CHAPTER IV

RESULTS AND DISCUSSION

4.1 Effect of initial cell concentration to astaxanthin accumulation from *Haematococcus pluvialis*

4.1.1 Effect of initial cell concentration under control condition (indoor experiments)

The effect of cell concentration is twofold, i.e. on death rate and on astaxanthin production. Fundamentally, cells at different concentrations are exposed to different light intensities. At certain light intensity, diluted cells are exposed more to light and this could significantly affect the death rate of the alga. For this case, Figure 4.1 illustrates that cell survival rate varied inversely with the initial cell concentration at all light intensity levels. In other words, too much light intensity (at all intensities employed in this work) could be fatal to cells. Nevertheless, cells at high concentration could provide shading for neighboring cells and reduce the death rate as observed from the experiment. This effect is called in this work as "mature shading".

The effect of cell concentration on astaxanthin induction was complicated as demonstrated in Table 4.1. At 0.65 klux and 25X dilution of initial cell medium, astaxanthin accumulation started to take place at about Day 8 where the maximum specific astaxanthin accumulation (per cell) of 1.69% dry weight occurred at Day 14. At different light intensity, for instance, 2.73 klux, astaxanthin accumulation slightly increased in all diluted cell concentrations and the maximum specific astaxanthin accumulation for the 10X cell concentrated dilution condition occurred at Day 12 where 5.33% dry weight was obtained. At 4.83 klux, the maximum specific astaxanthin accumulation was also that highest at 25X dilution (6.46 % dry weight cells at Day 10). At 6.50 klux, the maximum astaxanthin specific accumulation occurred at Day 8 with 10X cell dilution and the attainable astaxanthin concentration was 5.39 % dry weight.

To be able to select the most appropriate induction condition, it is proposed here to use the following equation:

$$\%Astaxanthin\ accumulation\ rate = \frac{X_2 - X_1}{t} = \frac{\Delta X}{t} \qquad (4.1)$$

where X_2 = Final %Astaxanthin

X_1 = Initial %Astaxanthin

t = time duration (day)

This equation describes the rate at which *Haematococcus pluvialis* induce and accumulate astaxanthin. Cell death rate could also be compared based on similar calculation. Figures 4.2 and 4.3 demonstrate the cell survival rate and the %astaxanthin obtained from the cultivation and the highest was found when the culture was diluted 10 times of the final harvested cell concentration (10X cell dilution) and 6.5 klux (6 fluorescent lamps) which yielded astaxanthin of 5.39% dry weight after 8 days of induction.

The results as described above demonstrate that the dilution of cell concentration could be quite important. Cells at diluted condition were more exposed to high light intensity which then led to a better astaxanthin accumulation. This was because cells are known to accumulate astaxanthin under unfavorable conditions like high light intensity, drought, salt and temperature stress, nutrient deprivation, etc. Under these unfavorable conditions, some compounds called reactive oxygen species (ROS) are produced in the chloroplast (Mittler, 2002) and this plays a direct role in the induction of carotenoid biosynthesis gene expression (Boussiba, 1999). It has been suggested that O_2 is probably the most effective ROS species involved in astaxanthin accumulation (Lu et al., 1998). Kobayashi and coworker (1992) demonstrated that ROS other than O_2 may also be involved in astaxanthin accumulation. However, under adequately high light intensity, cells could die.

4.1.2 Effect of initial cell concentration under local environmental condition (outdoor experiments)

This set of experiments were carried out twice at outdoor conditions. Due to its nature, the two environments were quite different and resulted in two different results. For both sets, the induction of astaxanthin from *Haematococcus pluvialis* was examined subject to the different levels of initial cell concentration, in the range from 0X-25X dilution of the stock cell of $1x10^5$ cells/ml. The stock vegetative cell was obtained from the cultivation in the flat plate airlift photobioreactor as described in Issarapayup (2007) and was used here as an inoculum. This had the cell density of around $1x10^5$ cells/ml, and the dilution rate is the ratio of the distilled water used to mix with this stock cell culture. It should also be noted that the average temperature at noon for this first set of experiments was around 30-34°C and the light intensity during the same measuring period was approx. 100-120 klux. In the second set of experiments, the average temperature at noon was 28-32 °C and the light intensity was 70-110 klux. In both cases, the cell number slightly increased with cell dilution rate. In these cell dilution experiments, it was anticipated hat there was enough nutrients left for further growth, therefore cell growth was observed, although only slightly.

It was observed that, in the control experiments with no cell dilution, the two different experiments led to two different results. In the first experiment, cells slightly increased with time, and in the second, the opposite was found. This could be due to the differences in temperature and light intensity in both experiments, as demonstrated in Figures 4.4 and 4.5.

Figures 4.6 and 4.7 illustrate the effect of diluted cell concentration for both sets of experiments. Similarly, both sets demonstrate that astaxanthin accumulated at significant levels. During the first set, diluted cells (10X) provided the best result where the maximum astaxanthin concentration was 1.56%dry weight or 0.26 mg/10^6 cells after 14 days of induction period. Dilution allowed cells to be exposed to higher light intensity and this ended up with more astaxanthin accumulation. Too strong or not enough light intensity could result in a lower level of induction as observed here. In the second set of experiment, the best results were observed for the control condition (without cell dilution) with the maximum astaxanthin concentration was

1.14 %dry weight or 0.19 mg/10^6 cells. This result was not expected as this experiment was subject to lower light intensity and lower temperature and therefore the opposite outcome was anticipated. However, it was observed that cell death was more significant in this case than in the first experiment. Therefore less cells remained and diluting cells could even allow more light to pass through and lower level of survival became more visible. It should be noted that the reason why more cell death was observed in the second experiment still could not be drawn from this experiment.

Although it could not be concluded from this work the best outdoor condition for the induction of astaxanthin, the results illustrate that this induction was possible with a relatively good level of astaxanthin accumulation when compared to the levels reported in literature, e.g. Harker (1995) who increased the light intensity replaced the cell dilution. These result also in accordance to the result of Qinglin (2007) increased light intensity from 60 µmol photon/m^2 s to 360 µmol photon/m2. The maximum astaxanthin was 2.94 %g astaxanhin/ g cell (the data was not shown) in the 25X dilution cell concentration at 18 days.

4.2 Effect of medium concentration to astaxanthin accumulation from *Haematococcus pluvialis*

4.2.1 Effect of medium concentration under control condition (indoor experiments)

The effect of medium concentration was examined at various light intensities. It was observed that a sudden change of environmental conditions, e.g. medium concentration and light intensity could have notable impact on survival rate of algae, and this explains why, during the first few days, the number of algae cells violently dropped at all light intensities. After that, the number of cells still dropped but only slightly. This is possible because cells might not survive the medium starvation condition or the high light intensity at long duration (Figure 4.8). Diluting the medium concentration also exerted some significant influence on the death rate, where the survival rate was low at high dilution rate. It could be that, in such condition, there was not enough nutrient for the growth of the alga.

Table 4.3 demonstrates an effect of medium concentrations on astaxanthin accumulation. The results could be described according to the employed light

intensity as follows. At 0.65 klux, astaxanthin concentration slightly increased in all cases until the maximum astaxanthin of 1.36% dry weight was reached after 18 days of induction and at 15 times dilution of final spent medium concentration (15X medium dilution). At 2.73 klux, astaxanthin concentration increased at all conditions, but in a much higher scale for diluted medium concentration than for control condition (without dilution). The maximum astaxanthin accumulation occurred at 10X medium dilution at Day 12 which was 2.21% dry weight. At 4.83 klux, astaxanthin drastically increased at Day 8 in 10X and 15X medium dilution experiments. This increase was not found in the control culture. The maximum astaxanthin concentration of 2.58% dry weight was obtained after 16 days of induction in 10X medium dilution. The highest accumulation of astaxanthin was found at the light intensity of 6.50 klux, particularly at 10X and 15X medium dilutions. The attainable maximum astaxanthin concentration occurred 10X medium dilution was 3.81% dry weight after 6 days of induction. This level of accumulation was satisfactorily and provided high scale up potential.

