

## CHAPTER IV

### RESULTS AND DISCUSSION

#### 4.1 The growth of yeast in monoculture.

##### 4.1.1 The growth of *Candida utilis*

*Candida utilis* was cultivated in YM medium at OTR about 4 mMol O<sub>2</sub>/l.h, 150 rpm (Milan Dostalek 1983 ). The growth, protein of biomass and glucose consumption were measured. The results are presented in Table 4.1 and figure 4.1. It can be seen that most of glucose was used after 24 hour of cultivation. The maximum specific growth rate of 0.083 h<sup>-1</sup>, the yield of biomass of 0.495 g/g and the percent of theoretical biomass yield of 97 % were achieved.

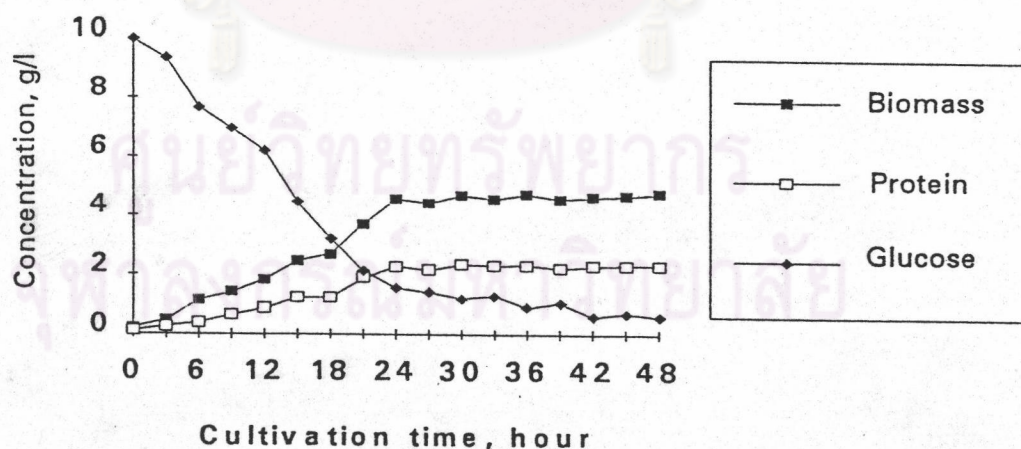


Fig. 4.1 The growth of *C. utilis* 5001 in YM medium

The cultivation of *C. utilis* for used as starter was carried out in three groups

Each group with 5 observations and results are shown in figure 4.2. The average dry weight of the three repetitions are 4.55 g/l, 4.53 g/l, 4.52 g/l respectively (Table 4.2). The results of calculation of Anova is shown in Table 4.3. The critical value of F for two degrees of freedom (2 and 12) were given as 3.08 from S.Critical Table value of the F-distribution. Since the computed value of F (0.12) does not exceed the critical value ( $F_{0.05,2,12} = 3.08$ ). Thus the average dry weight of biomass of *C. utilis* in YM medium are not significantly different at level of 0.05.

Table 4.1 The growth of *Candida utilis* 5001 in YM medium

No.	hour	Biomass g/l	Protein g/l	Glucose g/l
1	0	0.21	0.11	9.99
2	3	0.47	0.25	9.36
3	6	1.14	0.39	7.68
4	9	1.4	0.65	6.95
5	12	1.82	0.86	6.21
6	15	2.45	1.22	4.43
7	18	2.68	1.23	3.2
8	21	3.71	1.88	2.11
9	24	4.55	2.27	1.54
10	27	4.41	2.15	1.4
11	30	4.68	2.35	1.2
12	33	4.55	2.29	1.29
13	36	4.73	2.31	0.9
14	39	4.54	2.22	1.05
15	42	4.61	2.28	0.6
16	45	4.66	2.31	0.7
17	48	4.75	2.32	0.6

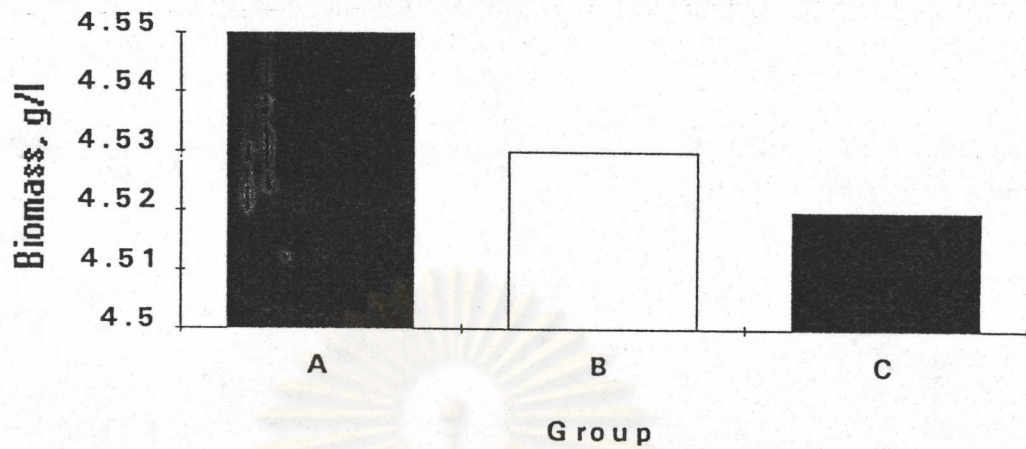


Fig. 4.2 Average dry weight of biomass of *C. utilis* after 24 hour of cultivation.

It can be concluded that these averages of dry weight of biomass are equal and they do not depend on the amount of observations. In this condition of cultivation, the culture contains approximately 300-400 million cell per ml as determined by hemocytometer counts. The growth characteristics of *C. utilis* was considered suitable to be used for the experiments of mixed culture in SCP production.

Table 4.2 Average dry weight of biomass of *C. utilis* after 24 hour of cultivation.

Observations	group A g/l	group B g/l	group C g/l
1	4.55	4.57	4.37
2	4.61	4.39	4.51
3	4.48	4.62	4.67
4	4.57	4.58	4.55
5	4.54	4.51	4.52
average	4.55	4.53	4.52

Table 4.3 Anova Table for *Candida utilis*~ for 24 hr.

Source	DF	SS	MS
Treatment	2	1.75E-03	8.52E-04
Error	12	8.70E-02	7.25E-03
Total	14	8.87E-2	-

F- value = 0.12

D.F. 2, 12

F- table = 3.08

0.12 &lt; 3.08

#### 4.1.2 The growth of *Endomycopsis fibuligera*

*E. fibuligera* was cultivated in yeast starch medium at OTR about 4 mMol O<sub>2</sub>/l.h at 150 rpm (Milan Dostalek 1983). The growth, protein content, accumulation of glucose and starch consumption were measured. The results are presented in figure 4.3 and Table 4.4.

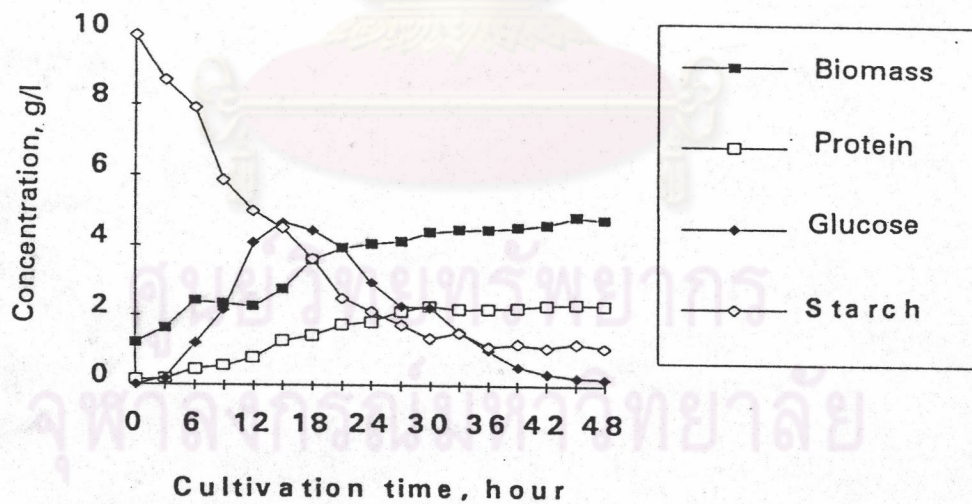
Fig. 4.3 The growth of *E. fibuligera* 5097 in YM medium

Table 4.4 The growth of *E. fibuligera* 5097 in yeast starch medium

No.	hour	pH	Biomass g/l	Protein g/l	Glucose g/l	Starch g/l
1	0	6.65	1.23	0.15	0.00	10
2	3	5.82	1.65	0.18	0.20	8.71
3	6	5.57	2.41	0.47	1.20	7.92
4	9	5.23	2.36	0.56	2.18	5.87
5	12	4.77	2.27	0.81	4.09	5.00
6	15	3.34	2.78	1.29	4.68	4.50
7	18	2.54	3.61	1.45	4.45	3.65
8	21	2.56	3.39	1.73	3.96	2.51
9	24	2.77	4.06	1.84	2.95	2.12
10	27	2.54	4.13	2.14	2.29	1.76
11	30	2.41	4.39	2.29	2.25	1.36
12	33	2.35	4.47	2.19	1.56	1.53
13	36	2.24	4.49	2.21	1.01	1.14
14	39	2.25	4.54	2.25	0.55	1.20
15	42	2.27	4.63	2.32	0.35	1.12
16	45	2.35	4.84	2.35	0.24	1.22
17	48	2.27	4.77	2.31	0.20	1.12

The study on cultivation of *E. fibuligera* for used as starter was carried out in three groups. Each group has 5 observations and results are shown in figure 4.6. The average dry weight of group A1, group B1 and group C1 are 4.59 g/l, 4.59 g/l, 4.55 g/l respectively (Table 4.5). The results of calculation of Anova is shown in Table 4.6.

Table 4.5 Average dry weight of biomass of *E. fibuliger* after 39 h. of cultivation.

Observations	group A1 g/l	group B1 g/l	group C1 g/l
1	4.68	4.61	4.57
2	4.58	4.63	4.51
3	4.55	4.44	4.53
4	4.55	4.59	4.52
5	4.59	4.67	4.62
average	4.59	4.59	4.55

Table 4.6 Anova Table of biomass of *E.fibuliger* in yeast starch media for 39 h.

Source	DF	SS	MS
Treatment	2	5.12E-03	2.56E-03
Error	12	5.05E-02	4.21E-03
Total	14	5.56E-02	-

F- value = 0.61

D.F. 2, 12

F- table = 3.08

0.61 < 3.08

The critical value of F for two degrees of freedom 2 and 12 was given as 3.08 from the Table of S.critical values of the F-distribution. Since the computed value of F is 0.61 which does not exceeds the critical value ( $F_{.05,2,12} = 3.08$ ), therefore, the average dry weight of biomass of *E. fibuligera* in YM medium are not different at the level of significant of 0.05. The conclusion is thus these values of average dry weight of biomass are equal and they do not depend on the number of observations.

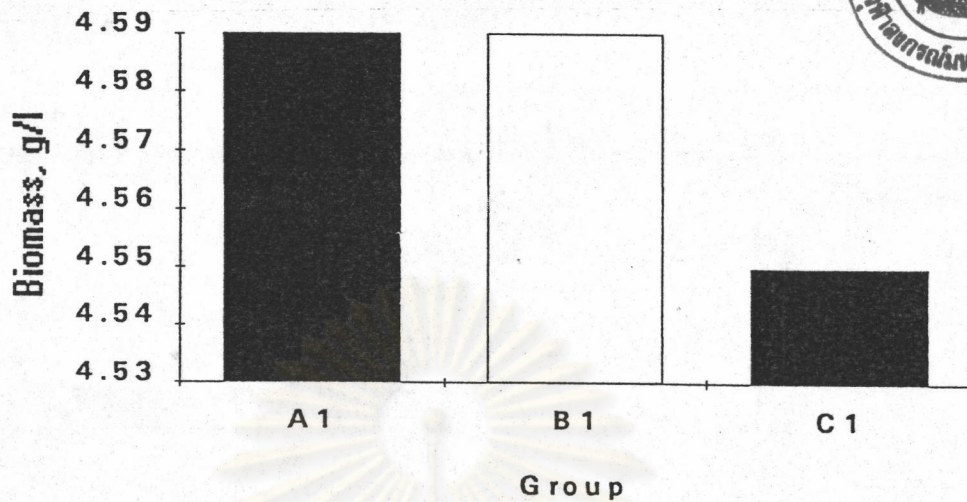


Fig.4.4 The average dry weight of biomass of *E. fibuliger* after 39 h. of cultivation.

The maximum specific growth rate of  $0.082 \text{ h}^{-1}$ , the yield of biomass of  $0.473 \text{ g/g}$  and the percent of theoretical biomass yield of  $92.7 \%$  were achieved (Table 4.7).

Table 4.7 Kinetics parameter of *C. utilis* 5001 and *E. fibuligera* 5097

Yeast	$U_{max}$	$q_s$	$Y_{x/s}$	Y%
<i>C. utilis</i> 5001	0.083	0.459	0.167	97
<i>E. fibuligera</i> 5097	0.082	0.473	0.173	92.7

$U_{max}$ : The maximum specific growth rate  $\text{h}^{-1}$

$q_s$  : The specific biomass production rate  $\text{g/g.h}$

$Y_{x/s}$  : The biomass yield  $\text{g/g}$

Y% : The percent of theoretical biomass yield %

In this condition of cultivation, the culture contains approximately 400-450 million cell per ml as determined by hemocytometer counts. The growth characteristics of *E. fibuligera* was considered suitable to be used for the experiments of mixed culture in SCP production.

## 4.2 The growth of yeast in the mixed culture

### 4.2.1 Effect of the beginning time of mixing

Figure 4.5 and Table 18 showed the results of experiment which used ratio of *C. utilis* to *E. fibuligera* as 1:1 by volume. The final concentration of biomass reached a highest value of 4.55 g/l for the introduction of *C. utilis* at 18 th hour and a lowest value of 2.62 g/l at the 0th hour. When mixed inoculum of two yeast at the 0th hour of cultivation, amylase activity of *E. fibuligera* appeared to be retarded because growth of *C. utilis* predominated in the culture. This interpretation agrees with the report by S.A. Lemmel 1979 who examined the growth of *C. utilis* and *E. fibuligera* in mixed, batch cultures. Mean while addition of *C. utilis* at the 18th hour it did not limit the activity of amylase because large quantity of enzyme had been produced sufficiently. For the 24th hour, the time was too long that substrate concentration was reduced due to the consumption by *E. fibuligera*.

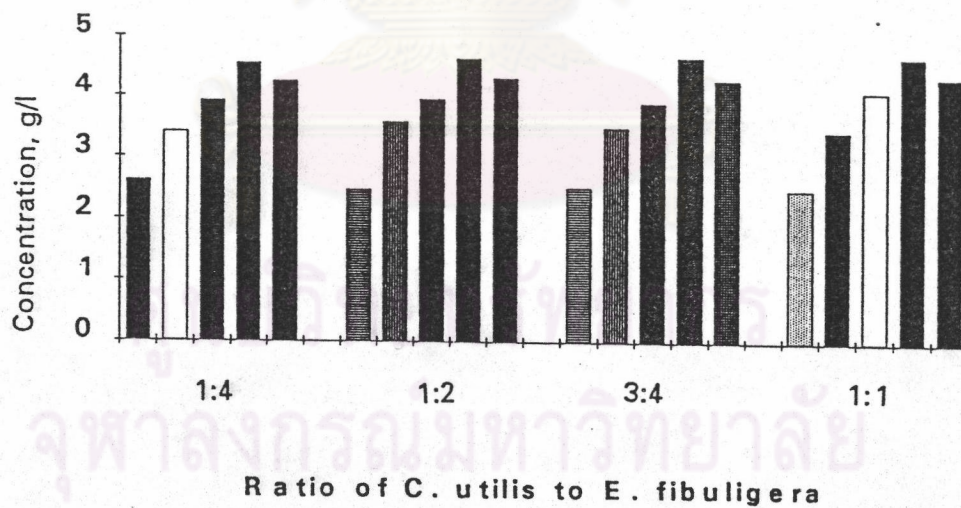


Fig. 4.5 Variations of biomass with time for all volume ratio of starter.

*C. utilis* and *E. fibuligera* was mixed also with a volume ratio of 1:2, 1:3 and 1:4. It can be seen from figure 4.5 that biomass yield from mixed culture is the same for one addition time being highest at 18th hour. The biomass yielded



does not depend on the ratio of the two yeast

Table 4.8 Variations of biomass dry weight (g/l) with time for all volume ratio of starter

Ratio Candida:Endomycopsis	The addition time				
	0 h	6 h	12 h	18 h	24 h
1:1	2.62	3.41	3.91	4.55	4.25
1:2	2.47	3.57	3.95	4.63	4.31
1:3	2.51	3.49	3.89	4.66	4.29
1:4	2.49	3.45	4.10	4.68	4.35

In all cases, amylase production and concentration of biomass may have been limited by early time of addition of *C. utilis*. Thus all the results presented should be primarily attributed to the growth of *C. utilis* due to its predominance. According to the results, for early the addition of *C. utilis* is probably not good and efficient approach for this SCP production process. An alternative would be a two-stage cultivation which would cultivate *E. fibuligera* first for a period of time until sufficient biomass is obtained to produce amylase as reported in Symba process (Jarl 1969). The second stage would be a mixed culture at an appropriate time, in which *C. utilis* would be predominant. The information gained from this study indicates an optimum addition time of *C. utilis* to be at 18th hour.

Table 4.9 Anova Table for the effect of addition time and ratio of stater

Source	DF	SS	MS
Treatment	19	33.05	1.740
Blocks	2	0.00	0.003
Error	38	0.35	0.009
Total	59	33.41	-

F- value = 186.4    F- table = 1.84    D.F. 19, 38    239.3 > 1.84

The statistical analyses was used here to determine the confidence of the data. (Table 4.9). For any ratio of *C. utilis* to *E. fibuligera*, it was observed that a high concentration of biomass was reached at 18th hour of addition time. The variations of the quantity of biomass is not significant, or they are equal at significant level 0.05.

