected growth and oil contents of jatropha endosperm suspended cells and increased of sucrose concentrations could improve growth and oil contents as well. Similarly, Kim *et al.* (1999) who studied the effects of sugar concentration on camptothecin production in suspension cultures of *Camptotheca acuminate,* reported that the highest of camptothecin was obtained when increased sucrose concentration in media which gave highest camptothecin than low sucrose concentration. In addition, several studies have been reported that the high sucrose concentration increased the secondary metabolite production. For example, the production anthocyanin in *Daucascorota* which increased by use high sucrose concentrations (Rajendran *et al.*, 1992). In consideration to the FW/DW ratio, the result revealed that FW/DW ratio was also increased by increased sucrose concentration and 35 g/L of sucrose gave highest the ratio. From the results we can conclude that the oil yield was related to sucrose concentration. When comparing to the FW/DW ratio, the results were similar to Park and Kim (1993) who studied in plant cell suspension of *Thalictrum rugusum,* which explained that the ratio of fresh weight to dry weight (FW/DW) was important to determining the theoretical maximum cell concentration in high density of plant cell suspension, which might be rerated oil yield of *J. curcas* L. in vitro. In addition, the result on temperature studied found that decreasing temperature promoted growth and oil yield in *J. curcas* L. Similar research in fresh water microalgae *Scenedesmus* sp. LX1 reported that the optimal temperature production microalgae biomass and lipid was 20°C after 15 days of batch cultivation (Xin *et al*., 2011). Moreover, the combination of sucrose concentration and temperature could improve growth and oil content of jatropha endosperm suspended cell. From the results, we recommended that used 35 g/L sucrose and 20°C of culture temperature are optimum for oil production in *J. curcas* L. endosperm suspended cell. Consistent with Zárate *et al*.(2013)who reported that the application of abiotic stress resulted in larger yields of stearidonic and α-linolenic acids, 60 and 35%, respectively. In the other research, the application of osmotic stress employing sorbitol showed no positive influence on the fatty acid yields. Furthermore, the combination of a lower culture temperature and glucose did not show a cumulative boosting effect on the yield, although this carbon source was similarly attractive. In plant organ, there are very few studies addressing the distribution of lipid classes in roots, although one describes the phosphoglycerol (PG)

content in the thylakoid membranes and its relative increase, together with unsaturation enrichment of fatty acid (FA) esterified in phosphoglycerol (PG) against low temperatures, both in monocotyledons and dicotyledons (Xu *et al.*, 2003and Umura *et al*., 2006). From this study, although the lowest and high sucrose concentration gave high oil content, but the suitable condition we choose set up and for studies the oil production of jatropha endosperm suspended cell in a modified 1 L bubble column reactor were 20°C on MS liquid media supplemented with 35 g/L sucrose, 10 µM NAA, pH 5.6±0.02 under dark condition for 20 days and it found that the oil content production was 57% w/dw at 20 days after culture.

Part 3 Study on oil production from endosperm cells of Physic Nut (*J.curcas* L.) in a bubble column bioreactor.

A bioreactor refers to any manufactured or engineered device or system that supports a biologically active environment. Suspension bioreactors can use a wider in variety organism including microorganism and plant in which the environmental conditions inside the bioreactor such as temperature, nutrient concentrations, pH and dissolved gases (O_2) are affect to the growth and productivity of these organisms. Therefore, choosing of bioreactor type and condition of the culture are important to the cultured success. A Bubble column reactor is a bioreactor which is an apparatus used for gas - liquid reactions. The introduction of gas takes place at bottom of the colomn and causes a turbulent stream to enable an optimum gas exchange and the liquid can be in parallel flow or counter current.

The study of the oil production from the endosperm cells of *J.curcas* L. was conducted in 1 of Liter modified bubble column bioreactor (figure22A).The bioreactor, contained liquid MS medium supplemented with 10µM 1-Naphthaleneacetic acid (NAA) alone and 35 g/L of sucrose, pH 5.6±0.2. The culture was incubated under the dark condition, at 20±3 ºC with0.3 L/min of air flow rate for 20 days. The growth and oil contents were evaluated at the last day of the cultured with three replications. The results were as follow

On the last day of the culture time, the results were shown that the endosperm cells of *J.curcas* L. could grow in 1 of Liter modified bubble column bioreactor,

contained liquid MS medium supplemented with 10µM 1-Naphthaleneacetic acid (NAA) alone and 35 g/L of sucrose, pH 5.6±0.2, in the dark condition at 20±3 ºC and 0.3 L/min of air flow rate (showed in figure 22B). In addition, the growth of endosperm cells presented fresh weight (FW), dry weight (DW), the number of cell and packed cell volume (PCV) equaled (mean±standard error of the mean, SEM) 76.8813±0.8083 g/L, 5.8800±0.6513 g/L, 18.8±0.3 x 10^4 cell/cm^3 and 1.11±0.10 cm^3/cm^3respectively. Moreover, the endosperm cells could produce oil in the bioreactor; the oil content was 57.594% (w/w). This result indicated that it is possible to be produce oil from the endosperm cells of *J. curcas* L. in the bioreactor.

Figure 22 Culture in 1 liter of modified bubble column bioreactor.

a. 1 liter of modified bubble column bioreactor.
b. The endosperm cells of *J. curcas* L. in 1 liter of modified bubble column bioreactor at day 20th of the culture.

From the results, it was found that 1 Litter of modified bubble column reactor could be used for cultured jatropha endosperm cell. Although the use of bubble column reactor for plant cell culture were still not applicable and need to be further developed, but it was important for produce industrially valuable products of microorganism such as enzyme, protein, antibiotics, etc. (Nigar *et al.*, 2005). However our experiment was first reported on this type rector for plant cell suspension culture and lipid production. Although, the experiment had many problem in cultured such as the control a contamination, the problem for harvested and difficult in sampling. However, the oil component produced in our reactor was quite high and the modified reactor system for

growth and production of oil component from *J. curcas* L. need be further investigated and develop.