Equation 4.1 was again employed to find the most suitable condition for the induction of astaxanthin for indoor experiments, and the results indicated that the 10X medium dilution and 6.5 klux was best where 3.81% dry weight could be achieved within 6 days of induction. Cell and astaxanthin concentrations time profiles are shown in Figures 4.9 and 4.10, respectively.

The results as described above suggested that, in most cases, astaxanthin accumulated better in diluting cell and medium conditions than in the control condition. However, it appeared that the cells also died more significantly in experiments with diluting cell/medium conditions than in the control. The death rate became quite significant for the diluting medium condition for the experiments in this work. As stated earlier, diluting medium might result in an inadequate nutrient supply for the cell growth. In addition, cells in diluting cell conditions became more exposed to light at high intensity which might also induce higher level of cell death. Since the survival rate in the diluting cell concentration was better than in the diluting medium condition, it could be concluded that the cell dilution did not exhibit undesirable conditions for the growth, and the effect of too much light exposure became more significant than the effect of inadequate nutrient.

4.2.2 Effect of medium concentration under local environmental condition (outdoor experiments)

This work was done by diluting medium concentrations in the range from 0X-40X of the final spent nutrient concentration when the vegetative cells were harvested. Under the normal experimental condition here, the average temperature at noon was 33-35°C and the light intensity was 110-150 klux. Figure 4.11 illustrates that the number of algal cells slightly increased in the control medium concentrated condition (no dilution) whereas a slight drop in cell density was observed at diluting medium conditions. This could be due to the fact that nutrients were present in the highest quantity in the controlled experiment which allowed cell growth. On the other hand, cells slightly decreased with time in the medium dilution condition as they did not have enough nutrient concentration to grow and finally died. The effect of medium dilution to astaxanthin concentration is depicted in Figure 4.12.

The best condition in this case was at Day 14 in the 10X medium dilution condition where astaxanthin reached the maximum of 0.36 mg/10^6 cells or 2.24 % dry weight.

4.3 Effect of light intensity

4.3.1 In diluted initial cell concentration

Cell concentration drastically dropped in the cell dilution culture. Especially, cell in diluted cell concentration were more drop at low light intensity (0.65-2.73 klux) and they reached a plateau at high light intensity (4.83-6.5 klux) Astaxanthin accumulation was found to exhibit opposite trend to that of cell concentration, i.e. astaxanthin concentration increased as cell concentration decreased. Light intensity seemed to have the major role in this induction mechanism as more astaxanthin was observed with stronger light intensity (see Figure 4.13).

Continuous lighting also seemed to be one significant factor, and astaxanthin concentration in the indoor condition with constant lighting (5.39% dry weight) was markedly higher than that obtained from the outdoor condition (1.56%dry weight), despite a much lower light intensity achievable in indoor environment. It was possible that this occurred as in the indoor condition, light was supplied continuously (24 hours) whereas the outdoor condition only provided illumination for 12 hours. This

finding was consistent with that of Dominguez-Bocanegra (2003), who reported that continuous illumination could produce higher level of astaxanthin than 12 h light/12 h dark cycle. Similar results could also be found in Kaobayashi et al. (1992).

4.3.2 In diluted spent medium concentration

Cell concentration slightly increased at low light intensity and decreased at high light intensity at all conditions examined in this section. For instance, in 10X and 15X medium dilutions, cells rapidly increased in low light intensity (0.65-2.73 klux) and rapidly decreased in high light intensity (4.83-6.5 klux). Astaxanthin accumulation was observed in all cases. This increase was more significant for cases with harsh conditions that cells could not grow, and was only slight for milder conditions that cells still could grow. Light intensity in particular could again be a major parameter as more astaxanthin accumulation was obtained at higher than at lower intensity as illustrated in Figure 4.14. However, opposite results were observed with the cell survival rate. Similar to the discussion in the previous section, astaxanthin accumulation in the indoor condition (3.81% dry weight) was higher than that in the outdoor condition (2.24%dry weight), and this was believed to be due to the availability of light. In indoor condition, light was supplied at lower intensity, but high enough to induce astaxanthin, and this light was continuously supplied. On the other hand, a much higher light intensity from outdoor condition might be able to stimulate astaxanthin accumulation during the short time period but in a longer time scale, the accumulation of astaxanthin was lower.

4.4 Astaxanthin production in airlift photobioreactor

The induction of astaxanthin in the 2.7L airlift photobioreactor was done with the most appropriate conditions obtained from the experiments in 1.5L bubble column, i.e. 10 times diluted initial cell concentration and 10 times diluted medium concentration. Other conditions included the light intensity of 4.83 klux, the air volumetric flowrate of 20 cm^3/ml. This was equivalent to the superficial gas velocity of 1.28 cm/s which was higher than that for the bubble column (0.40 cm/s) (to minimize precipitation).

Cell concentration during the first stage of experiments suddenly dropped as illustrated in Figure 4.15 due primarily to the drastic change in the environmental conditions. Cell precipitation was expected to be one of the key factors that caused the death of the cell. This could be due to the inadvertent defect in the design of the airlift system where the dead zone existed at the bottom of the reactor and this allowed the cells to be captured and remained still (Figure 4.16).

Figure 4.17 shows that astaxanthin concentration slightly increased and the maximum astaxanthin concentration of 4.79 mg astaxanthin/l was achieved at Day 16 of the induction period. This was equivalent to 2.16% dry weight. However, the calculation shows that the highest productivity of 8.18 mg/l at Day 2. This astaxanthin concentration was lower than that in bubble column which was believed to be due to the existence of cell precipitation in the airlift system. Cell precipitation does not allow adequate light to pass through and therefore no astaxanthin accumulation occurred. The bubble column, on the other hand, was designed with the cone bottom which then prevented the cell precipitation in this case.

In brief, the performance of the bubble column used in this work was superior to that in the airlift system. Cell precipitation in the airlift was anticipated to be the main cause. Due to laboratory constrains, the adjustment of the design of the airlift was not possible in the course of this research. However, it is proposed that the bottom of the airlift should be re-designed, for instance, airlift with taper or cone bottom.

4.5 Economical analysis of astaxanthin production for *H. pluvialis*

Economical analysis of astaxanthin production with the methods employed in this experiment is displayed in Tables 4.4 and 4.5. The analysis was based on the production of 1 kg of astaxanthin. The results indicated that the operation of 25X cell dilution (or approx. 100,000 cells/ml) and 2.73 klux was the most attractive in terms of economical return. For the dilution of spent medium concentration, the dilution at 15X with (4.83 klux) became the most attractive option, while the dilution at 15X (0.65 klux) was the worst. It should be noted that the length of service pump and lamps was assumed to be 5 and 1 years, respectively, and the cost of astaxanthin was at 7,000 $/kg (data from www.israe121c.org, visited in 2009).