#### 4.2.2 The study on the use of molasses as vitamins in mixed culture for SCP production.

The result of measurement of biomass concentration, protein formation and glucose uptake rates in the medium M1 are presented in Figure 4.6 and Table 4.10. The maximum rate of protein formation (0.04 g/l.h) takes place between the 12th and the 39th hour of cultivation. The higher concentration of glucose reduces the rate of starch hydrolysis and hinders the growth of *E. fibuligera*.. The concentration of glucose reached a peak at the 15th hour. The maximum glucose accumulation rate was 0.52 g/l.h.

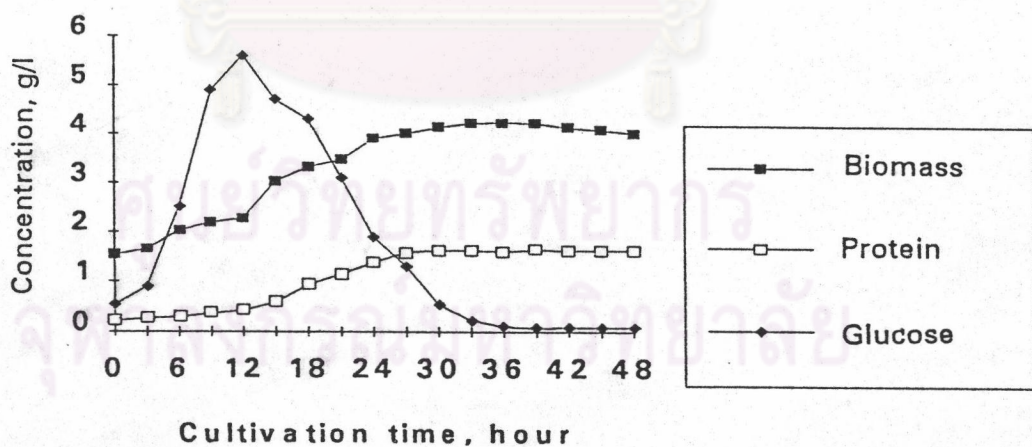


Fig. 4.6l The growth of mixed culture of *C. utilis* and *E. fibuligera* in medium M1.

The concentration of glucose decreased after the 12th hour. Glucose was consumed at a nearly constant rate of 0.29 g/l.h and its concentration at the end of the cultivation period was 0.09 g/l.

Table 4.10 The growth of mixed culture of *C. utilis* and *E. fibuligera* in medium M1.

No.	hour	Biomass g/l	Protein g/l	Glucose g/l
1	0	1.53	0.22	0.54
2	3	1.65	0.27	0.90
3	6	2.02	0.30	2.50
4	9	2.19	0.38	4.90
5	12	2.28	0.43	5.60
6	15	3.03	0.59	4.70
7	18	3.32	0.94	4.30
8	21	3.47	1.15	3.10
9	24	3.91	1.38	1.90
10	27	4.01	1.58	1.30
11	30	4.14	1.62	0.54
12	33	4.23	1.63	0.23
13	36	4.24	1.60	0.11
14	39	4.22	1.64	0.08
15	42	4.14	1.62	0.09
16	45	4.09	1.63	0.09
17	48	4.02	1.63	0.09

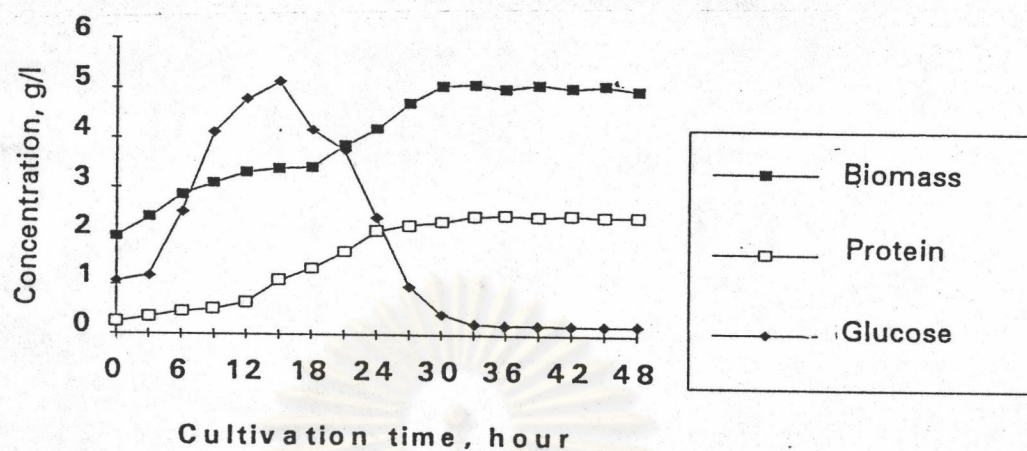


Fig.4.7 The growth of mixed culture of *C. utilis* and *E. fibuligera* in medium M2.

Similarly, for the growth of mixed culture in medium M2, the maximum protein formation rate, 0.06 g/l.h, the maximum glucose accumulation rate, 0.33 g/l.h, the maximum glucose consumption rate, 0.27 g/l.h were obtained. The numerical results are shown in figure 4.7 and Table 4.11.

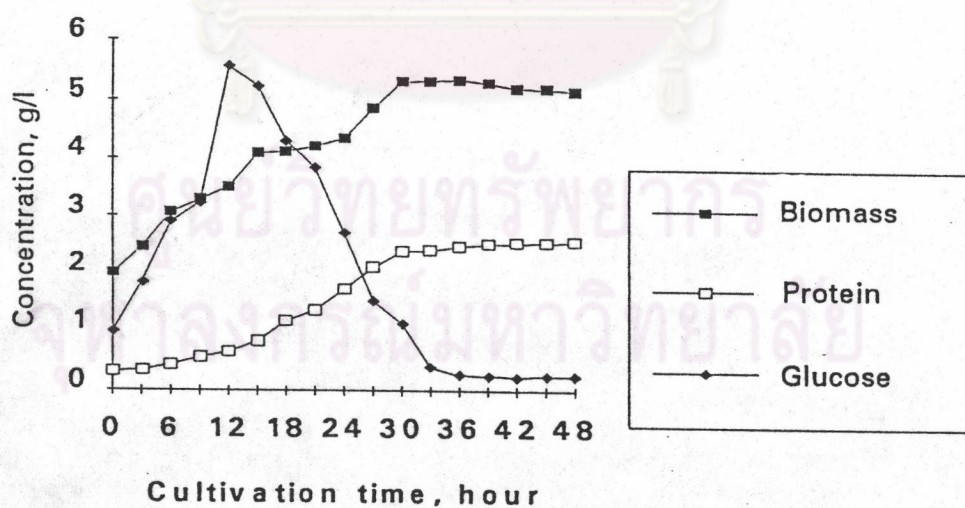


Fig.4.8 The growth of mixed culture of *C. utilis* and *E. fibuligera* in medium M3.

Table 4.11 The growth of mixed culture of *C. utilis* and *E. fibuligera* in medium M2.

No.	hour	Biomass g/l	Protein g/l	Glucose g/l
1	0	2.01	0.24	1.08
2	3	2.39	0.35	1.20
3	6	2.86	0.47	2.50
4	9	3.11	0.52	4.13
5	12	3.32	0.65	4.78
6	15	3.40	1.10	5.13
7	18	3.42	1.35	4.18
8	21	3.87	1.69	3.76
9	24	4.20	2.12	2.39
10	27	4.72	2.25	0.99
11	30	5.07	2.31	0.42
12	33	5.09	2.43	0.22
13	36	5.01	2.45	0.21
14	39	5.10	2.43	0.20
15	42	5.02	2.45	0.19
16	45	5.08	2.43	0.19
17	48	4.98	2.44	0.19

Similarly, for the growth of mixed culture in medium M3, the maximum protein formation rate, 0.06 g/l.h, the maximum glucose accumulation rate, 0.41 g/l.h, the maximum glucose consumption rate, 0.25 g/l.h were obtained. The numerical results are shown in figure 4.8 and Table 4.12

Table 4.12 The growth of mixed culture of *C. utilis* and *E. fibuligera* in medium M3.

No.	hour	Biomass g/l	Protein g/l	Glucose g/l
1	0	2.21	0.23	1.62
2	3	2.45	0.34	1.85
3	6	3.06	0.44	2.90
4	9	3.29	0.57	3.24
5	12	3.51	0.66	5.56
6	15	4.09	0.84	5.24
7	18	4.12	1.18	4.30
8	21	4.21	1.37	3.84
9	24	4.35	1.73	2.71
10	27	4.87	2.11	1.52
11	30	5.32	2.38	1.14
12	33	5.33	2.41	0.40
13	36	5.35	2.48	0.27
14	39	5.30	2.52	0.25
15	42	5.21	2.56	0.24
16	45	5.20	2.55	0.25
17	48	5.17	2.54	0.26

The concentration of biomass reached a highest value of 4.24 g/l after 36 hour of cultivation. At the same time, concentration of protein reached 1.64 g/l. Therefore, the ratio of biomass to cassava was 0.402 g/g (figure 4.10) and protein in biomass (dry weight) was 38 % (figure 4.11). The ratio of protein produced at any time to the maximum protein (1.64 g/l) that can be obtained increases gradually reaching 1 at the 39th hour of cultivation.

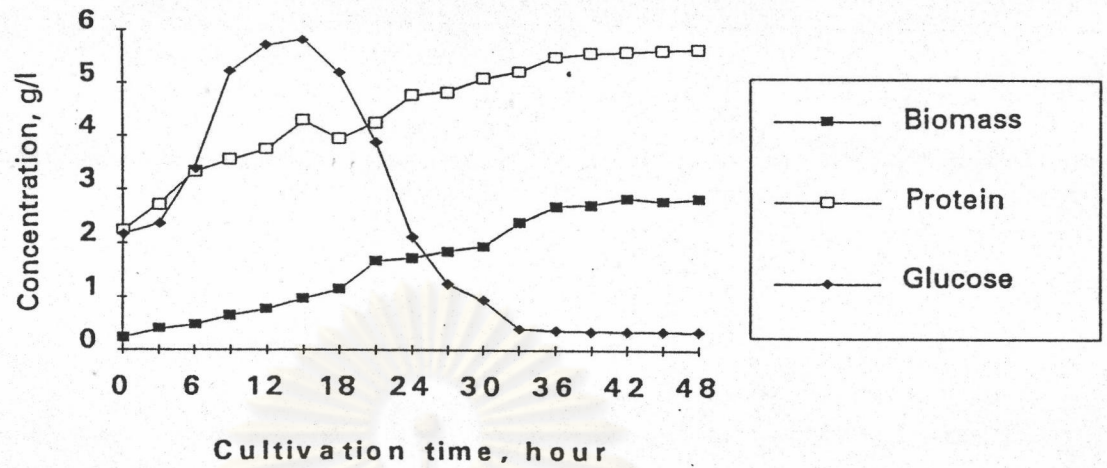


Fig. 4.9 The growth of mixed culture of *C. utilis* and *E. fibuligera* in medium M4.

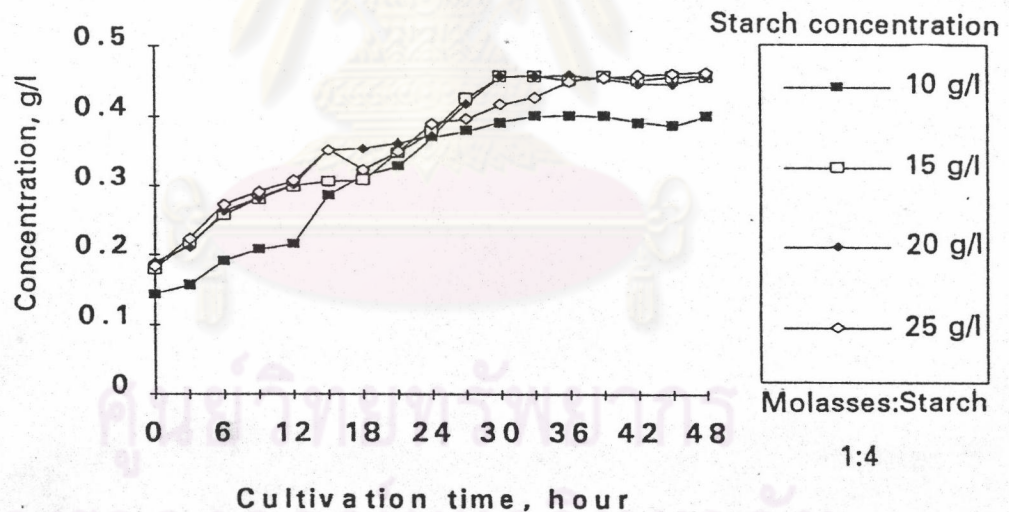


Fig. 4.10 Ratio of biomass to cassava in mixed culture of *C. utilis* and *E. fibuligera* in medium M1, M2, M3, M4.

The ratio of biomass to cassava was 0.402 g/g (figure 4.10) and protein in biomass (dry weight) was 38 % (figure 4.11). The ratio of protein produced at any time to the maximum protein (1.64 g/l) that can be obtained increases gradually reaching 1 at the 39th hour of cultivation. The maximum rate of

protein formation (0.04 g/l.h) takes place between the 12th and the 39th hour of cultivation.

Similarly, for the growth of mixed culture in medium M4, the maximum protein formation rate, 0.07 g/l.h, the maximum glucose accumulation rate, 0.29 g/l.h, the maximum glucose consumption rate, 0.30 g/l.h were obtained. The numerical results are shown in Table 4.13

Table 4.13 The growth of mixed culture of *C. utilis* and *E. fibuligera* in medium M4.

No.	hour	Biomass g/l	Protein g/l	Glucose g/l
1	0	2.24	0.24	2.16
2	3	2.72	0.39	2.35
3	6	3.32	0.47	3.39
4	9	3.55	0.65	5.22
5	12	3.75	0.77	5.70
6	15	4.29	0.97	5.82
7	18	3.94	1.14	5.18
8	21	4.26	1.66	3.88
9	24	4.76	1.72	2.11
10	27	4.83	1.85	1.25
11	30	5.08	1.95	0.94
12	33	5.22	2.38	0.41
13	36	5.49	2.69	0.37
14	39	5.56	2.72	0.36
15	42	5.59	2.84	0.35
16	45	5.62	2.79	0.35
17	48	5.65	2.83	0.34



The ratio of biomass dry weight to cassava in mixed culture using medium M1, M2, M3, M4 was relatively stable after 33 hour of cultivation. The numerical results are presented in Table 4.14

Table 4.14 Ratio of dry weight to cassava in mixed culture of *C. utilis* and *E. fibuligera* in medium M1, M2, M3, M4.

No.	hour	M1 g/g	M2 g/g	M3 g/g	M 4 g/g
1	0	0.145	0.181	0.190	0.184
2	3	0.157	0.216	0.211	0.224
3	6	0.192	0.258	0.263	0.273
4	9	0.208	0.281	0.283	0.292
5	12	0.216	0.300	0.302	0.308
6	15	0.287	0.307	0.352	0.353
7	18	0.315	0.309	0.355	0.324
8	21	0.329	0.349	0.362	0.350
9	24	0.371	0.379	0.374	0.391
10	27	0.380	0.426	0.419	0.397
11	30	0.393	0.458	0.458	0.418
12	33	0.401	0.459	0.459	0.429
13	36	0.402	0.452	0.460	0.451
14	39	0.401	0.459	0.455	0.457
15	42	0.393	0.453	0.448	0.460
16	45	0.388	0.458	0.448	0.462
17	48	0.401	0.459	0.459	0.465

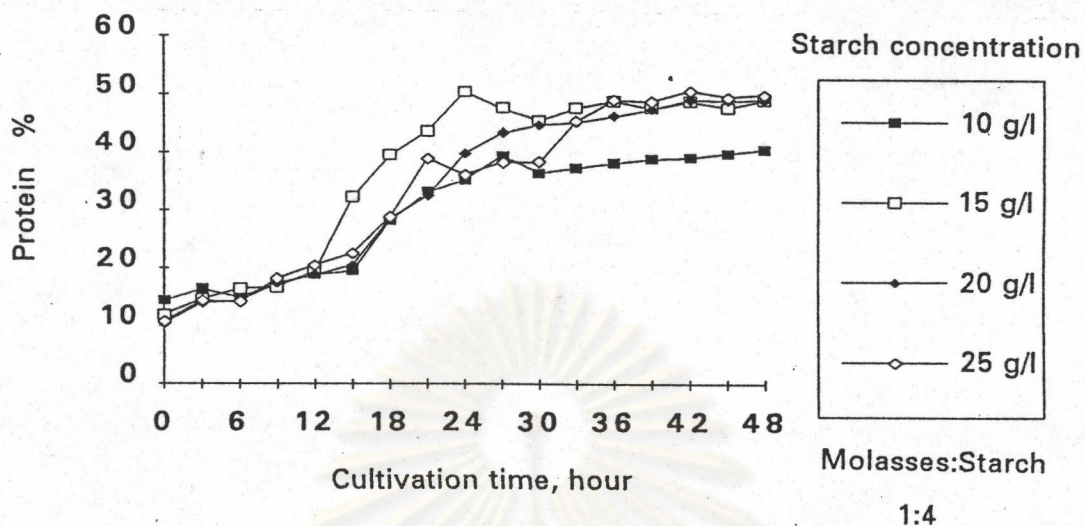


Fig. 4.11 Protein content in biomass of mixed culture of *C. utilis* and *E. fibuligera* in medium M1, M2, M3, M4.

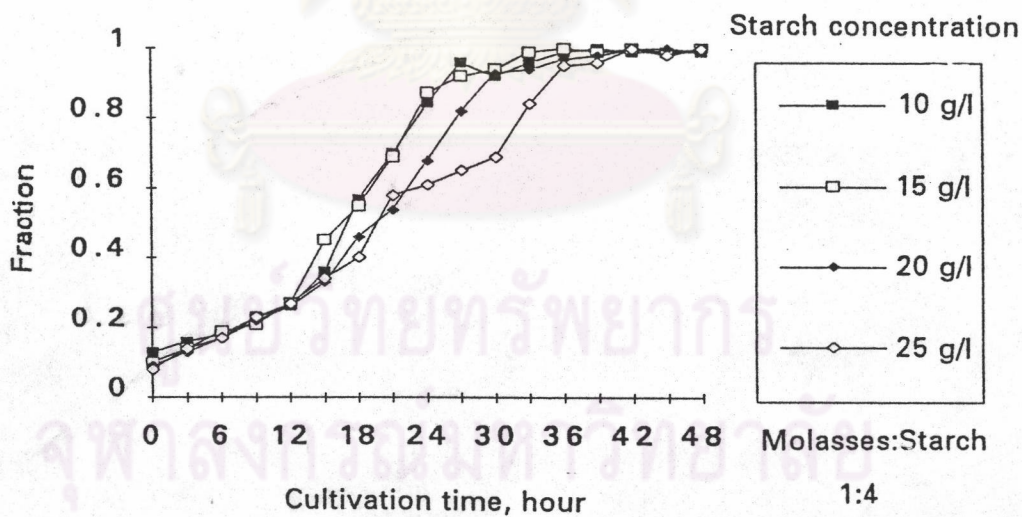


Fig. 4.12 Ratio of protein produced at any time to maximum protein obtained in mixed culture in medium M1, M2, M3, M4.

The protein content in biomass of mixed culture of *C. utilis* and *E. fibuligera* in medium, M1, M2, M3, M4 was relatively stable after 36 hour of cultivation. The numerical results are presented in Table 4.15 and figure 4.11.

Table 4.15 Protein content in biomass of mixed culture of *C. utilis* and *E. fibuligera* in medium M1, M2, M3, M4.

No.	hour	M1 %	M2 %	M3 %	M4 %
1	0	14.38	11.94	10.41	10.71
2	3	16.36	14.64	13.88	14.34
3	6	14.85	16.43	14.38	14.16
4	9	17.35	16.72	17.33	18.31
5	12	18.86	19.58	18.80	20.53
6	15	19.47	32.35	20.54	22.61
7	18	28.31	39.47	28.64	28.93
8	21	33.14	43.67	32.54	38.97
9	24	35.22	50.48	39.77	36.13
10	27	39.40	47.67	43.33	38.30
11	30	36.47	45.56	44.74	38.39
12	33	37.35	47.74	45.22	45.59
13	36	38.21	48.90	46.36	49.00
14	39	38.86	47.65	47.55	48.92
15	42	39.13	48.80	49.14	50.81
16	45	39.85	47.83	49.04	49.64
17	48	40.55	49.00	49.13	50.09

The formation of cell mass protein in mixed culture of *C. utilis* and *E. fibuligera* in medium M1, M2, M3, M4 was relatively stable about 39 hour of cultivation. The numerical results are presented in Table 4.16

Table 4.16 Ratio of protein produced at any time to maximum protein obtained in mixed culture in medium M1, M2, M3, M4.

No.	hour	M1 g/g	M2 g/g	M3 g/g	M4 g/g
1	0	.013	0.10	0.09	0.08
2	3	0.16	0.14	0.13	0.14
3	6	0.18	0.19	0.17	0.17
4	9	0.23	0.21	0.22	0.23
5	12	0.26	0.27	0.26	0.27
6	15	0.36	0.45	0.33	0.34
7	18	0.57	0.55	0.46	0.40
8	21	0.70	0.69	0.54	0.58
9	24	0.84	0.87	0.68	0.61
10	27	0.96	0.92	0.82	0.65
11	30	0.92	0.94	0.93	0.69
12	33	0.96	0.99	0.94	0.84
13	36	0.99	1.00	0.97	0.95
14	39	1.00	0.99	0.98	0.96
15	42	0.99	1.00	1.00	1.00
16	45	0.99	0.99	1.00	0.98
17	48	0.99	1.00	0.99	1.00

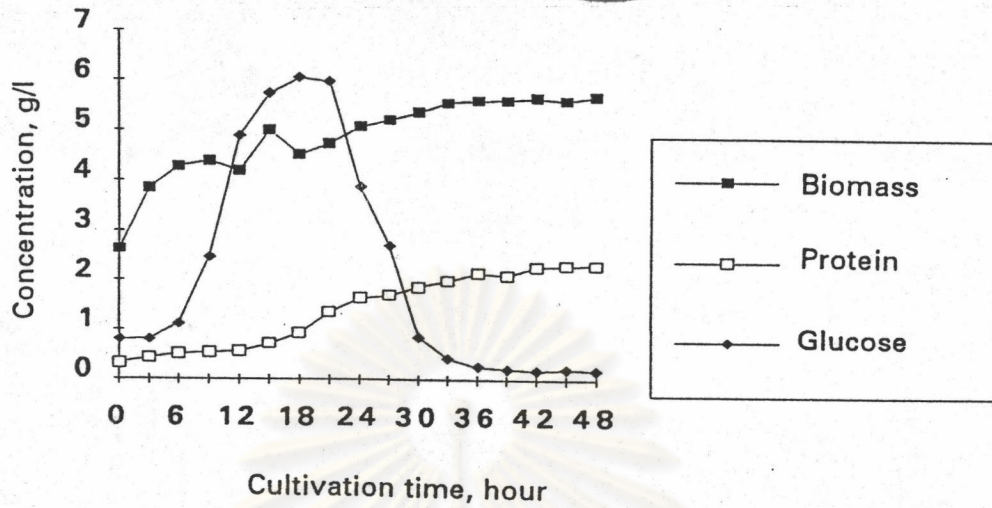


Fig. 4.13 The growth of mixed culture of *C. utilis* and *E. fibuligera* in medium M5.

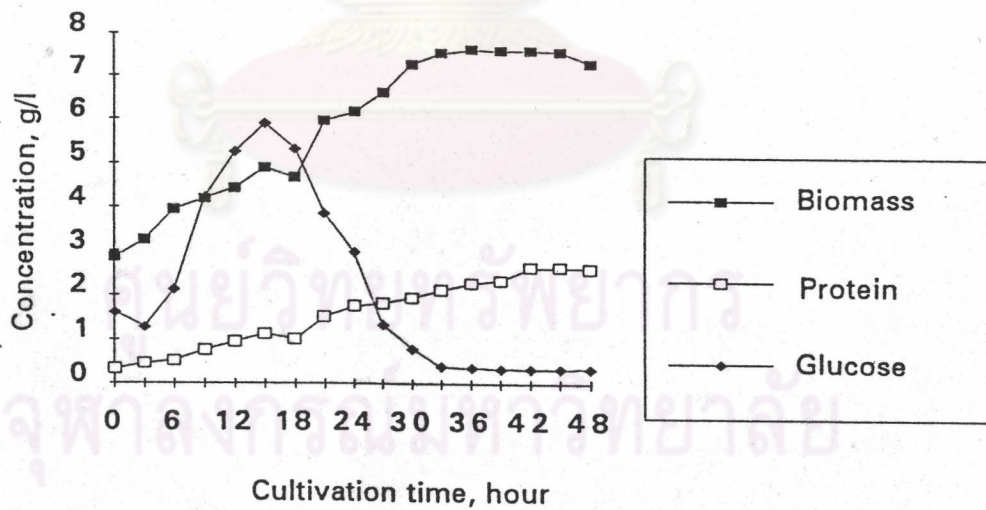


Fig. 4.14 The growth of mixed culture of *C. utilis* and *E. fibuligera* in medium M6

For the growth of mixed culture in medium M5, the maximum protein formation rate, 0.07 g/l.h, the maximum glucose accumulation rate, 0.29 g/l.h,

the maximum glucose consumption rate, 0.30 g/l.h were obtained. The numerical results are shown in Table 4.17

Table 4.17 The growth of mixed culture of *C. utilis* and *E. fibuligera* in medium M5.

No.	hour	Biomass g/l	Protein g/l	Glucose g/l
1	0	2.63	0.33	0.81
2	3	3.85	0.43	1.12
3	6	3.95	0.53	2.12
4	9	4.19	0.76	4.22
5	12	4.44	0.95	5.25
6	15	4.89	1.12	5.88
7	18	4.66	1.01	5.32
8	21	5.95	1.52	3.85
9	24	6.14	1.75	2.97
11	30	5.35	1.87	0.86
12	33	5.55	1.99	0.42
13	36	5.59	2.15	0.26
14	39	5.60	2.11	0.22
15	42	5.64	2.29	0.19
16	45	5.59	2.31	0.21
17	48	5.68	2.32	0.20

Similarly, for the growth of mixed culture in medium M6 , the maximum protein formation rate, 0.06 g/l.h, the maximum glucose accumulation rate, 0.42 g/l.h, the maximum glucose consumption rate, 0.36 g/l.h were obtained.

The numerical results are shown in Table 4.18

Table 4.18 The growth of mixed culture of *C. utilis* and *E. fibuligera* in medium M6

No.	hour	Biomass g/l	Protein g/l	Glucose g/l
1	0	2.84	0.34	1.62
2	3	3.25	0.45	1.28
3	6	3.95	0.53	2.12
4	9	4.19	0.76	4.22
5	12	4.44	0.95	5.25
6	15	4.89	1.12	5.88
7	18	4.66	1.01	5.32
8	21	5.95	1.52	3.85
9	24	6.14	1.75	2.97
10	27	6.61	1.83	1.34
11	30	7.29	1.95	0.80
12	33	7.55	2.11	0.39
13	36	7.63	2.28	0.37
14	39	7.61	2.35	0.35
15	42	7.61	2.63	0.34
16	45	7.59	2.63	0.34
17	48	7.31	2.62	0.35

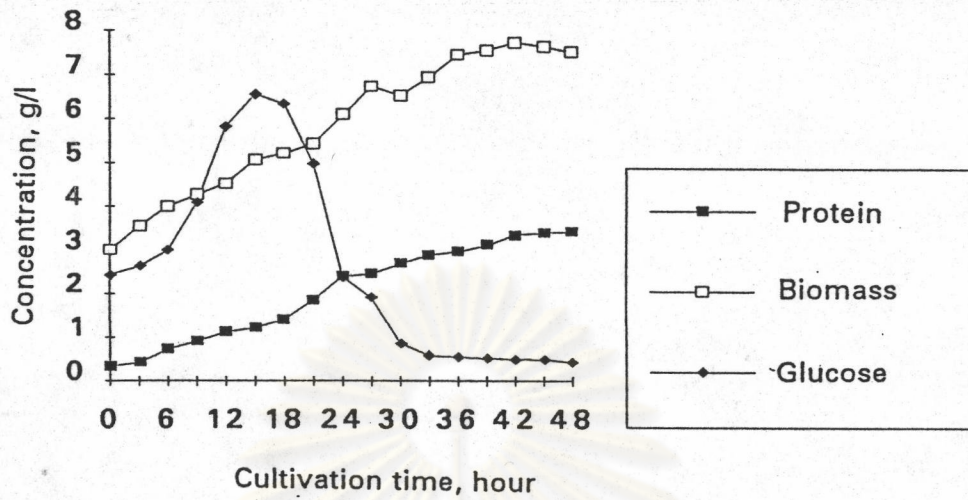


Fig. 4.15 The growth of mixed culture of *C. utilis* and *E. fibuligera* in medium M7

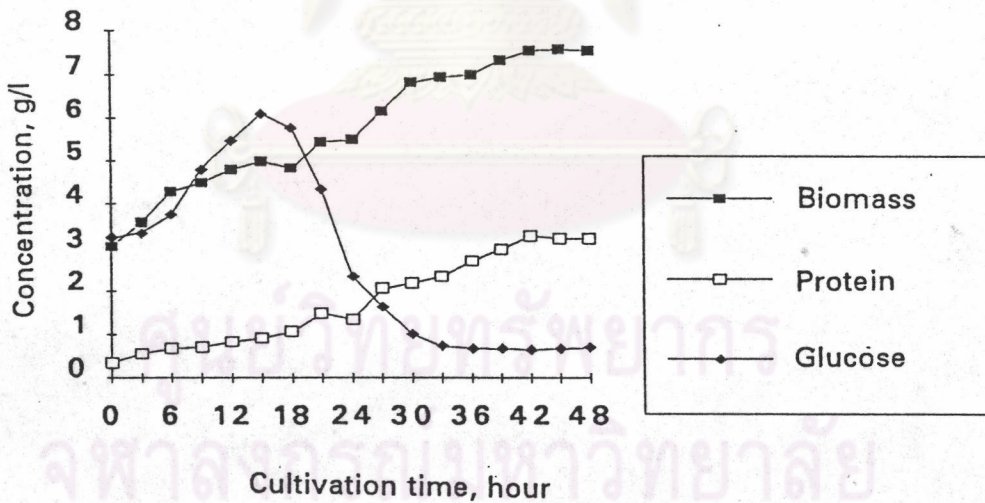


Fig. 4.16 The growth of mixed culture of *C. utilis* and *E. fibuligera* in medium M8

Similarly, for the growth of mixed culture in medium M7, the maximum protein formation rate, 0.09 g/l.h, the maximum glucose accumulation rate, 0.40 g/l.h, the maximum glucose consumption rate, 0.33 g/l.h were obtained. The numerical results are shown in Table 4.19



Table 4.19 The growth of mixed culture of *C. utilis* and *E. fibuligera* in medium M7

No.	hour	Biomass g/l	Protein g/l	Glucose g/l
1	0	2.99	0.34	2.43
2	3	3.55	0.42	2.63
3	6	4.01	0.72	2.99
4	9	4.27	0.91	4.10
5	12	4.53	1.11	5.82
6	15	5.07	1.21	6.56
7	18	5.22	1.40	6.34
8	21	5.43	1.86	4.96
9	24	5.55	2.38	3.22
10	27	6.73	2.45	1.90
11	30	6.53	2.69	0.85
12	33	6.95	2.88	0.58
13	36	7.46	2.96	0.54
14	39	7.56	3.11	0.52
15	42	7.73	3.35	0.50
16	45	7.65	3.39	0.49
17	48	7.51	3.43	0.43

Similarly, for the growth of mixed culture in medium M8, the maximum protein formation rate, 0.09 g/l.h, the maximum glucose accumulation rate, 0.23 g/l.h, the maximum glucose consumption rate, 0.30 g/l.h were obtained. The numerical results are shown in Table 4.20

Table 4.20 The growth of mixed culture of *C. utilis* and *E. fibuligera* in medium M8

No.	hour	Biomass g/l	Protein g/l	Glucose g/l
1	0	3.03	0.34	3.24
2	3	3.59	0.56	3.35
3	6	4.29	0.66	3.75
4	9	4.48	0.71	4.80
5	12	4.79	0.82	5.46
6	15	4.98	0.91	6.10
7	18	4.83	1.05	5.76
8	21	5.43	1.49	4.33
9	24	5.48	1.34	2.34
10	27	6.16	2.05	1.65
11	30	6.82	2.19	0.99
12	33	6.95	2.33	0.72
13	36	7.01	2.70	0.68
14	39	7.33	2.98	0.66
15	42	7.56	3.28	0.65
16	45	7.58	3.23	0.66
17	48	7.56	3.22	0.69

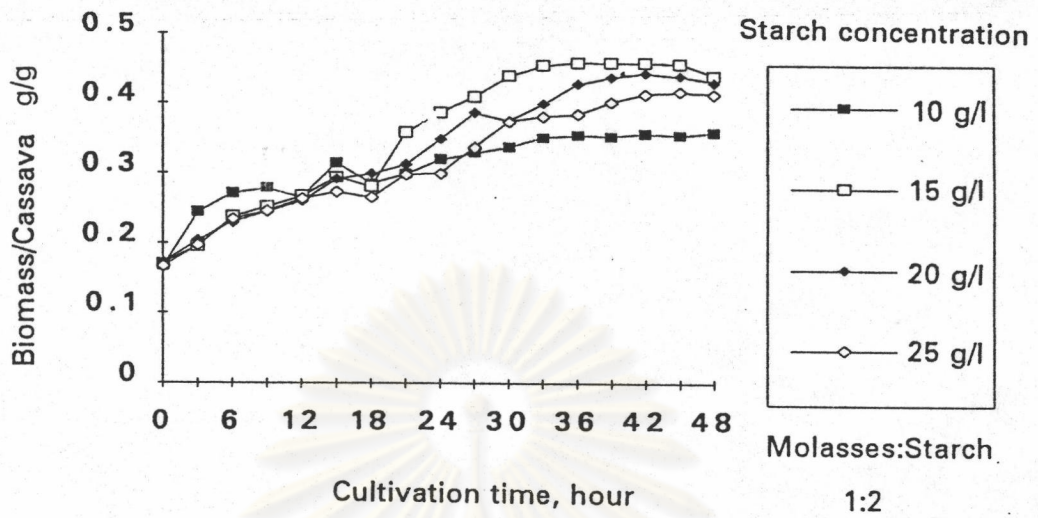


Fig. 4.17 Ratio of dry weight to cassava in mixed culture of *C. utilis* and *E. fibuligera* in medium M5, M6 , M7, M8.

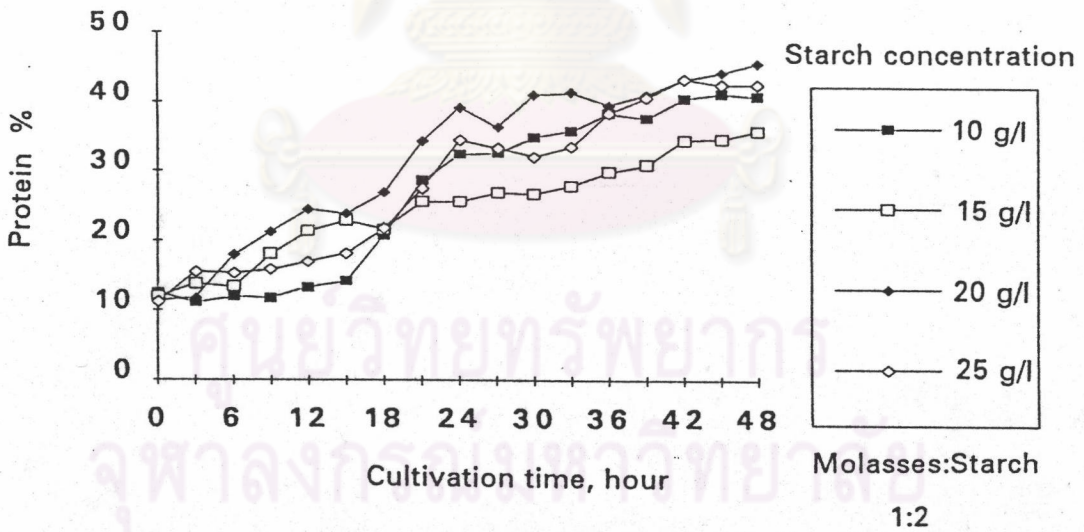


Fig. 4.18 Protein content in biomass of mixed culture of *C. utilis* and *E. fibuligera* in medium M5, M6 , M7, M8.