Table 4.1 Maximum astaxanthin accumulation from the induction in bubble columns at various light intensities

Units	650 lux (ambient condition)				2.73 klux (2 fluorescent lamps)				4.83 klux (4 fluorescent lamps)				6.5 klux (6 fluorescent lamps)			
	DCC	DC10	DC20	DC25	DCC	DC10	DC20	DC25	DCC	DC10	DC20	DC25	DCC	DC10	DC20	DC25
Average cell concentration ($x10^4$ cells/ml)	9.31	2.48	2.08	1.49	9.07	0.52	0.83	1.182	8.73	0.99	0.38	0.36	8.46	1.12	0.77	0.52
Maximum astaxanthin concentration (mg/l)	9.85	44.5	53.3	66.4	17.9	56.8	95.7	104.4	16.19	64	62.5	88.2	21.2	92	77.4	103
Maximum astaxanthin productivity (mg/l day)	2.66	0.38	0.23	0.19	2.57	0.58	0.42	0.47	2.82	0.77	0.74	0.33	3.82	1.15	0.76	0.62
Maximum astaxanthin content (mg/10^6 cells)	0.11	0.21	0.22	0.28	0.2	1.17	0.64	0.46	0.19	0.65	0.73	1.05	0.28	0.91	0.53	0.87
Maximum % astaxanthin (% g astaxanthin/g cell)	0.7	1.26	1.32	1.69	1.2	5.3	3.9	2.79	1.13	3.95	3.65	6.46	1.71	5.39	3.24	5.3
Maximum % astaxanthin accumulation rate (% g astaxanthin/day g cell)	0.05	0.09	0.11	0.12	0.09	0.44	0.28	0.279	0.063	0.49	0.46	0.65	0.12	0.67	0.23	0.53

Remark DCC is the control condition where the initial cell concentration was fixed at $1x10^5$ cells/ml
DC10 is 10X dilution of final cell concentration (growth stage) or $1x10^4$ cells/ml
DC20 is 20X dilution of final cell concentration (growth stage) or $5x10^3$ cells/ml
DC25 is 25X dilution of final cell concentration (growth stage) or $4x10^3$ cells/ml

Table 4.2 Effect of initial cell dilution on the induction of astaxanthin at outdoor condition

Astaxanthin concentration (mg/l)									
DCC		DC5		DC10		DC20		DC25	
I	II	I	II	I	II	I	II	I	II
2.70	0.00	2.42	0.00	2.20	0.00	5.56	0.00	1.81	0.00
2.49	2.98	3.99	0.07	8.74	0.83	2.82	4.62	2.89	0.36
3.75	3.47	10.53	2.55	14.09	4.30	12.36	10.33	12.01	13.64
4.97	7.73	10.77	13.11	22.07	9.83	30.64	20.88	34.05	26.37
2.01	6.86	19.44	27.71	26.99	19.44	153.54	44.51	52.84	69.19
11.53	10.40	14.25	18.86	31.54	41.94	47.47	52.17	94.48	62.14
15.11	11.78	31.97	18.59	45.09	37.03	97.98	74.14	72.71	130.06
17.25	10.64	44.22	16.42	90.39	39.34	75.94	91.04	105.31	131.42
17.12	9.61	45.30	14.74	34.25	39.67	74.86	86.92	107.12	137.65
16.60	8.45	32.08	13.42	46.10	34.65	86.42	85.48	120.31	135.30

Remarks

DCC is controlled cell concentration or $1x10^5$ cells/ml
DC5 is 5X dilution of final cell concentration (growth stage) or $5x10^4$ cells/ml
DC10 is 10X dilution of final cell concentration (growth stage) or $1x10^4$ cells/ml
DC20 is 20X dilution of final cell concentration (growth stage) or $5x10^3$ cells/ml
DC25 is 25X dilution of final cell concentration (growth stage) or $4x10^3$ cells/ml

Table 4.3 Maximum astaxanthin accumulation from the induction in bubble column at various light intensities

Units	650 lux (ambient condition)				2.73 klux (2 fluorescent lamps)				4.83 klux (4 fluorescent amps)				6.5 klux (6 fluorescent lamps)			
	DMC	DM5	DM10	DM15	DMC	DM5	DM10	DM15	DMC	DM5	DM10	DM15	DMC	DM5	DM10	DM15
Average cell concentration ($x10^4$ cells/ml)	2.29	0.73	0.26	0.10	4.10	0.96	1.06	0.31	1.67	0.93	1.13	1.24	1.31	1.03	0.24	0.20
Maximum astaxanthin concentrations (mg/l)	2.77	1.80	0.71	0.24	4.36	2.15	2.75	0.64	2.36	6.18	5.80	5.69	3.88	4.70	1.50	0.92
Maximum astaxanthin productivity (mg/l day)	0.33	0.12	0.06	0.04	0.72	0.21	0.23	0.25	0.33	0.34	0.58	0.57	0.46	0.61	0.25	0.14
Maximum astaxanthin content (mg/10^6 cells)	0.10	0.22	0.15	0.25	0.14	0.29	0.36	0.16	0.28	0.44	0.51	0.61	0.34	0.44	0.62	0.38
Maximum % astaxanthin (% g astaxanthin/g cell)	0.63	1.37	0.89	1.49	0.86	1.76	2.21	0.96	0.85	1.33	2.58	2.08	2.08	2.70	3.81	2.34
Maximum % astaxanthin accumulation rate (% g astaxanthin/day g cell)	0.04	0.08	0.07	0.09	0.05	0.10	0.18	0.05	0.09	0.07	0.16	0.26	0.12	0.15	0.64	0.13

Remark DMC is no dilution of final spent medium concentration
DM 5 is 5 times dilution of final spent medium concentration (with distilled water)
DM10 is 10 times dilution of final spent medium concentration (with distilled water)
DM15 is 15 times dilution of final spent medium concentration (with distilled water)

Table 4.4 Economical analysis for astaxanthin production from the induction with diluting initial cell concentration in 1.5L bubble column photobioreactor

		0.65 klux				2.73 klux			
		DCC	DC10	DC20	DC25	DCC	DC10	DC20	DC25
Total astaxanthin (kg)	[A]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Total volume of cultivation (l)	[B]	101.52	224.72	446.43	375.94	55.74	239.23	208.77	227.79
Cultivation time (days)	[C]	1421.32	314.61	267.86	210.53	780.38	287.08	146.14	91.12
Number of cultivation	[D]	101.52	224.72	446.43	375.94	55.74	239.23	208.77	227.79
Volume of water (l)	[E]	91.37	20.22	20.09	13.53	50.17	21.53	9.39	8.20
Volume of nutrient (l)	[F]	10.15	2.25	2.23	1.50	5.57	2.39	1.04	0.91
Volume of cell (l)	[G]	25.38	0.56	0.28	0.15	13.94	0.60	0.13	0.09
Total volume of water (l)	[H = ExD]	91.37	202.25	401.79	338.35	50.17	215.31	187.89	205.01
Total volume of nutrient (l)	[I = FxD]	10.15	22.47	44.64	37.59	5.57	23.92	20.88	22.78
Total volume of cell (l)	[J = GxD]	25.38	5.62	5.58	3.76	13.94	5.98	2.61	2.28
Cost of water, 0.06 THB/l (THB)	[K =Hx0.06]	5.48	12.13	24.11	20.30	3.01	12.92	11.27	12.30
Cost of nutrient, 1 THB/l (THB)	[L = Ix1]	10.15	22.47	44.64	37.59	5.57	23.92	20.88	22.78
Cost of cell, 564 THB/l (THB)	[M = Jx564]	14314.72	3168.54	3147.32	2120.30	7859.53	3373.21	1471.82	1284.74
Power of air compressor (W)	[N = Dx0.8]	81.22	179.78	357.14	300.75	44.59	191.39	167.01	182.23
Power of light source (W)	[O]	0.00	0.00	0.00	0.00	1114.83	4784.69	4175.37	4555.81
Total electrical unit (units)	[P = (N+O)x(Cx24)÷1000]	27.29	60.40	102.86	101.05	389.57	1433.11	1459.04	1137.13
Electrical cost, 3 THB per unit (THB)	[Q = Px3]	81.87	181.21	308.57	303.16	1168.70	4299.33	4377.12	3411.39
Cost of pump[a] (BTH)	[R = 0.0132xCxD]	18.76	41.53	70.71	69.47	10.30	37.89	38.58	30.07
Cost of lamp[b] (BTH)	[S]	0.00	0.00	0.00	0.00	128.29	471.96	480.50	374.49
Total operating cost (THB)	[T = K+L+M+Q+R+S]	14430.98	3284.80	3263.59	2236.56	7975.80	3489.47	1588.08	1401.00

Remark DCC is controlled cell concentration or $1x10^5$ cells/ml, DC10 is 10X dilution of final cell concentration (growth stage) or $1x10^4$ cells/ml, DC20 is 20X dilution of final cell concentration (growth stage) or $5x10^3$ cells/ml, DC25 is 25X dilution of final cell concentration (growth stage) or $4x10^3$ cells/ml

[a] Length of service is 5 years., [b] Length of service is 1 year.