The ratio of biomass dry weight to cassava in mixed culture using medium M5, M6 , M7, M8 was relatively stable after 36 hour of cultivation. The numerical results are presented in Table 4.21

Table 4.21 Ratio of dry weight to cassava in mixed culture of *C. utilis* and *E. fibuligera* in medium M5, M6, M7, M8.

No.	hour	M5 g/g	M6 g/g	M7 g/g	M8 g/g
1	0	0.166	0.171	0.172	0.166
2	3	0.244	0.196	0.204	0.197
3	6	0.271	0.238	0.230	0.235
4	9	0.278	0.252	0.245	0.246
5	12	0.265	0.267	0.260	0.263
6	15	0.315	0.294	0.291	0.273
7	18	0.285	0.280	0.299	0.265
8	21	0.299	0.358	0.312	0.298
9	24	0.320	0.387	0.349	0.300
10	27	0.329	0.410	0.386	0.338
11	30	0.338	0.439	0.375	0.374
12	33	0.351	0.454	0.399	0.381
13	36	0.354	0.459	0.428	0.384
14	39	0.353	0.458	0.438	0.402
15	42	0.357	0.458	0.443	0.414
16	45	0.354	0.457	0.439	0.416
17	48	0.359	0.440	0.431	0.414

The protein content in biomass of mixed culture of *C. utilis* and *E. fibuligera* in medium, M5, M6, M7, M8 was relatively stable after 36 hour of cultivation. The numerical results are presented in Table 4.22

Table 4.22 Protein content in biomass of mixed culture of *C. utilis* and *E. fibuligera* in medium M5, M6, M7, M8.

No.	hour	M5 %	M6 %	M7 %	M8 %
1	0	12.55	11.97	11.37	11.22
2	3	11.17	13.85	11.83	15.60
3	6	11.92	13.42	17.96	15.38
4	9	11.85	18.14	21.31	15.85
5	12	13.37	21.40	24.50	17.12
6	15	14.26	22.90	23.87	18.27
7	18	20.84	21.67	26.82	21.74
8	21	28.54	25.55	34.25	27.44
9	24	32.41	25.62	39.31	34.49
10	27	32.50	26.87	36.40	33.28
11	30	34.95	26.75	41.19	32.11
12	33	35.86	27.95	41.44	33.53
13	36	38.46	29.88	39.68	38.52
14	39	37.68	30.88	41.14	40.65
15	42	40.60	34.56	43.34	43.39
16	45	41.32	34.65	44.31	42.61
17	48	40.85	35.84	45.67	42.59

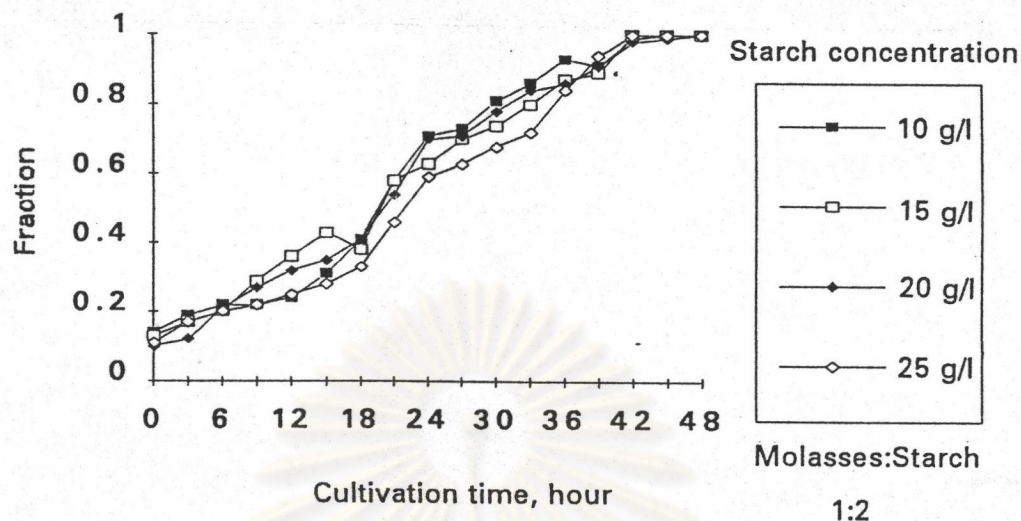


Fig. 4.19 Ratio of protein produced at any time to maximum protein obtained in mixed culture in medium M5, M6, M7, M8.

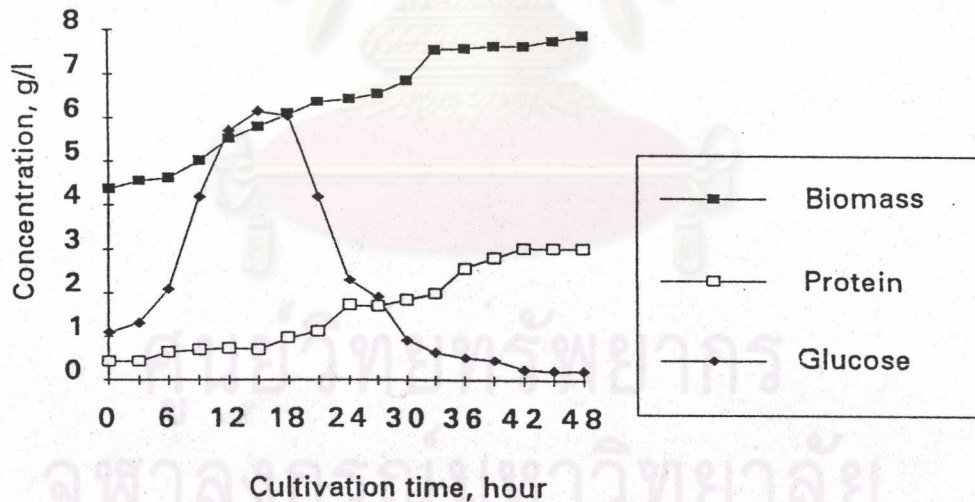


Fig. 4.20 The growth of mixed culture of *C. utilis* and *E. fibuligera* in medium M 9.

The formation of cell mass protein in mixed culture of *C. utilis* and *E. fibuligera* in medium M5, M6, M7, M8 was relatively stable about 39 hour of cultivation. The numerical results are presented in Table 4.23

Table 4.23 Ratio of protein produced at any time to maximum protein obtained in mixed culture in medium M5, M6, M7, M8.

No.	hour	M5 g/g	M6 g/g	M7 g/g	M8 g/g
1	0	0.14	0.13	0.10	0.11
2	3	0.19	0.17	0.12	0.17
3	6	0.22	0.20	0.21	0.20
4	9	0.22	0.29	0.27	0.22
5	12	0.24	0.36	0.32	0.25
6	15	0.31	0.43	0.35	0.28
7	18	0.41	0.38	0.41	0.33
8	21	0.58	0.58	0.54	0.46
9	24	0.71	0.63	0.70	0.59
10	27	0.73	0.70	0.71	0.63
11	30	0.81	0.74	0.78	0.68
12	33	0.86	0.80	0.84	0.72
13	36	0.93	0.87	0.86	0.84
14	39	0.91	0.89	0.91	0.94
15	42	0.99	1.00	0.98	1.00
16	45	1.00	1.00	0.99	1.00
17	48	1.00	1.00	1.00	1.00

For the growth of mixed culture in medium M9, the maximum protein formation rate, 0.08 g/l.h, the maximum glucose accumulation rate, 0.40 g/l.h, the maximum glucose consumption rate, 0.31 g/l.h were obtained. The numerical results are shown in Table 4.24

Table 4.24 The growth of mixed culture of *C. utilis* and *E. fibuligera* in medium M9.

No.	hour	Biomass g/l	Protein g/l	Glucose g/l
1	0	4.36	0.43	1.08
2	3	4.55	0.43	1.32
3	6	4.60	0.65	2.08
4	9	4.99	0.69	4.18
5	12	5.51	0.73	5.70
6	15	5.80	0.70	6.14
7	18	6.08	0.96	6.04
8	21	6.36	1.12	4.18
9	24	6.42	1.73	2.31
10	27	6.56	1.69	1.90
11	30	6.85	1.86	0.92
12	33	7.56	1.99	0.63
13	36	7.59	2.59	0.51
14	39	7.65	2.81	0.45
15	42	7.65	3.04	0.26
16	45	7.77	3.02	0.21
17	48	7.88	3.04	0.22

For the growth of mixed culture in medium M10, the maximum protein formation rate, 0.09 g/l.h, the maximum glucose accumulation rate, 0.34 g/l.h, the maximum glucose consumption rate, 0.32 g/l.h were obtained. The



results are shown in Table 4.25 and figure 4.21

Table 4.25 The growth of mixed culture of *C. utilis* and *E. fibuligera* in medium M10.

No.	hour	Biomass g/l	Protein g/l	Glucose g/l
1	0	3.75	0.43	2.16
2	3	4.25	0.63	2.22
3	6	4.97	0.77	2.69
4	9	4.96	0.89	3.98
5	12	4.59	0.91	5.52
6	15	5.20	1.12	6.60
7	18	6.07	1.93	6.24
8	21	6.35	2.00	3.66
9	24	6.76	2.15	1.99
10	27	6.57	2.69	1.66
11	30	6.83	2.92	0.92
12	33	7.16	2.99	0.87
13	36	7.35	3.05	0.81
14	39	7.55	3.10	0.75
15	42	7.95	3.05	0.61
16	45	7.87	3.12	0.53
17	48	7.88	3.14	0.46

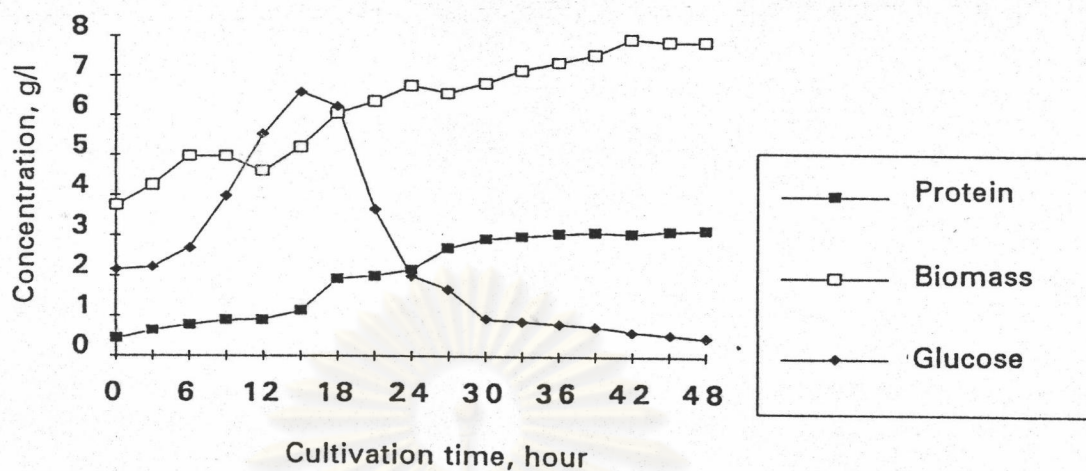


Fig. 4.21 The growth of mixed culture of *C. utilis* and *E. fibuligera* in medium M10.

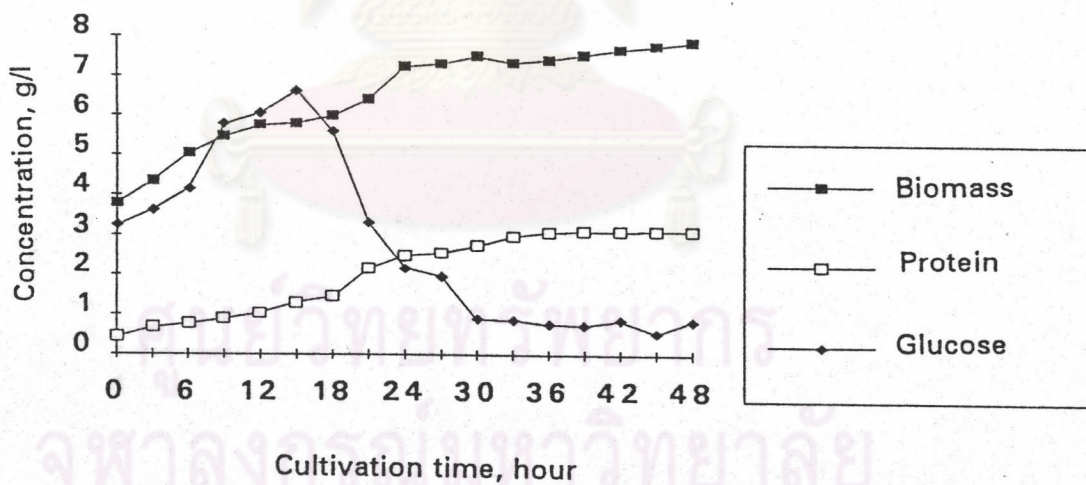


Fig. 22. The growth of mixed culture of *C. utilis* and *E. fibuligera* in medium M11.

For the growth of mixed culture in medium M11, the maximum protein formation rate, 0.08 g/l.h, the maximum glucose accumulation rate, 0.25 g/l.h,

the maximum glucose consumption rate, 0.38 g/l.h were obtained. The numerical results are shown in Table 4.26

Table 4.26 The growth of mixed culture of *C. utilis* and *E. fibuligera* in medium M11.

No.	hour	Biomass g/l	Protein g/l	Glucose g/l
1	0	3.78	0.43	3.24
2	3	4.35	0.66	3.62
3	6	5.03	0.76	4.14
4	9	5.46	0.89	5.78
5	12	5.75	1.04	6.07
6	15	5.80	1.31	6.64
7	18	5.99	1.46	5.59
8	21	6.43	2.15	3.32
9	24	7.24	2.49	2.17
10	27	7.32	2.55	1.96
11	30	7.51	2.75	0.89
12	33	7.35	2.98	0.87
13	36	7.43	3.09	0.76
14	39	7.56	3.11	0.75
15	42	7.69	3.11	0.86
16	45	7.77	3.13	0.53
17	48	7.88	3.12	0.85

For the growth of mixed culture in medium M12, the maximum protein formation rate, 0.07 g/l.h, the maximum glucose accumulation rate, 0.33 g/l.h,

the maximum glucose consumption rate, 0.42 g/l.h were obtained. The numerical results are shown in Table 4.27

Table4.27 The growth of mixed culture of *C. utilis* and *E. fibuligera* in medium M12.

No.	hour	Biomass g/l	Protein g/l	Glucose g/l
1	0	4.48	0.43	4.32
2	3	4.88	0.66	4.62
3	6	5.68	1.02	5.16
4	9	5.49	0.99	6.78
5	12	6.52	1.14	8.12
6	15	6.67	1.55	8.60
7	18	6.67	2.11	7.41
8	21	7.04	2.32	6.40
9	24	7.16	2.63	3.32
10	27	7.32	2.65	2.16
11	30	7.41	2.95	1.66
12	33	7.36	2.99	0.97
13	36	7.39	2.94	0.96
14	39	7.55	3.12	0.75
15	42	8.50	3.27	0.88
16	45	8.87	3.12	0.63
17	48	9.37	3.25	0.59

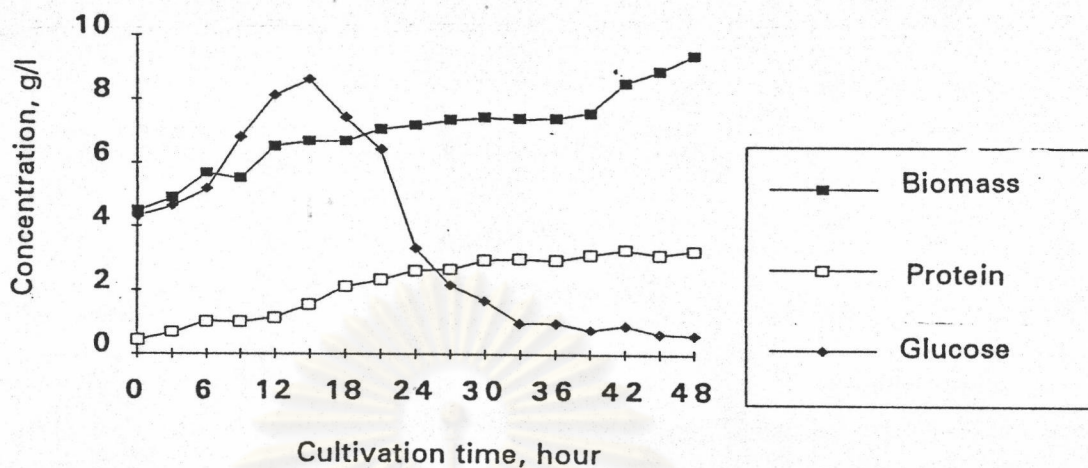


Fig. 4.23 The growth of mixed culture of *C. utilis* and *E. fibuligera* in medium M12.

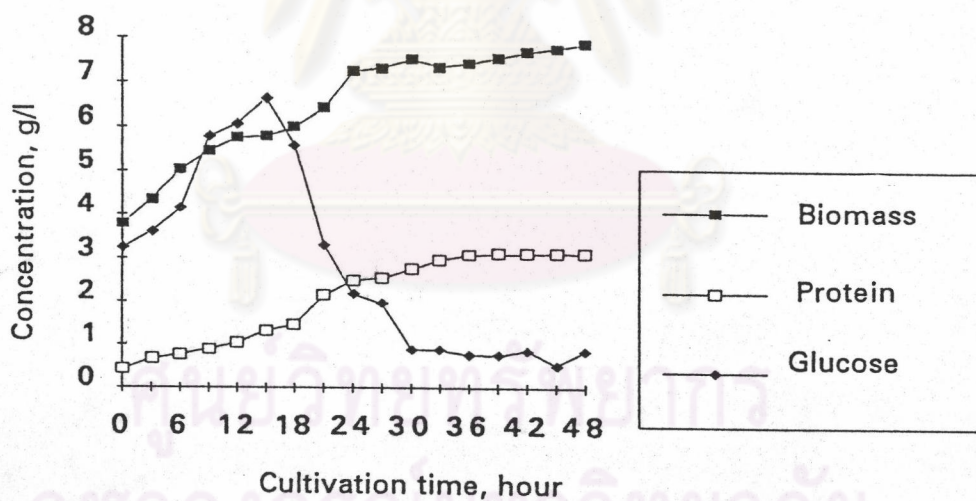


Fig. 4.24 Ratio of dry weight to cassava in mixed culture of *C. utilis* and *E. fibuligera* in medium M9, M10, M11, M12

The ratio of biomass dry weight to cassava in mixed culture using medium M9, M10, M11, M12 was relatively stable after 36 hour of

cultivation. The results are presented in figure 4.24 and Table 4.28

Table 4.28 Ratio of dry weight to cassava in mixed culture of *C. utilis* and *E. fibuligera* in medium M9, M10, M11, M12

No.	hour	M9 g/g	M10 g/g	M11 g/g	M12 g/g
1	0	0.207	0.169	0.163	0.184
2	3	0.216	0.192	0.187	0.201
3	6	0.218	0.224	0.216	0.234
4	9	0.237	0.224	0.235	0.226
5	12	0.261	0.207	0.247	0.268
6	15	0.275	0.235	0.250	0.274
7	18	0.288	0.274	0.258	0.274
8	21	0.302	0.287	0.277	0.289
9	24	0.305	0.305	0.312	0.294
10	27	0.311	0.306	0.315	0.301
11	30	0.325	0.315	0.323	0.305
12	33	0.359	0.323	0.316	0.303
13	36	0.360	0.332	0.320	0.304
14	39	0.363	0.341	0.325	0.310
15	42	0.363	0.359	0.331	0.350
16	45	0.369	0.355	0.334	0.365
17	48	0.374	0.356	0.339	0.385

The protein content in biomass of mixed culture of *C. utilis* and *E. fibuligera* in medium, M9, M10, M11, M12 was relatively stable after 39 hour of cultivation. The numerical results are presented in Table 4.29



Table 4.29 Protein content in biomass of mixed culture of *C. utilis* and *E. fibuligera* in medium M9, M10, M11, M12

No.	hour	M9 %	M10 %	M11 %	M12 %
1	0	9.86	11.47	11.38	9.60
2	3	9.45	14.82	15.17	13.52
3	6	14.13	15.49	15.11	17.96
4	9	13.83	17.94	16.30	18.03
5	12	13.25	19.83	18.09	17.48
6	15	12.07	21.54	22.59	23.24
7	18	15.79	23.56	24.37	31.63
8	21	17.61	28.36	33.44	32.95
9	24	26.95	31.81	34.39	36.73
10	27	25.76	33.73	34.84	36.20
11	30	27.15	34.62	36.62	39.81
12	33	26.32	38.97	40.54	40.63
13	36	34.12	41.50	41.59	39.78
14	39	36.73	41.19	41.14	41.19
15	42	39.74	38.36	40.44	38.47
16	45	38.87	39.64	40.28	35.17
17	48	38.58	39.85	39.59	34.69

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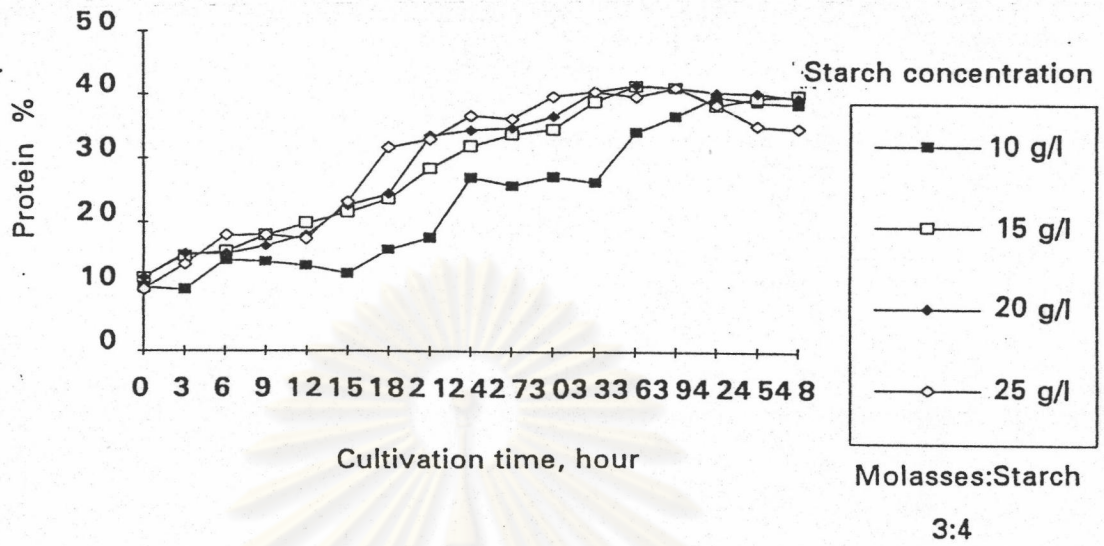


Fig. 4.25 Protein content in biomass of mixed culture of *C. utilis* and *E. fibuligera* in medium M9, M10, M11, M12

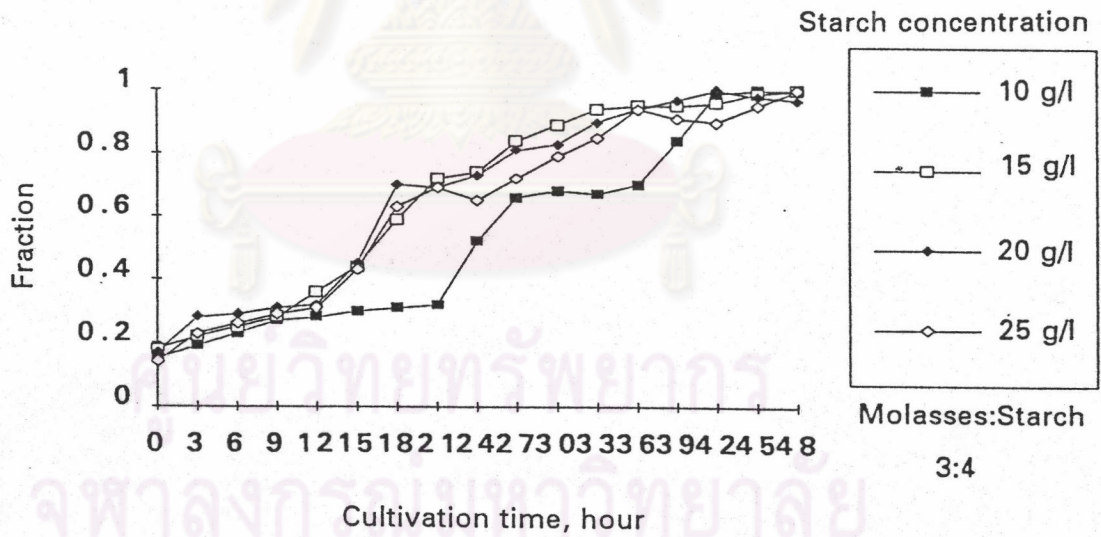


Fig. 26. Ratio of protein produced at any time to maximum protein obtained in mixed culture in medium M9, M10, M11, M12.

The formation of cell mass protein in mixed culture of *C. utilis* and *E. fibuligera* in medium M9, M10, M11, M12 was relatively stable about 39 hour of cultivation. The results are presented in figure 4.26 and Table 4.30.



Table 4.30 Ratio of protein produced at any time to maximum protein obtained in mixed culture in medium M9, M10, M11, M12.

No.	hour	M9 g/g	M10 g/g	M11 g/g	M12 g/g
1	0	0.14	0.14	0.14	0.13
2	3	0.14	0.20	0.21	0.20
3	6	0.21	0.24	0.24	0.31
4	9	0.23	0.28	0.28	0.30
5	12	0.24	0.29	0.33	0.35
6	15	0.23	0.36	0.42	0.47
7	18	0.32	0.45	0.47	0.65
8	21	0.37	0.57	0.69	0.71
9	24	0.57	0.68	0.80	0.80
10	27	0.56	0.73	0.81	0.81
11	30	0.61	0.77	0.88	0.90
12	33	0.65	0.89	0.95	0.91
13	36	0.85	0.97	0.99	0.90
14	39	0.92	0.99	0.99	0.95
15	42	1.00	0.97	0.99	1.00
16	45	0.99	0.99	1.00	0.95
17	48	1.00	1.00	1.00	0.99

For the growth of mixed culture in medium M13, the maximum protein formation rate, 0.09 g/l.h, the maximum glucose accumulation rate, 0.63 g/l.h, the maximum glucose consumption rate, 0.46 g/l.h were obtained. The results are shown in figure 4.27 and Table 4.31.

Table 4.31 The growth of mixed culture of *C. utilis* and *E. fibuligera* in medium M13.

No.	hour	Biomass g/l	Protein g/l	Glucose g/l
1	0	6.38	0.54	1.35
2	3	6.48	0.71	1.62
3	6	6.57	0.84	2.58
4	9	6.99	0.99	6.28
5	12	7.23	1.05	7.72
6	15	7.44	1.12	9.18
7	18	7.62	1.15	8.52
8	21	7.77	1.18	6.64
9	24	7.79	1.93	3.15
10	27	7.83	2.45	2.32
11	30	7.92	2.50	1.15
12	33	7.96	2.49	0.91
13	36	8.65	2.58	0.90
14	39	8.78	3.11	0.72
15	42	9.27	3.65	0.75
16	45	9.57	3.69	0.67
17	48	9.58	3.70	0.65

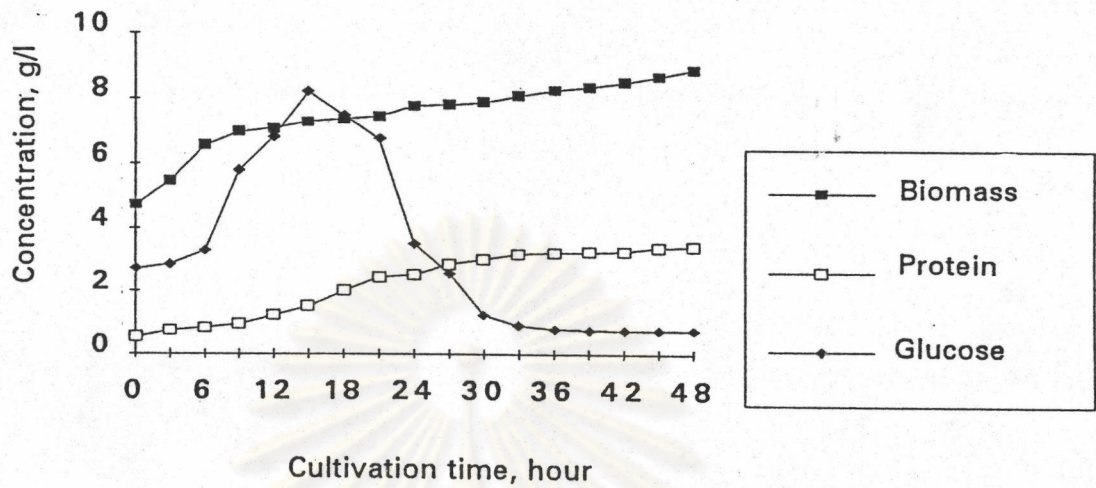


Fig. 4.28 The growth of mixed culture of *C. utilis* and *E. fibuligera* in medium M14.

For the growth of mixed culture in medium M14, the maximum protein formation rate, 0.08 g/l.h, the maximum glucose accumulation rate, 0.45 g/l.h, the maximum glucose consumption rate, 0.41 g/l.h were obtained. The results are shown in figure 4.28 and Table 4.32

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Table 4.32 The growth of mixed culture of *C. utilis* and *E. fibuligera* in medium M14.

No.	hour	Biomass g/l	Protein g/l	Glucose g/l
1	0	4.72	0.54	2.70
2	3	5.47	0.75	2.85
3	6	6.56	0.85	3.28
4	9	6.98	0.95	5.78
5	12	7.08	1.24	6.84
6	15	7.29	1.51	8.20
7	18	7.37	2.01	7.49
8	21	7.45	2.45	6.76
9	24	7.77	2.53	3.51
10	27	7.79	2.86	2.57
11	30	7.88	3.02	1.25
12	33	8.08	3.19	0.90
13	36	8.25	3.23	0.81
14	39	8.35	3.24	0.77
15	42	8.49	3.27	0.75
16	45	8.67	3.39	0.75
17	48	8.88	3.41	0.74

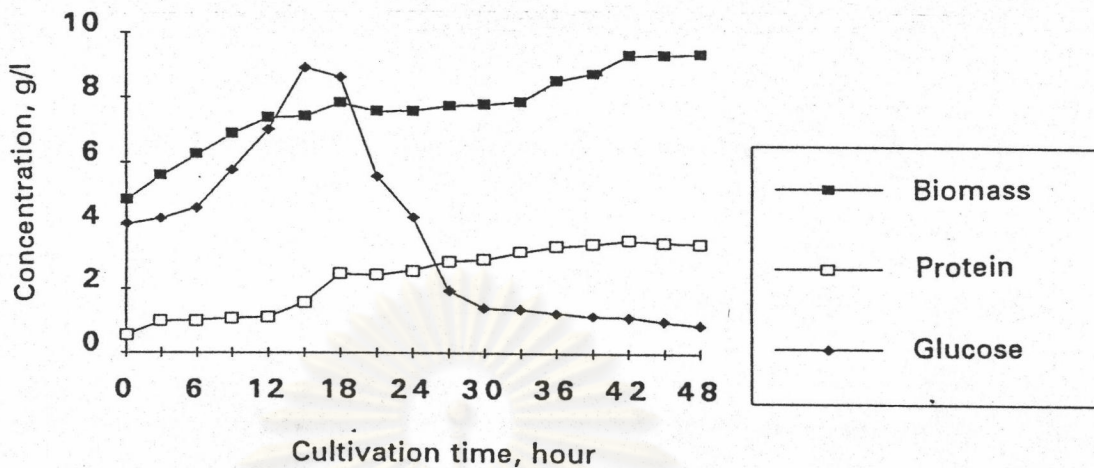


Fig. 4.29 The growth of mixed culture of *C. utilis* and *E. fibuligera* in medium M15.

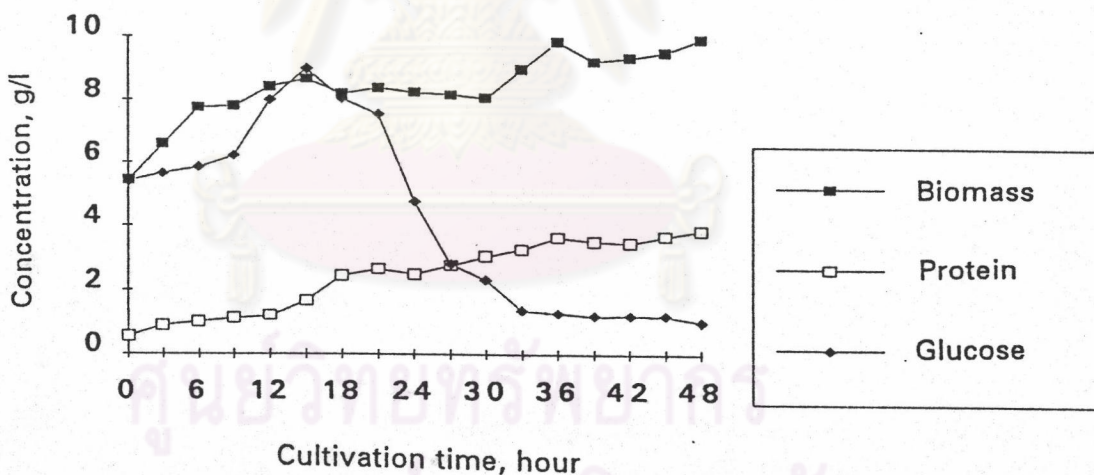


Fig. 4.30 The growth of mixed culture of *C. utilis* and *E. fibuligera* in medium M16.

For the growth of mixed culture in medium M15, the maximum protein formation rate, 0.09 g/l.h, the maximum glucose accumulation rate, 0.39 g/l.h,

the maximum glucose consumption rate, 0.42 g/l.h were obtained. The numerical results are shown in Table 4.33

Table 4.33 The growth of mixed culture of *C. utilis* and *E. fibuligera* in medium M15.

No.	hour	Biomass g/l	Protein g/l	Glucose g/l
1	0	4.79	0.55	4.05
2	3	5.58	0.99	4.23
3	6	6.24	1.02	4.57
4	9	6.87	1.09	5.77
5	12	7.35	1.13	6.98
6	15	7.41	1.58	8.90
7	18	7.81	2.47	8.61
8	21	7.56	2.44	5.55
9	24	7.57	2.59	4.28
10	27	7.74	2.87	1.95
11	30	7.77	2.94	1.41
12	33	7.87	3.18	1.37
13	36	8.51	3.35	1.26
14	39	8.75	3.44	1.15
15	42	9.31	3.55	1.14
16	45	9.32	3.49	0.99
17	48	9.34	3.46	0.89

For the growth of mixed culture in medium M16, the maximum protein formation rate, 0.10 g/l.h, the maximum glucose accumulation rate, 0.28 g/l.h, the maximum glucose consumption rate, 0.42 g/l.h were obtained. The

numerical results are shown in Table 4.34

Table: 4.34 The growth of mixed culture of *C. utilis* and *E. fibuligera* in medium M16.