Table 4.4 (continued) Economical analysis for astaxanthin production from the induction with diluting initial cell concentration in 1.5L bubble column photobioreactor

		4.83 klux				6.5 klux			
		DCC	DC10	DC20	DC25	DCC	DC10	DC20	DC25
Total astaxanthin (kg)	[A]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Total volume of cultivation (l)	[B]	61.77	209.64	319.49	303.95	47.10	108.70	395.26	243.31
Cultivation time (days)	[C]	1111.80	167.71	223.64	121.58	659.44	86.96	276.68	97.32
Number of cultivation	[D]	61.77	209.64	319.49	303.95	47.10	108.70	395.26	243.31
Volume of water (l)	[E]	55.59	18.87	14.38	10.94	42.39	9.78	17.79	8.76
Volume of nutrient (l)	[F]	6.18	2.10	1.60	1.22	4.71	1.09	1.98	0.97
Volume of cell (l)	[G]	15.44	0.52	0.20	0.12	11.78	0.27	0.25	0.10
Total volume of water (l)	[H = ExD]	55.59	188.68	287.54	273.56	42.39	97.83	355.73	218.98
Total volume of nutrient (l)	[I = FxD]	6.18	20.96	31.95	30.40	4.71	10.87	39.53	24.33
Total volume of cell (l)	[J = GxD]	15.44	5.24	3.99	3.04	11.78	2.72	4.94	2.43
Cost of water, 0.06 THB/l (THB)	[K =Hx0.06]	3.34	11.32	17.25	16.41	2.54	5.87	21.34	13.14
Cost of nutrient, 1 THB/l (THB)	[L = Ix1]	6.18	20.96	31.95	30.40	4.71	10.87	39.53	24.33
Cost of cell, 564 THB/l (THB)	[M = Jx564]	8709.08	2955.97	2252.40	1714.29	6641.54	1532.61	2786.56	1372.26
Power of air compressor (W)	[N = Dx0.8]	49.41	167.71	255.59	243.16	37.68	86.96	316.21	194.65
Power of light source (W)	[O]	2470.66	8385.74	12779.55	12158.05	2826.19	6521.74	23715.42	14598.54
Total electrical unit (units)	[P = (N+O)x(Cx24)÷1000]	1088.67	1642.26	4379.81	2976.29	962.26	1268.87	8074.62	3550.36
Electrical cost, 3 THB per unit (THB)	[Q = Px3]	3266.02	4926.79	13139.42	8928.88	2886.78	3806.61	24223.87	10651.09
Cost of pump[a] (BTH)	[R = 0.0132xCxD]	14.68	22.14	59.04	40.12	8.70	11.48	73.04	32.12
Cost of lamp[b] (BTH)	[S]	365.56	551.45	1470.67	999.39	325.24	428.87	2729.17	1200.00
Total operating cost (THB)	[T = K+L+M+Q+R+S]	8825.34	3072.24	2368.66	1830.55	6757.81	1648.87	2902.83	1488.53

Remark DCC is controlled cell concentration or $1x10^5$ cells/ml, DC10 is 10X dilution of final cell concentration (growth stage) or $1x10^4$ cells/ml, DC20 is 20X dilution of final cell concentration (growth stage) or $5x10^3$ cells/ml, DC25 is 25X dilution of final cell concentration (growth stage) or $4x10^3$ cells/ml

[a] Length of service is 5 years., [b] Length of service is 1 year.

Table 4.5 Economical analysis for astaxanthin production from diluting medium concentration in 1.5 L bubble column photobioreactor

		0.65 klux				2.73 klux			
		DMC	DM5	DM10	DM15	DMC	DM5	DM10	DM15
Total astaxanthin (kg)	[A]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Total volume of cultivation (l)	[B]	361.01	555.56	1408.45	4166.67	229.36	465.12	363.64	1562.50
Cultivation time (days)	[C]	6498.19	2000.00	1690.14	4444.44	4128.44	1674.42	436.36	1875.00
Number of cultivation	[D]	361.01	555.56	1408.45	4166.67	229.36	465.12	363.64	1562.50
Volume of water (l)	[E]	0.00	88.89	126.76	258.33	0.00	74.42	32.73	96.88
Volume of nutrient (l)	[F]	361.01	22.22	14.08	18.33	229.36	18.60	3.64	6.98
Volume of cell (l)	[G]	90.25	27.78	35.21	69.44	57.34	23.26	9.09	26.04
Total volume of water (l)	[H = ExD]	0.00	444.44	1267.61	3875.00	0.00	372.09	327.27	1453.13
Total volume of nutrient (l)	[I = FxD]	361.01	111.11	140.85	277.78	229.36	93.02	36.36	104.17
Total volume of cell (l)	[J = GxD]]	90.25	138.89	352.11	1041.67	57.34	116.28	90.91	390.63
Cost of water, 0.06 THB/l (THB)	[K = Hx0.06]	0.00	26.67	76.06	232.50	0.00	22.33	19.64	87.19
Cost of nutrient, 1 THB/l (THB)	[L = Ix1]	361.01	111.11	140.85	277.78	229.36	93.02	36.36	104.17
Cost of cell, 526 THB/l (THB)	[M = Jx526]	47472.92	73055.56	185211.27	547916.67	30160.55	61162.79	47818.18	205468.75
Power of air compressor (W)	[N = Cx0.8]	288.81	444.44	1126.76	3333.33	183.49	372.09	290.91	1250.00
Power of light source (W)	[O]	0.00	0.00	0.00	0.00	4587.16	9302.33	7272.73	31250.00
Total electrical unit (units)	[P = (N+O)x(Cx24)÷1000]	124.77	192.00	324.51	1280.00	2060.92	4179.35	2178.33	14040.00
Electrical cost, 3 THB per unit (THB)	[Q = Px3]	374.30	576.00	973.52	3840.00	6182.75	12538.05	6534.98	42120.00
Cost of pump[a] (BTH/time)	[R = 0.0132xCxD]	85.78	132.00	223.10	880.00	54.50	110.51	57.60	371.25
Cost of lamp[b] (BTH)	[S]	0.00	0.00	0.00	0.00	678.72	1376.37	717.38	4623.75
Total operating cost (THB)	[T =K+L+M+Q+R+S]	48294.01	73901.33	186624.79	553146.94	37305.87	75303.07	55184.15	252775.10

Remark DCC is controlled cell concentration or $1x10^5$ cells/ml, DC10 is 10X dilution of final cell concentration (growth stage) or $1x10^4$ cells/ml, DC20 is 20X dilution of final cell concentration (growth stage) or $5x10^3$ cells/ml, DC25 is 25X dilution of final cell concentration (growth stage) or $4x10^3$ cells/ml

[a] Length of service is 5 years., [b] Length of service is 1 year.

Table 4.5 (continued) Economical analysis for astaxanthin production from diluting medium concentration in 1.5 L bubble column photobioreactor.