No.	hour	Biomass g/l	Protein g/l	Glucose g/l
1	0	5.42	0.55	5.40
2	3	6.57	0.89	5.65
3	6	7.75	1.01	5.83
4	9	7.77	1.11	6.21
5	12	8.42	1.19	7.98
6	15	8.65	1.67	8.98
7	18	8.19	2.45	8.03
8	21	8.38	2.66	7.52
9	24	8.23	2.50	4.77
10	27	8.17	2.78	2.81
11	30	8.06	3.06	2.32
12	33	8.98	3.29	1.36
13	36	9.86	3.64	1.28
14	39	9.25	3.54	1.19
15	42	9.34	3.47	1.20
16	45	9.52	3.69	1.19
17	48	9.93	3.87	1.01

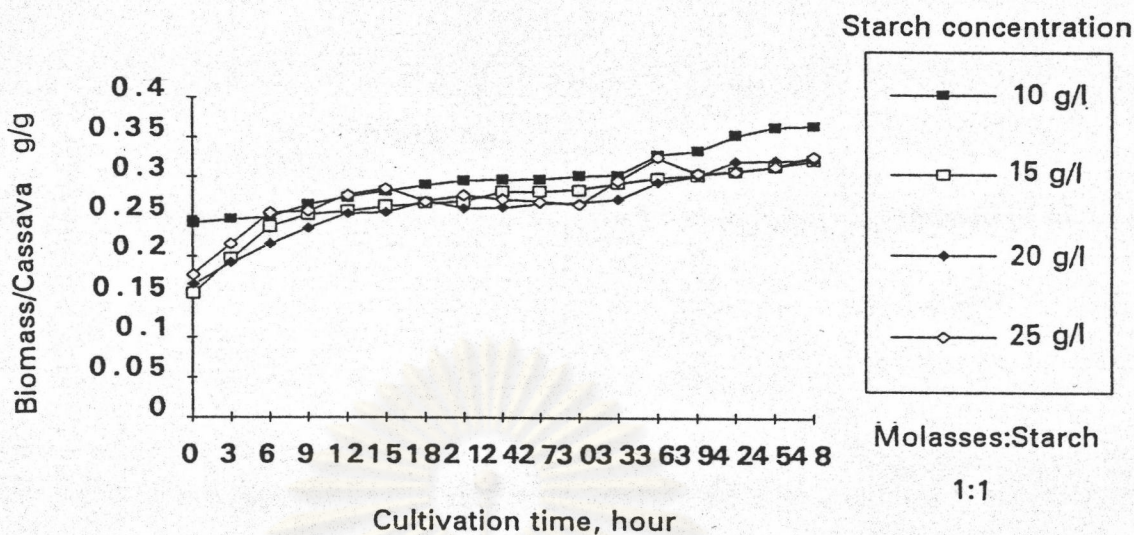


Fig. 4.31 Ratio of dry weight to cassava in mixed culture of *C. utilis* and *E. fibuligera* in medium M13, M14 M15 M16

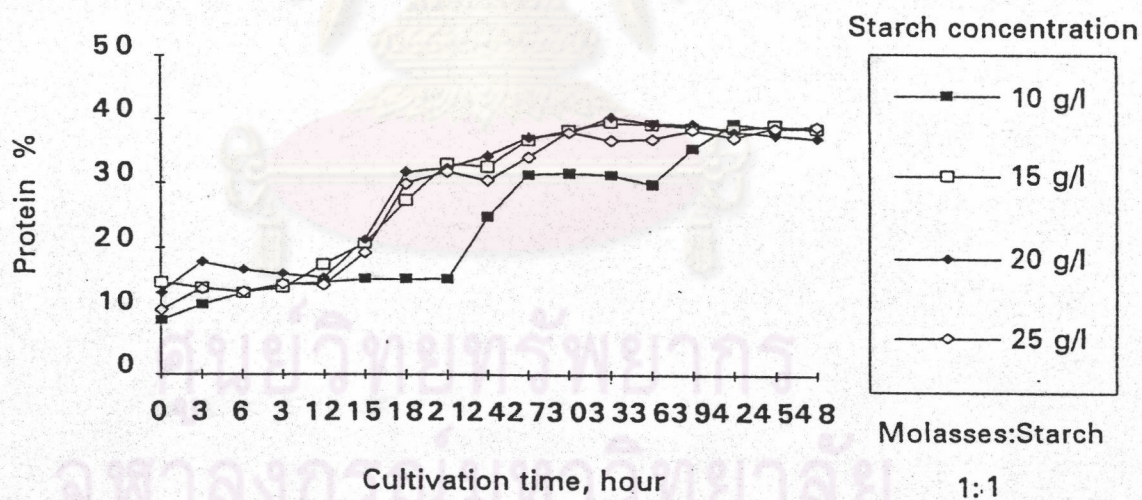


Fig. 4.32 Protein content in biomass of mixed culture of *C. utilis* and *E. fibuligera* in medium M13, M14, M15, M16.

The ratio of biomass dry weight to cassava in mixed culture using medium M13, M14 M15, M16 was relatively stable after 36 hour of



cultivation. The numerical results are presented in Table 4.35

Table 4.35 Ratio of dry weight to cassava in mixed culture of *C. utilis* and *E. fibuligera* in medium M13, M14 M15 M16

No.	hour	M13 g/g	M14 g/g	M15 g/g	M16 g/g
1	0	0.242	0.154	0.165	0.178
2	3	0.246	0.197	0.192	0.216
3	6	0.249	0.237	0.215	0.255
4	9	0.265	0.252	0.236	0.256
5	12	0.274	0.256	0.253	0.277
6	15	0.282	0.263	0.255	0.285
7	18	0.289	0.266	0.269	0.269
8	21	0.295	0.269	0.260	0.276
9	24	0.296	0.281	0.261	0.271
10	27	0.297	0.281	0.266	0.269
11	30	0.301	0.284	0.267	0.265
12	33	0.302	0.292	0.271	0.295
13	36	0.328	0.298	0.293	0.324
14	39	0.333	0.302	0.301	0.304
15	42	0.352	0.306	0.320	0.307
16	45	0.363	0.313	0.321	0.313
17	48	0.364	0.321	0.322	0.327

The protein content in biomass of mixed culture of *C. utilis* and *E. fibuligera* in medium, M13, M14, M15, M16 was relatively stable after 39 hour of cultivation. The numerical results are presented in Table 4.36

Table 4.36 Protein content in biomass of mixed culture of *C. utilis* and *E. fibuligera* in medium M13, M14, M15, M16.

No.	hour	M13 %	M14 %	M15 %	M16 %
1	0	8.46	14.52	12.94	10.15
2	3	10.96	13.71	17.74	13.55
3	6	12.79	12.96	16.53	13.03
4	3	14.16	13.61	15.87	14.29
5	12	14.52	17.51	15.37	14.13
6	15	15.05	20.71	21.32	19.31
7	18	15.09	27.27	31.63	29.91
8	21	15.19	32.89	32.28	31.74
9	24	24.78	32.56	34.21	30.38
10	27	31.29	36.71	37.08	34.03
11	30	31.57	38.32	37.84	37.97
12	33	31.28	39.48	40.41	36.64
13	36	29.83	39.15	39.37	36.92
14	39	35.42	38.80	39.31	38.27
15	42	39.37	38.52	38.13	37.15
16	45	38.56	39.10	37.45	38.76
17	48	38.62	38.40	37.04	38.97

of cultivation(Figure 4.33). The numerical results are presented in Table 4.37

Table 4.37 Ratio of protein produced at any time to maximum protein obtained in mixed culture in medium M13, M14, M15, M16.

No.	hour	M13 g/g	M14 g/g	M15 g/g	M16 g/g
1	0.15	0.18	0.17	0.14	0.13
2	3	0.19	0.22	0.28	0.23
3	6	0.23	0.25	0.29	0.26
4	9	0.27	0.28	0.31	0.29
5	12	0.28	0.36	0.32	0.31
6	15	0.30	0.44	0.45	0.43
7	18	0.31	0.59	0.70	0.63
8	21	0.32	0.72	0.69	0.69
9	24	0.52	0.74	0.73	0.65
10	27	0.66	0.84	0.81	0.72
11	30	0.68	0.89	0.83	0.79
12	33	0.67	0.94	0.90	0.85
13	36	0.70	0.95	0.94	0.94
14	39	0.84	0.95	0.97	0.91
15	42	0.99	0.96	1.00	0.90
16	45	1.00	0.99	0.98	0.95
17	48	1.00	1.00	0.97	1.00

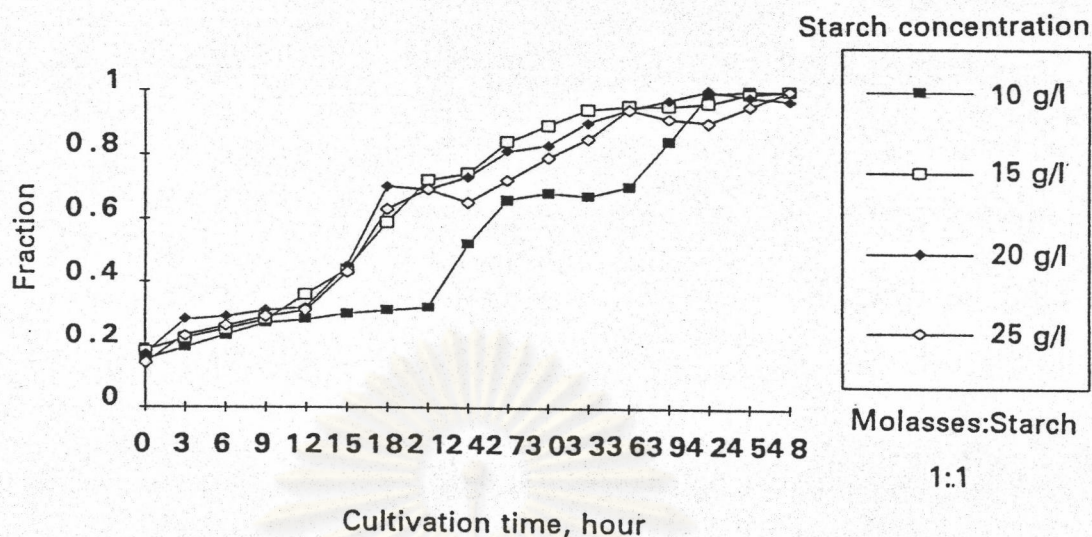


Fig. 4.33 Ratio of protein produced at any time to maximum protein obtained in mixed culture in medium M13, M14, M15, M16.

#### 4.3 Harvesting of the culture after 39h of cultivation

##### 4.3.1 The average dry weights of biomass

The average dry weights of biomass at the 39th hour of cultivation were higher with higher concentration of dry cassava in medium (25 g/l). The high values varied between 77 g/l and 8.89 g/l (figure 4.34 and Table 4.38). The statistical analyses were calculated and presented in Table 4.40. These values of average dry weight of biomass depends on the concentration of dry cassava and molasses. The difference of the high values, 8.77 g/l, 8.42 g/l, 8.75 g/l, 8.89 g/l in medium M4, M8, M12 and M16 respectively is not significant or they are equal at level 0.05.

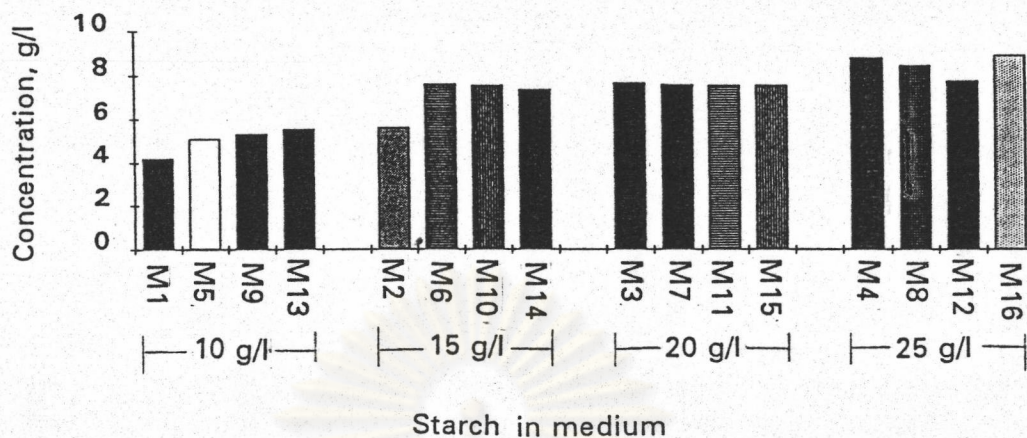


Fig. 34. Average of dry weight of biomass at the 39 th hour of cultivation

Table 4.38 Average d. weight of biomass at the 39th hour of cultivation

Dry cassava g/l	Ratio of molasses to dry cassava			
	1:4	1:2	3:4	1:1
10	4.21	5.09	5.32	5.56
15	5.60	7.61	7.53	7.33
20	7.65	7.56	7.55	7.54
25	8.77	8.42	8.75	8.89

Table 4.39 Anova table of average dry weight of biomass at the 39th hour of cultivation

Source	DF	SS	MS
Treatment	15	96.10	6.41
Blocks	2	0.43	0.21
Error	30	0.80	0.27
Total	47	97.33	-

F- value = 239.3                      D.F. 15, 30

F- table = 2.01                      239.3 > 2.01

#### 4.3.2 Ratio of dry weight to dry cassava

Ratio of dry weight of biomass to dry cassava at the 39th hour of cultivation were higher for the medium which had lower concentration of dry cassava (10 g/l and 15 g/l). The high values varied between 0.438 and 0.457, see figure 4.34 and Table 4.40. The statistical analyses were calculated and presented in, Table 4.41. These average dry weight of biomass depends on the concentration of dry cassava and molasses. The difference of the high values in medium M10, M9, M13 and M6 respectively is not significant or they are equal at level 0.05.

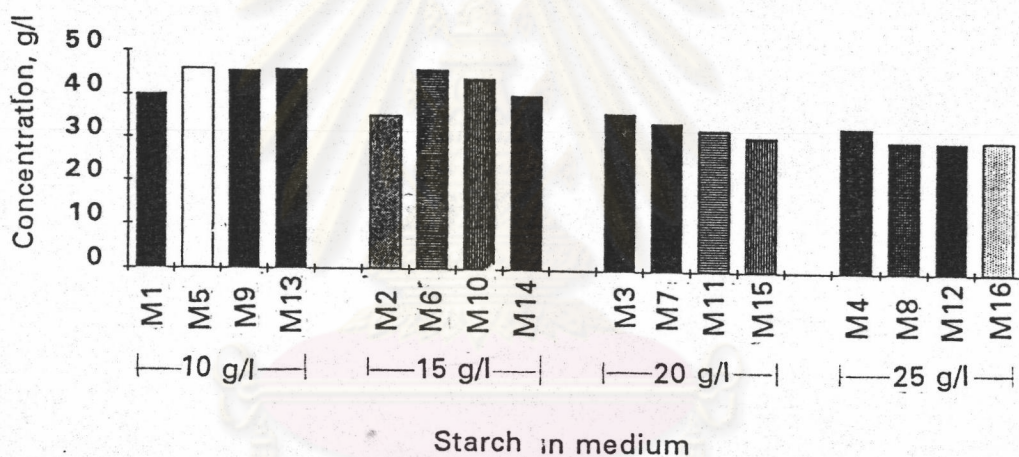


Fig. 4.35 Ratio of dry weight to dry cassava at the 39th hour of the cultivation.

Table 4.40 Ratio of dry weight to dry cassava at the 39th hour of cultivation

Dry cassava g/l	Ratio of molasses to dry cassava			
	1:4	1:2	3:4	1:1
10	40.1	45.9	45.5	45.7
15	35.3	45.8	43.8	40.2
20	36.3	34.1	32.5	31.0
25	33.2	30.2	30.1	30.4

Table 4.41 Anova Table of ratio of dry weight to dry cassava at the 39th hour of cultivation

Source	DF	SS	MS
Treatment	15	1763.13	117.54
Blocks	2	13.95	6.97
Error	30	64.86	2.16
Total	47	1841.94	-

F- value = 54.36                      D.F. 15, 30

F- table = 2.01                      54.36 > 2.01

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### 4.3.3 Protein percent of dry weight

The values of protein content of biomass dry weight at the 39th hour of cultivation were higher for medium which had lower concentration of dry cassava in medium (10 g/l). The high values varied between 47.6% and 48.8 %, see figure 4.36 and Table 4.42. The statistical analyses were calculated and presented in Table 4.43. These average dry weight of biomass depends on the concentration of dry cassava and molasses. The difference of the high values in medium M9, M5 and M13 respectively is not significant or they are equal at level 0.05.

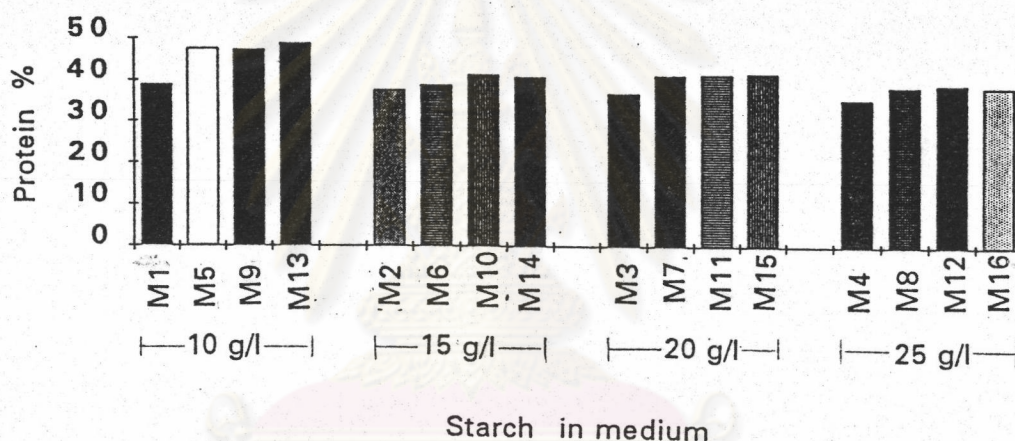


Fig. 4.36 Protein content of dry weight at the 39th hour of the cultivation.

Table 4.42 Protein content of dry weight at the 39th hour of cultivation

Dry cassava g/l	Ratio of molasses to dry cassava			
	1:4	1:2	3:4	1:1
10	38.9	47.6	47.3	48.8
15	37.6	38.8	41.4	40.7
20	36.7	41.2	41.3	41.6
25	35.4	38.5	39.1	38.4



Table 4.43 Anova table of protein content of d. weight at 39 h. of cultivation

Source	DF	SS	MS
Treatment	15	699.22	46.61
Blocks	2	1.29	0.65
Error	30	111.17	3.71
Total	811.67	811.67	-

F- value = 12.57                      D.F. 15, 30

F- table = 2.01                      12.57 > 2.01

#### 4.3.4 The total protein of dry weight

The values of total protein of dry weight at the 39th hour of cultivation were higher for medium which had molasses added at 0.8% ~1.1% (w/v). The high values varied between 3.10 g/l and 3.54 g/l, see figure 4.37 and Table 4.44. The statistical analyses were calculated and presented in Table 4.45. These average dry weight of biomass depends on the concentration of dry cassava and molasses. The difference of the high values between 3.10 g/l and 3.54 g/l in medium M4, M7, M10 and M13 respectively is not significant at level 0.05.