		4.83 klux				6.5 klux			
		DMC	DM5	DM10	DM15	DMC	DM5	DM10	DM15
Total astaxanthin (kg)	[A]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Total volume of cultivation (l)	[B]	425.53	161.81	206.19	362.32	257.73	200.00	666.67	1086.96
Cultivation time (days)	[C]	4255.32	582.52	329.90	193.24	4639.18	720.00	400.00	1304.35
Number of cultivation	[D]	425.53	161.81	206.19	362.32	257.73	200.00	666.67	1086.96
Volume of water (l)	[E]	0.00	25.89	18.56	22.46	0.00	32.00	60.00	67.39
Volume of nutrient (l)	[F]	425.53	6.47	2.06	1.62	257.73	8.00	6.67	4.86
Volume of cell (l)	[G]	106.38	8.09	5.15	6.04	64.43	10.00	16.67	18.12
Total volume of water (l)	[H = ExD]	0.00	129.45	185.57	336.96	0.00	160.00	600.00	1010.87
Total volume of nutrient (l)	[I = FxD]	425.53	32.36	20.62	24.15	257.73	40.00	66.67	72.46
Total volume of cell (l)	[J = GxD]]	106.38	40.45	51.55	90.58	64.43	50.00	166.67	271.74
Cost of water, 0.06 THB/l (THB)	[K = Hx0.06]	0.00	7.77	11.13	20.22	0.00	9.60	36.00	60.65
Cost of nutrient, 1 THB/l (THB)	[L = Ix1]	425.53	32.36	20.62	24.15	257.73	40.00	66.67	72.46
Cost of cell, 526 THB/l (THB)	[M = Jx526]	55957.45	21278.32	27113.40	47644.93	33891.75	26300.00	87666.67	142934.78
Power of air compressor (W)	[N = Cx0.8]	340.43	129.45	164.95	289.86	206.19	160.00	533.33	869.57
Power of light source (W)	[O]	17021.28	6472.49	8247.42	14492.75	15463.92	12000.00	40000.00	65217.39
Total electrical unit (units)	[P = (N+O)x(Cx24)÷1000]	4166.81	2852.04	3230.35	2838.26	6769.48	5253.12	5836.80	28549.57
Electrical cost, 3 THB per unit (THB)	[Q = Px3]	12500.43	8556.12	9691.05	8514.78	20308.45	15759.36	17510.40	85648.70
Cost of pump[a] (BTH/time)	[R = 0.0132xCxD]	56.17	38.45	43.55	38.26	61.24	47.52	52.80	258.26
Cost of lamp[b] (BTH)	[S]	1399.15	957.67	1084.70	953.04	2288.04	1775.52	1972.80	9649.57
Total operating cost (THB)	[T =K+L+M+Q+R+S]	70338.72	30870.68	37964.45	57195.39	56807.22	43932.00	107305.33	238624.42

Remark DCC is controlled cell concentration or $1x10^5$ cells/ml, DC10 is 10X dilution of final cell concentration (growth stage) or $1x10^4$ cells/ml, DC20 is 20X dilution of final cell concentration (growth stage) or $5x10^3$ cells/ml, DC25 is 25X dilution of final cell concentration (growth stage) or $4x10^3$ cells/ml

[a] Length of service is 5 years., [b] Length of service is 1 year.

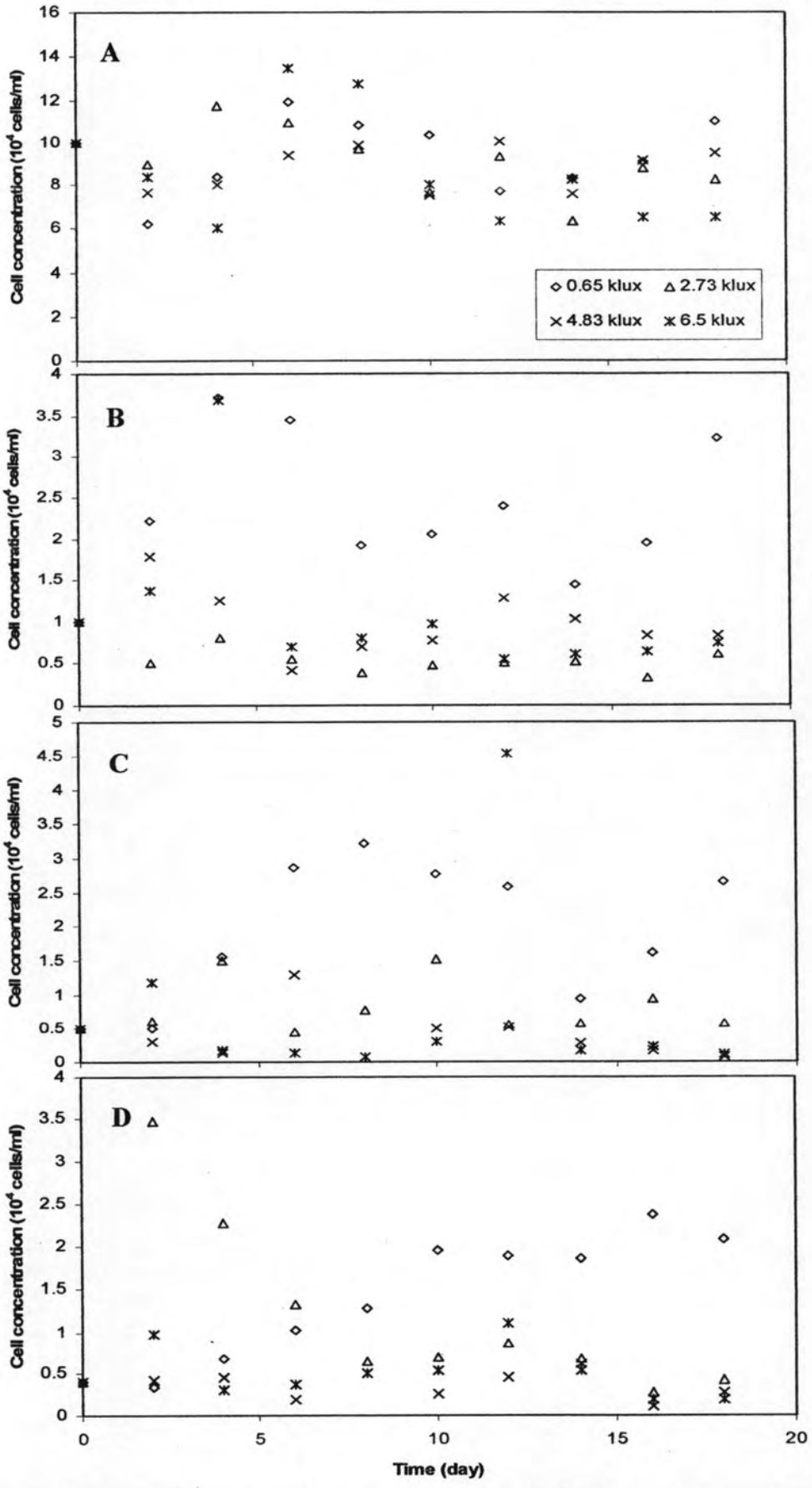

Figure 4.1 Cell concentration in the culture with dilution of final cell concentration (from the growth stage): A is control cell concentration; B is 10X cell dilution ($1x10^4$ cells/ml); C is 20X cell dilution ($5x10^3$ cells/ml); D is 25X cell dilution ($4x10^3$ cells/ml)

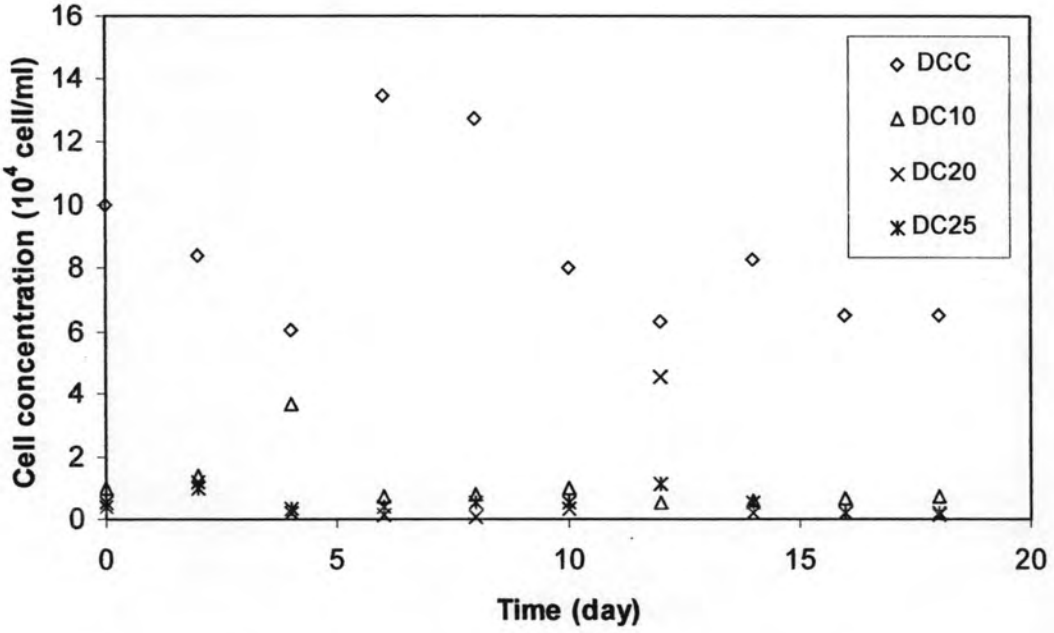

Figure 4.2 Cell density from indoor experiments at 6.5 klux: DCC is control cell concentration; DC10 is 10X cell dilution ($1x10^4$ cells/ml); DC20 is 20X cell dilution ($5x10^3$ cells/ml); DC25 is 25X cell dilution ($4x10^3$ cells/ml)

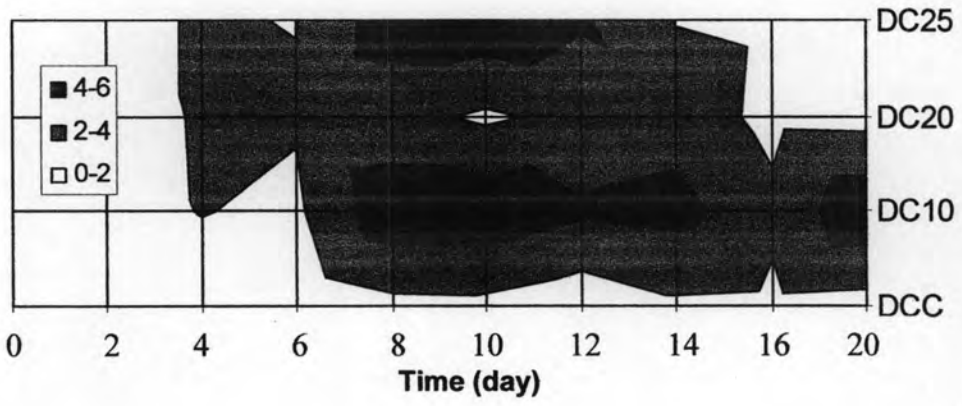

Figure 4.3 Effect of initial cell dilution on % dry weight at light intensity of 6.5 klux.: DCC is control cell concentration; DC10 is 10X cell dilution ($1x10^4$ cells/ml); DC20 is 20X cell dilution ($5x10^3$ cells/ml); DC25 is 25X cell dilution ($4x10^3$ cells/ml)

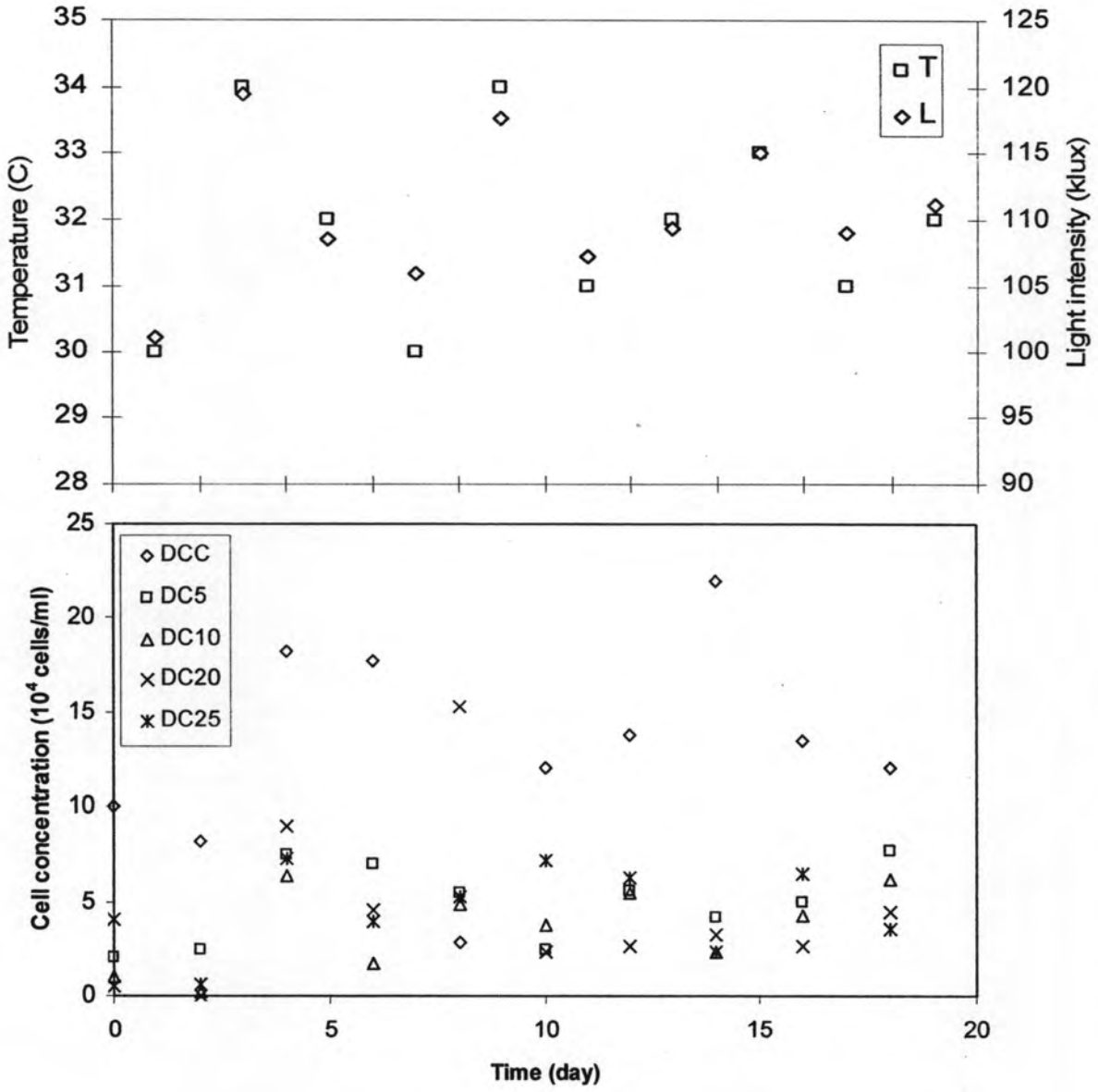

Figure 4.4 Cell density from the first set of outdoor experiments with different initial cell densities: DCC is control cell concentration; DC5 is 5X cell dilution ($2x10^4$ cells/ml); DC10 is 10X cell dilution ($1x10^4$ cells/ml); DC20 is 20X cell dilution ($5x10^3$ cells/ml); DC25 is 25X cell dilution ($4x10^3$ cells/ml); T: temperature; L: Light intensity.