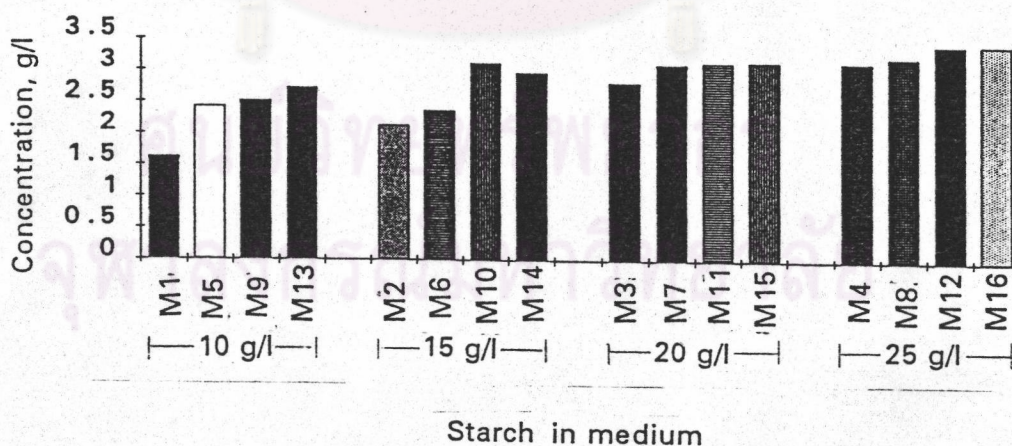


Fig. 4.37 Total protein of dry weight at the 39th hour of the cultivation.

Table 4.44 Total protein at 39 hour of fermentation

Dry cassava g/l	Ratio of molasses to dry cassava			
	1:4	1:2	3:4	1
1:1	1.63	2.42	2.51	2.71
15	2.13	2.36	3.11	2.96
20	2.81	3.10	3.13	3.15
25	3.14	3.24	3.44	3.54

Table 4.45 Anova Table of total protein at 39 hour of fermentation

Source	DF	SS	MS
Treatment	15	12.32	0.82
Blocks	2	1.62	0.81
Error	30	2.09	0.07
Total	47	16.04	-

F- value = 11.75

D.F. 15, 30

F- table = 2.01

11.75 > 2.01

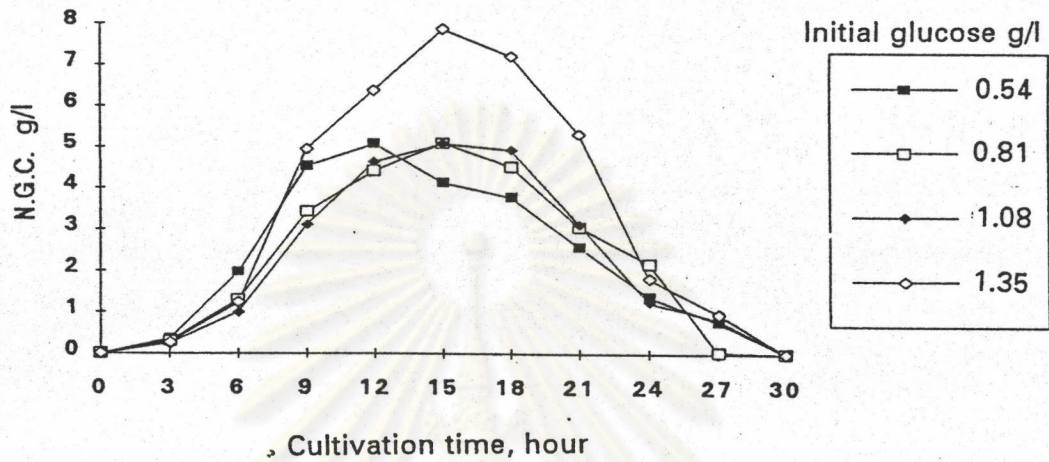
#### 4.3.5 Effect of initial sugar concentration on glucose formation

Data obtained from the experiment 3.3.4 were normalised and present in figure 4.41, 4.42, 4.43, 4.44. The normalised glucose concentration (N.G.C.) is calculated as the difference between the glucose concentration at any time and its initial value. It can be observed from the experimental results as follows :

(i) Glucose formation is high for cultivation time from 12 to 18 hour.

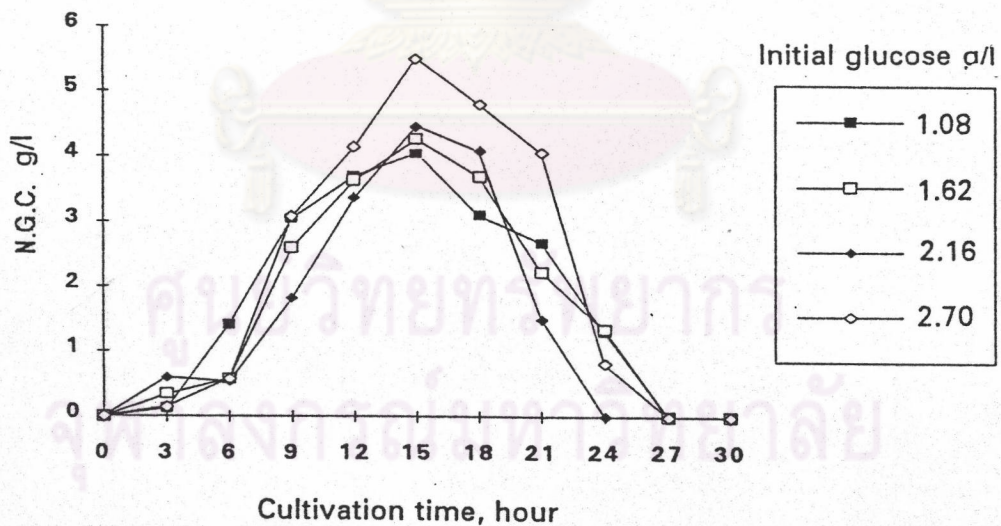
(ii) When the initial glucose is high but not exceeding 4.32 g/l, glucose formation is also high upto 8-9 g/l. For initial glucose concentration exceeding 4.32 g/l, *E. fibuligera* appears to consume the sugar than the starch.

(iii) Maximum glucose formation, appears to be at 15 hour, therefore it is the best time to introduce *C. utilis*. This is the time that glucose consumption by *E. fibuligera* alone.



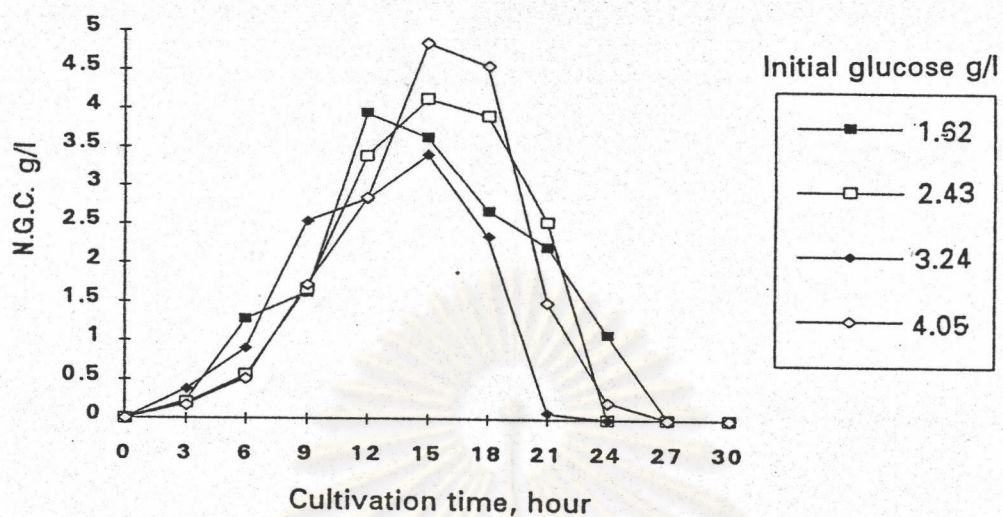
N.G.C. : Normalised glucose concentration

Fig. 4.38 Effect of initial glucose content on glucose formation using starch in medium 10 g/l



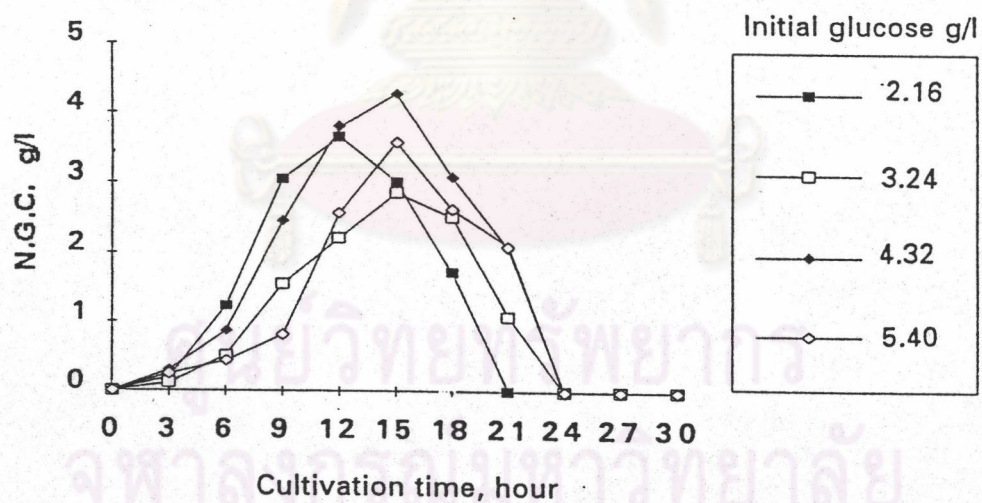
N.G.C. : Normalised glucose concentration

Fig. 4.39 Effect of initial glucose content on glucose formation using starch in medium 15 g/l



N.G.C. : Normalised glucose concentration

Fig. 4.40 Effect of initial glucose content on glucose formation using starch in medium 20 g/l



N.G.C. : Normalised glucose concentration

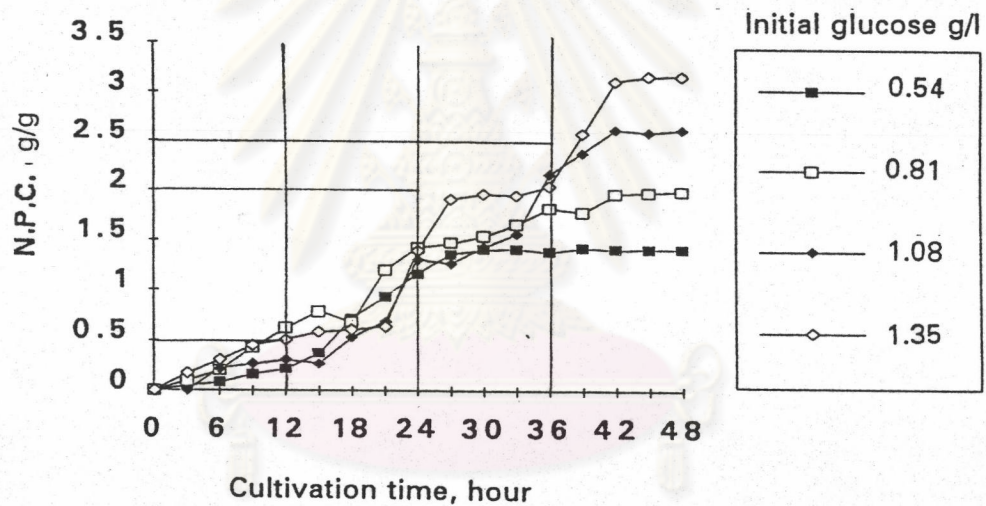
Fig. 4.41 Effect of initial starch content on glucose concentration in biomass product using starch in medium 25 g/l

#### 4.3.6 Effect of initial sugar concentration on protein production



Data obtained from the experiment 3.3.4 were normalised and present in figure 4.42, 4.43, 4.44, 4.45. The normalised protein concentration (N.P.C.) is calculated as the difference between the protein concentration at any time and its initial value. It can be observed from the experimental results as follows :

- (i) Initial glucose greater than 2 g/l yields higher than 2.5 g/l often 36 h.
- (ii) For initial starch concentration less than 2.5 g/l, protein production rate is higher reaching 2.5 g/l at about 32h provided that the initial glucose is also higher the 2 g/l.



N.P.C. : Normalised protein concentration

Fig. 4.42 Effect of initial sugar on protein production using starch in medium 10 g/l

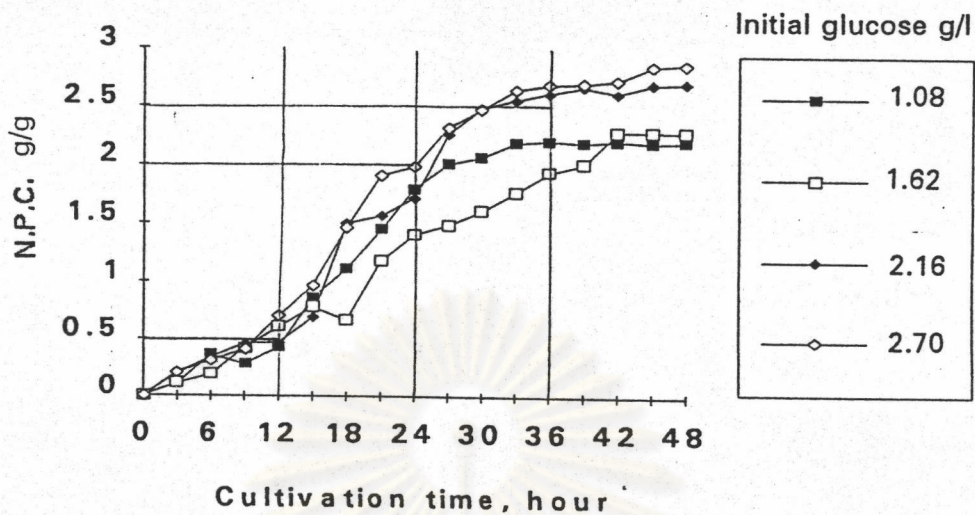


Fig. 4.43 Effect of initial sugar on protein production using starch in medium 15 g/l

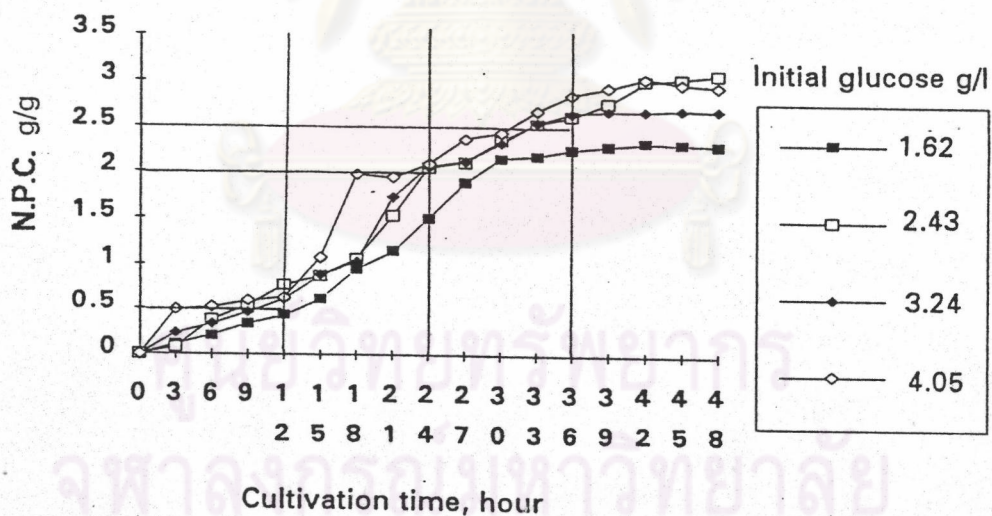
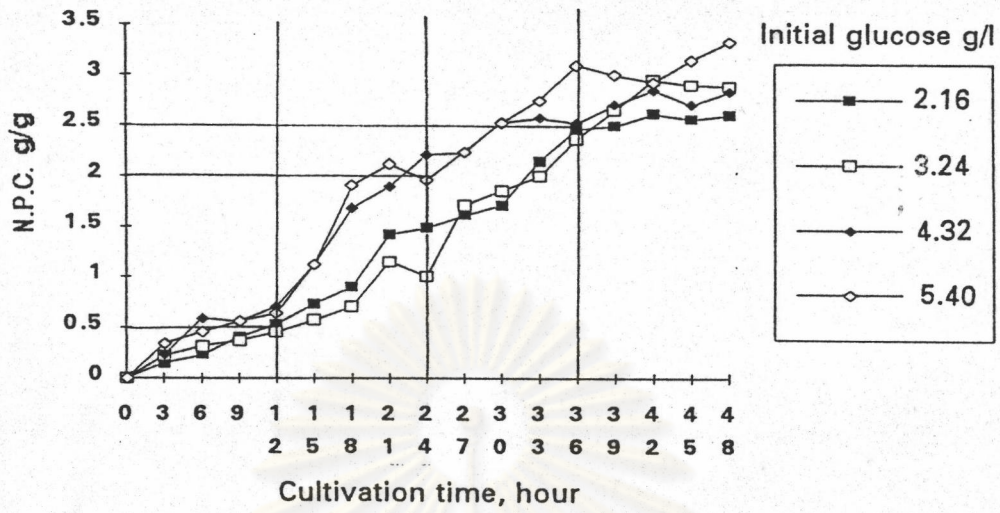


Fig. 4.44 Effect of initial sugar on protein production using starch in medium 20 g/l



N.P.C. : Normalised protein concentration  
Fig.4.45 Effect of initial rugar on protein production using starch in medium 25 g/l

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#### 4.4 The cultivation on 60 litre tank

##### 4.4.1 Experiments of the cultivation on 60 litre tank using medium M1

The study on cultivation for SCP production was performed with medium M1 which has the lowest concentration of starch in medium. This experiment was performed in order to confirm that the results which was obtained in flask of 250 ml, could be obtained also in the larger scale.

Table 4.46 The SCP production on 60 litter tank in medium M1

No.	hour	Biomass g/l	Protein g/l	Glucose g/l	Starch g/l
1	0	1.67	0.25	0.50	6.99
2	3	1.70	0.26	0.71	6.55
3	6	2.04	0.29	1.61	5.77
4	9	2.17	0.36	3.73	4.34
5	12	2.25	0.45	4.46	4.12
6	15	2.99	0.77	5.64	3.48
7	18	3.29	0.85	4.82	3.25
8	21	3.44	1.12	3.33	2.59
9	24	3.57	1.22	2.51	2.50
10	27	3.70	1.28	1.22	1.78
11	30	3.95	1.39	0.95	1.48
12	33	4.07	1.48	0.73	1.34
13	36	4.14	1.52	0.32	1.22
14	39	4.16	1.63	0.19	1.15
15	42	4.15	1.61	0.10	1.18
16	45	4.09	1.63	0.11	1.14
17	48	4.13	1.59	0.10	1.15



The broth in the large tank was agitated at 140 rpm which gave Reynold number (Re) of 25,130 and air flow rate of 8 l/min. The results are presented in figure 4.38 and Table 4.46. The comparison of the results presented in Table 4.47. The results were not significantly different. This means that the mixed culture of *C. utilis* and *E. fibuligera* can carried out under the condition as described in the experiment method.

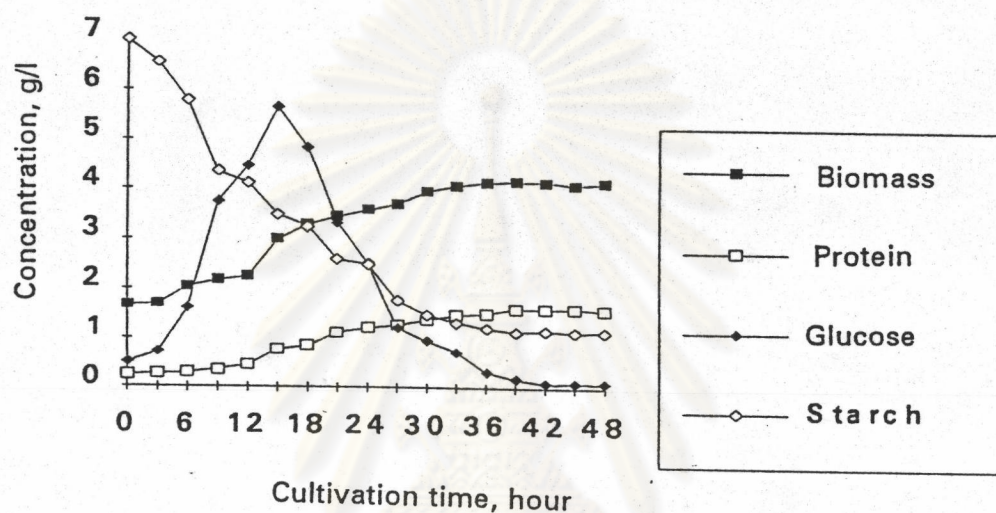


Fig. 4.46 The SCP production in 60 litre tank using medium M1.

Table 4.47 Comparison of the cultivation on 60 litre tank and flask of 250 ml in medium M1.

	Tank	Flask
Maximum protein formation rate g/l.h	0.036	0.044
Maximum glucose accumulation rate g/l.h	0.427	0.520
Maximum glucose consumption rate g/l.h	0.353	0.290
Maximum starch consumption rate g/l.h	0.164	-
Ratio of dry weight to cassava	0.416	0.422
Protein percent of dry weight %	39.18	38.86
Total of dry weight of biomass g/l	4.16	4.22
Total of protein of biomass g/l	1.63	1.64

#### 4.4.2 Experiments of the cultivation on 60 litre tank using medium M16

Table 4.48 The SCP production on 60 litter tank using medium M16

No.	hour	Biomass g/l	Protein g/l	Glucose g/l	Starch g/l
1	0	5.49	0.57	5.50	17.81
2	3	5.57	0.69	5.71	16.93
3	6	6.99	0.89	5.85	16.32
4	9	7.47	1.36	6.30	13.70
5	12	8.42	1.59	7.95	13.10
6	15	9.04	1.73	8.58	10.38
7	18	9.06	1.98	8.05	8.87
8	21	9.21	2.19	7.32	5.85
9	24	9.58	2.33	4.57	4.87
10	27	10.23	2.45	2.77	3.61
11	30	11.03	2.66	2.22	2.97
12	33	11.24	2.95	1.36	2.56
13	36	11.57	3.34	1.24	1.84
14	39	11.70	3.44	1.27	1.43
15	42	11.42	3.75	1.19	1.31
16	45	11.53	3.69	1.23	1.14
17	48	11.11	3.67	1.11	1.15

The study on cultivation for SCP production was performed with medium M16 which has the highest concentration of starch in medium. This experiment was performed in order to confirm that the results which was obtained in flask of 250 ml, could be obtained also in the larger scale. The broth in the large tank was agitated at 140 rpm which gave Reynold number (Re) of 25,130 and air flow rate of 8 l/min. The results are presented in figure 4.39 and Table 4.48.

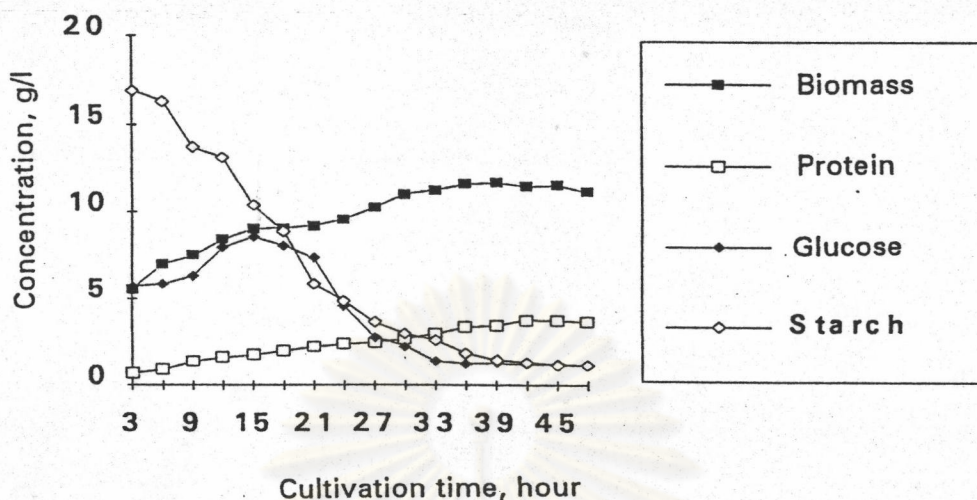


Fig. 4.47 The SCP production in 60 litre tank using medium M16.

The comparison of the results between the cultivation on 60 litre tank and flask of 250 ml in medium M16 is shown that the maximum glucose accumulation rate of the big tank, 0.280 g/l, was higher than  $G_{\text{max. acc.}}$  of flask. On the other hand, the maximum glucose consumption rate of the big tank, 0.401 g/l, was lower than  $G_{\text{max. cons.}}$  of flask, 0.42 g/l. These results showed that the growth of mixed culture on flask was better than the growth on big tank. Therefore, the maximum protein formation rate of culture on flask, 0.1 g/l, was higher than the  $P_{\text{max.}}$  of culture on tank, 0.073 g/l. The results of the growth of culture, total protein and total dry weight of biomass were 3.44 g/l and 11.70 g/l respectively. Total protein obtained using large tank is lower than flask by 0.1 g/l. This difference is not too significant in view of production in large scale. The significant difference of results was not observed. This indicates that the mixed culture of *C. utilis* and *E. fibuligera* can be carried out under the condition specified in the experimental method. The comparison of the results presented in Table 4.49. This means that the mixed culture of *C. utilis* and *E. fibuligera* can carried out under the condition as described in the experiment method.

Table 4.49 Comparison of the cultivation on tank and flask of 250 ml in medium M16.

	Tank	flask
Maximum protein formation rate g/l.h	0.073	0.100
Maximum glucose accumulation rate g/l.h	0.303	0.280
Maximum glucose consumption rate g/l.h	0.401	0.420
Maximum starch consumption rate g/l.h	0.555	-
Ratio of dry weight to cassava	0.468	0.370
Protein percent of dry weight %	30.25	38.37
Total of dry weight of biomass g/l	11.70	9.25
Total of protein of biomass g/l	3.44	3.55

#### 4.4.3 Experiments of the cultivation on 60 litre tank using medium M17, M18

As a result of the finding in the previous experiment, medium M17 and M18 were used as for SCP production on the large tank. The agitation speed and flow rate were the same as in the last experiment. The results are presented in figures 4.48, 4.49 and Tables 4.50, 4.51.

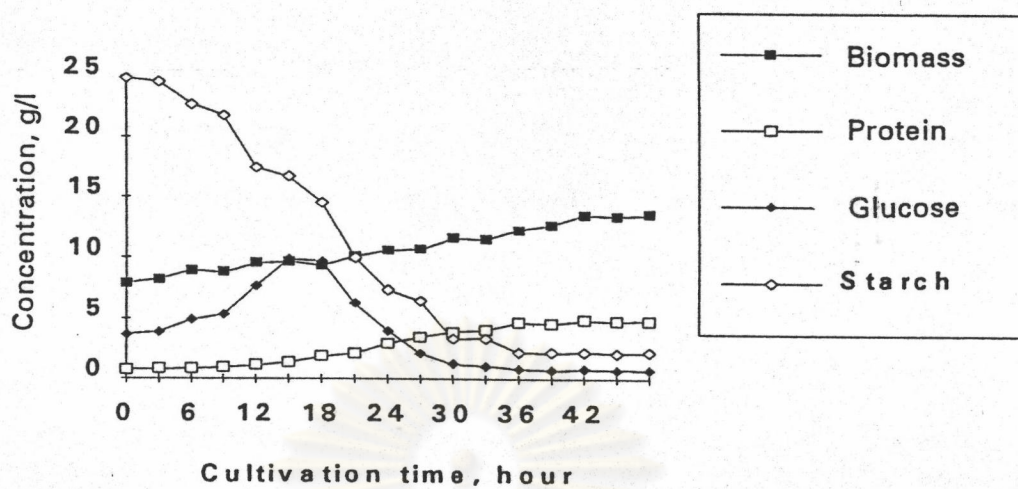


Fig. 48 The SCP production in 60 litre tank using medium M17.

Table 4.50 The SCP production on 60 litter tank in medium M17

No.	hour	Biomass g/l	Protein g/l	Glucose g/l	Starch g/l
1	0	7.91	0.79	3.67	24.85
2	3	8.23	0.80	3.86	24.54
3	6	8.96	0.84	4.87	22.70
4	9	8.77	0.96	5.28	21.76
5	12	9.49	1.12	7.67	17.40
6	15	9.65	1.34	9.88	16.64
7	18	9.33	1.85	9.67	14.50
8	21	10.05	2.11	6.19	9.98
9	24	10.58	2.93	3.99	7.34
10	27	10.64	3.48	2.09	6.38
11	30	11.55	3.85	1.23	3.28
12	33	11.47	4.02	1.10	3.34
13	36	12.25	4.65	0.82	2.22
14	39	12.67	4.56	0.75	2.15
15	42	13.51	4.84	0.80	2.18
16	45	13.35	4.79	0.77	2.14
17	48	13.52	4.82	0.75	2.15

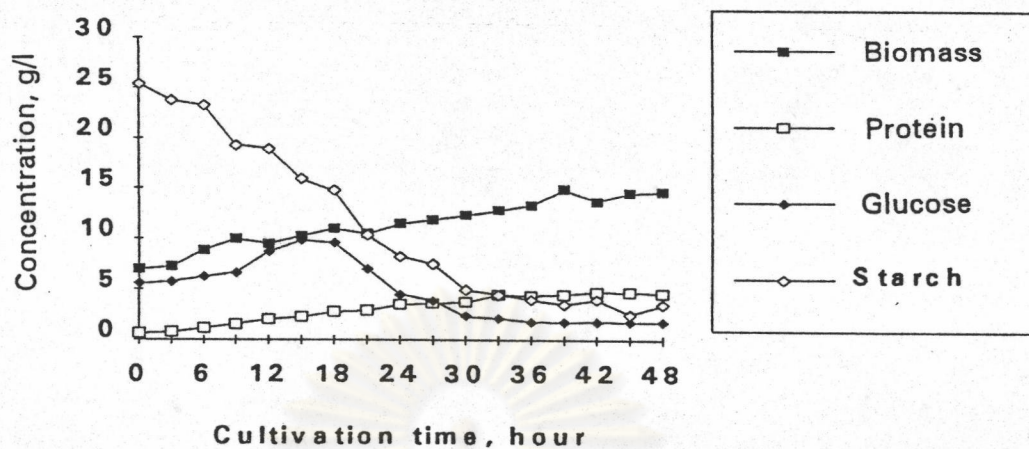


Fig. 49 The SCP production in 60 litre tank using medium M18.

Table 4.51 The SCP production on 60 litter tank in medium M18

No.	hour	Biomass g/l	Protein g/l	Glucose g/l	Starch g/l
1	0	6.97	0.65	5.55	25.34
2	3	7.23	0.75	5.76	23.70
3	6	8.86	1.14	6.25	23.23
4	9	9.97	1.57	6.66	19.29
5	12	9.49	1.99	8.65	18.94
6	15	10.26	2.25	9.79	15.87
7	18	10.95	2.73	9.53	14.74
8	21	10.45	2.86	7.01	10.34
9	24	11.38	3.46	4.51	8.25
10	27	11.87	3.65	3.89	7.47
11	30	12.28	3.73	2.34	4.99
12	33	12.79	4.49	2.12	4.52
13	36	13.35	4.39	1.82	3.99
14	39	14.87	4.51	1.75	3.54
15	42	13.72	4.76	1.80	3.99
16	45	14.54	4.72	1.77	2.56
17	48	14.63	4.65	1.75	3.45

Comparison of the characteristic rates in medium M17 and M18 is shown in Table 4.52. The maximum glucose accumulation rate of medium M17 (0.556 g/l.h) was higher than  $G_{\max.\text{acc.}}$  of medium M18. The maximum glucose consumption rate of medium M17 (0.576 g/l.h) was also higher than  $G_{\max.\text{cons.}}$  of medium M18 (0.426 g/l.h). The starch consumption rates were similar being 0.787 g/l.h and 0.760 g/l.h respectively.

These results showed that the growth of mixed culture in medium M17 was better than the growth in medium M18. Therefore, the maximum protein formation rate in M17 (0.134 g/l.h) was higher than the  $P_{\max.}$  in M18 (0.073 g/l.h). As the results of the growth of culture, total protein and total dry weight of biomass was 4.56 g/l and 12.67 g/l respectively. Total protein of culture in M17 was higher than total protein of M18 (0.5 g/l or 11.84 %). This difference is significant for the large scale of SCP production.

Table 4.52 Comparison of the cultivation on 60 litre tank using medium M17 and medium M18

Medium	M17	M18
Maximum protein formation rate g/l.h	0.134	0.093
Maximum glucose accumulation rate g/l.h	0.556	0.335
Maximum glucose consumption rate g/l.h	0.576	0.426
Maximum starch consumption rate g/l.h	0.787	0.760
Ratio of dry weight to cassava	0.362	0.425
Protein percent of dry weight %	35.99	30.33
Total of dry weight of biomass g/l	12.67	14.87
Total of protein of biomass g/l	4.56	4.51

#### 4.4.4 Experiments of the cultivation on 60 litre tank using medium M19.

The results are presented in figure 4.42 and Table 4.54. The comparison of rates between the cultivation on 60 litre tank tank using medium M17 and M19 is presented in Table 4.53.

These results showed that the growth of mixed culture in medium M17 and medium M19 was not significantly different. Therefore, the suitable medium for SCP production would be the medium which had molasses about 0.8 % ~ 1.1 % and concentration of dry cassava about 3.5 % of working volume. The concentration of dry cassava could not be higher than 3.5% in this case because the air compressor used can give the maximum air flow rate only about 10 l/min for continuous working of 24 hour per day.

Table 4.53 Comparison of the cultivation on 60 litre tank using medium M17 and medium M19

Medium	M17	M19
Maximum protein formation rate g/l.h	0.134	0.137
Maximum glucose accumulation rate g/l.h	0.556	0.600
Maximum glucose consumption rate g/l.h	0.576	0.486
Maximum starch consumption rate g/l.h	0.787	0.775
Ratio of dry weight to cassava	0.362	0.387
Protein percent of dry weight %	35.99	33.77
Total of dry weight of biomass g/l	12.67	13.56
Total of protein of biomass g/l	4.56	4.65



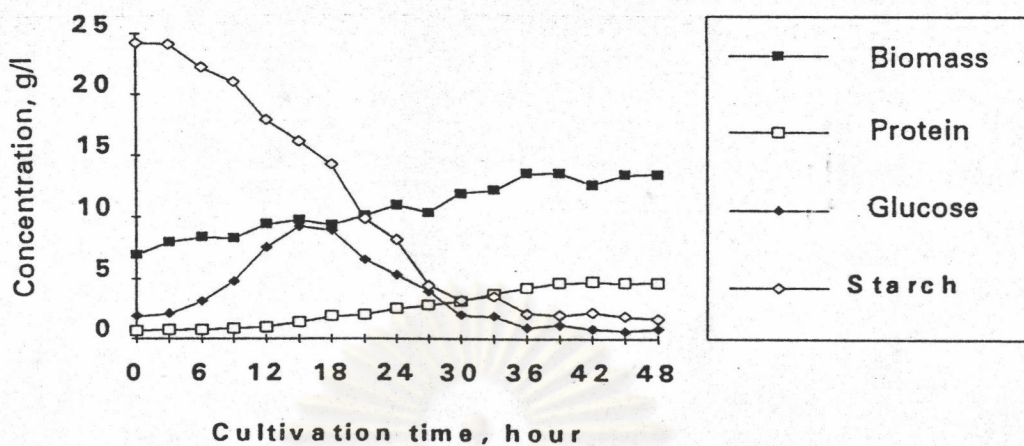


Fig. 4.50 The SCP production on 60 litre tank using medium M19.

Table 4.54 The SCP production on 60 litter tank using medium M19

No.	hour	Biomass g/l	Protein g/l	Glucose g.l	Starch g/l
1	0	6.92	0.66	1.84	24.25
2	3	7.98	0.71	2.04	24.14
3	6	8.35	0.79	3.11	22.20
4	9	8.27	0.85	4.77	20.97
5	12	9.39	0.99	7.56	17.90
6	15	9.74	1.35	9.24	16.14
7	18	9.34	1.84	8.89	14.24
8	21	10.14	1.98	6.57	9.88
9	24	10.94	2.54	5.28	8.14
10	27	10.38	2.78	3.98	4.48
11	30	11.95	3.15	1.95	3.18
12	33	12.17	3.79	1.84	3.54
13	36	13.55	4.25	0.92	2.12
14	39	13.56	4.65	1.15	1.95
15	42	12.61	4.74	0.84	2.18
16	45	13.45	4.66	0.67	1.84
17	48	13.51	4.71	0.85	1.65