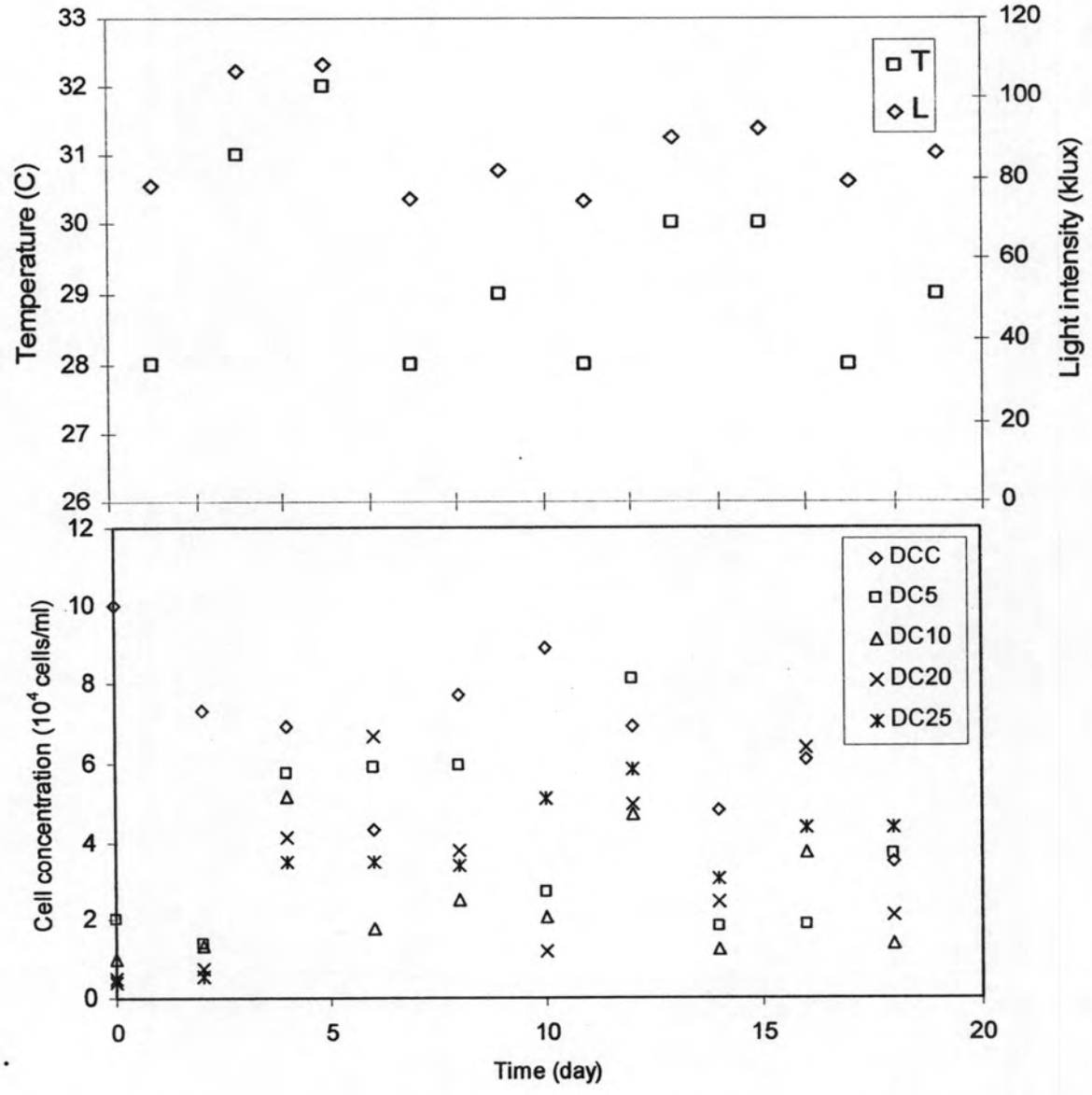

Figure 4.5 Cell density from the second set of outdoor experiments with different initial cell densities: DCC is control cell concentration; DC5 is 5X cell dilution ($2x10^4$ cells/ml); DC10 is 10X cell dilution ($1x10^4$ cells/ml); DC20 is 20X cell dilution ($5x10^3$ cells/ml); DC25 is 25X cell dilution ($4x10^3$ cells/ml); T: temperature; L: Light intensity.

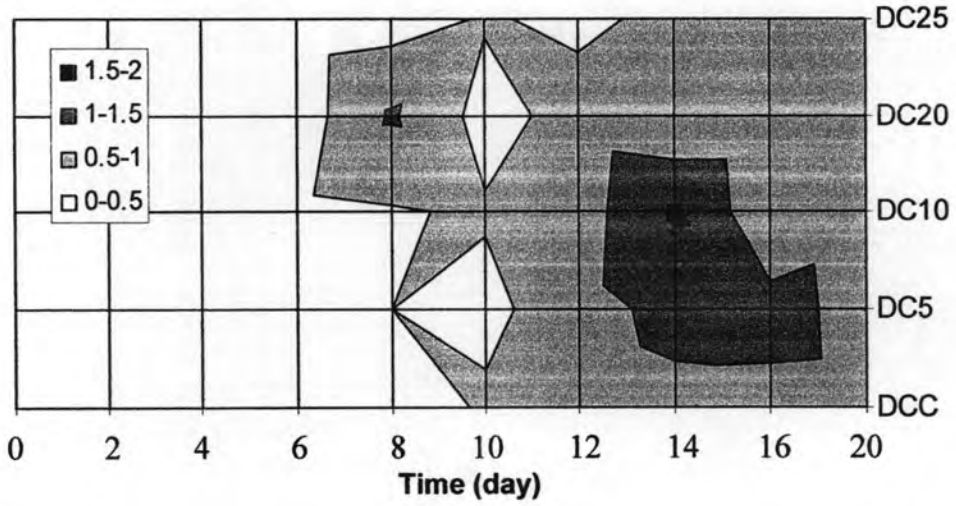

Figure 4.6 Effect of initial cell concentration on %astaxanthin from the first set of outdoor experiments: DCC is control cell concentration; DC5 is 5X cell dilution ($2x10^4$ cells/ml); DC10 is 10X cell dilution ($1x10^4$ cells/ml); DC20 is 20X cell dilution ($5x10^3$ cells/ml); DC25 is 25X cell dilution ($4x10^3$ cells/ml)

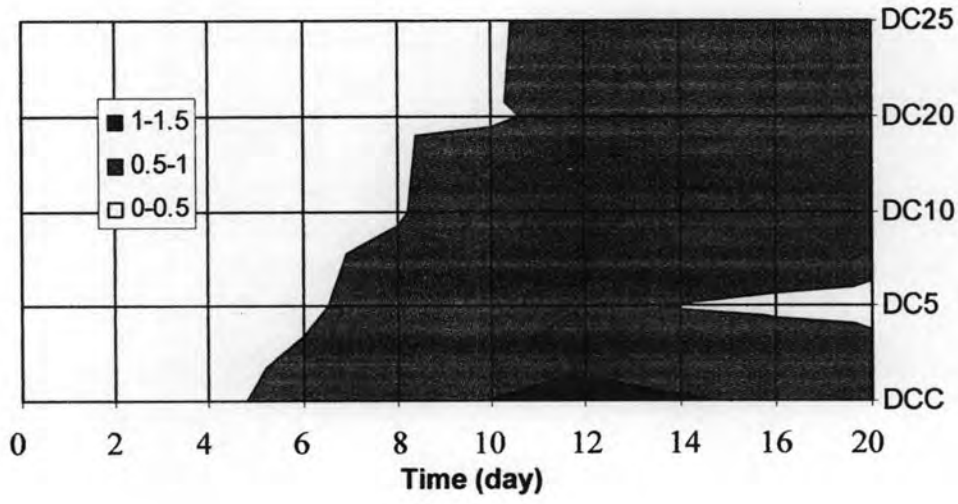

Figure 4.7 Effect of initial cell concentration on %astaxanthin from the second set of outdoor experiments: DCC is control cell concentration; DC5 is 5X cell dilution ($2x10^4$ cells/ml); DC10 is 10X cell dilution ($1x10^4$ cells/ml); DC20 is 20X cell dilution ($5x10^3$ cells/ml); DC25 is 25X cell dilution ($4x10^3$ cells/ml)

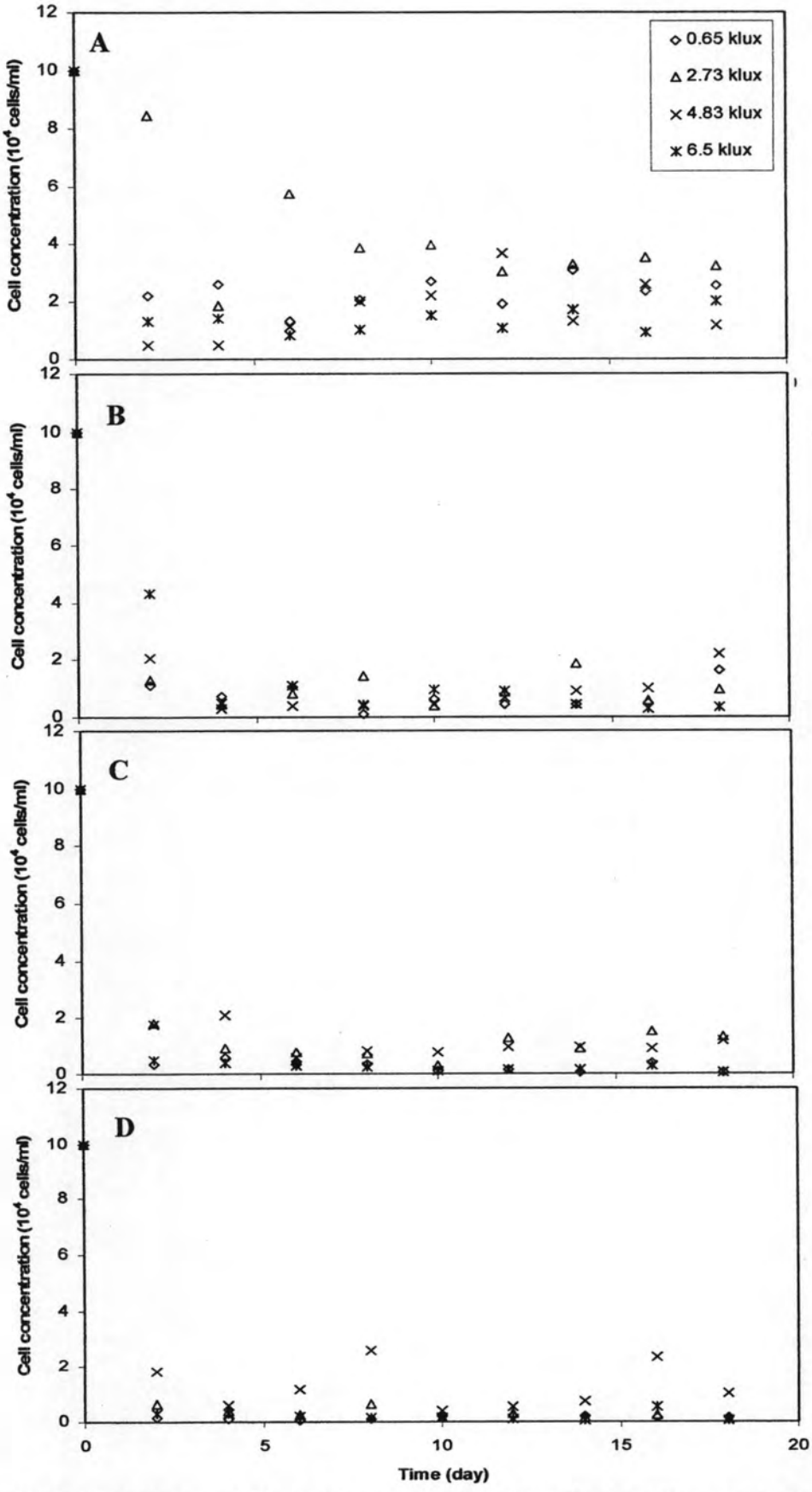

Figure 4.8 Cell concentration from the experiments with various medium concentrations and light intensities: A is control medium concentration; B is 5X dilution of the final spent medium concentration; C is 10X dilution of the final spent medium concentration; D is 15X dilution of the final spent medium concentration.

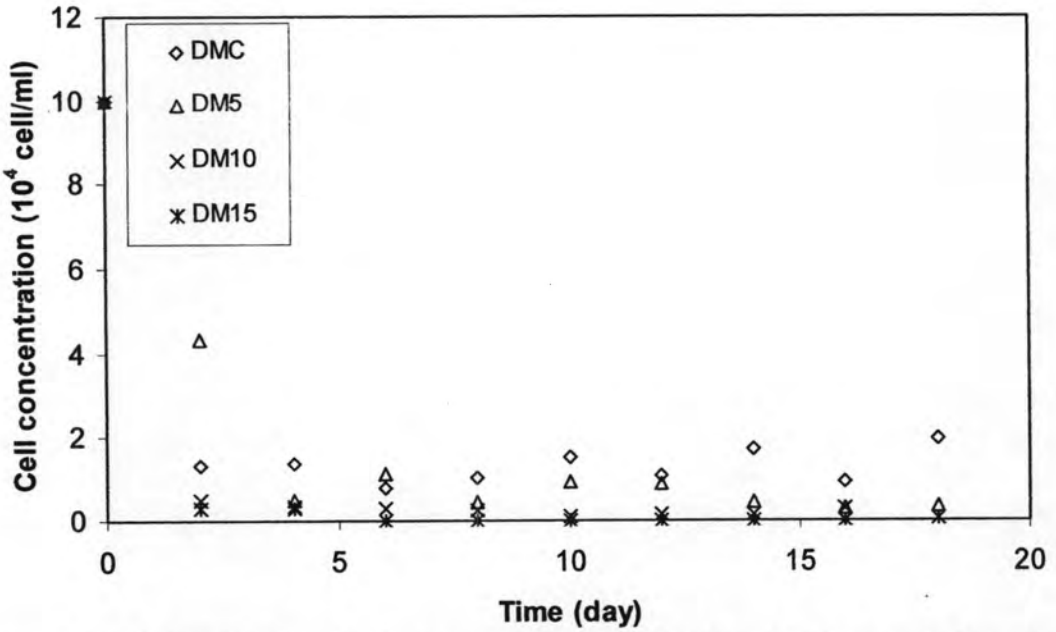

Figure 4.9 Cell density from indoor experiments at 6.5 klux: DMC is control medium concentration; DM5 is 5X dilution of the final spent medium concentration; DM10 is 10X dilution of the final spent medium concentration; DM15 is 15X dilution of the final spent medium concentration.

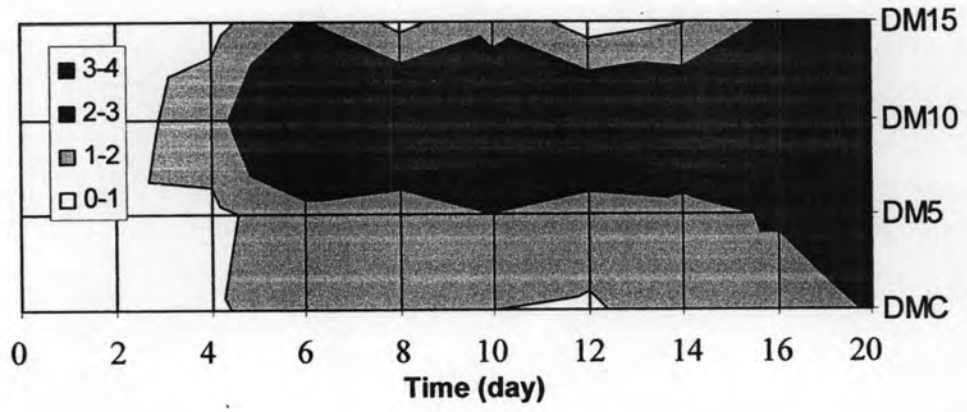

Figure 4.10 Effect of medium concentration on % dry weight at 6.5 klux: DMC is control medium concentration; DM5 is 5X dilution of the final spent medium concentration; DM10 is 10X dilution of the final spent medium concentration; DM15 is 15X dilution of the final spent medium concentration.

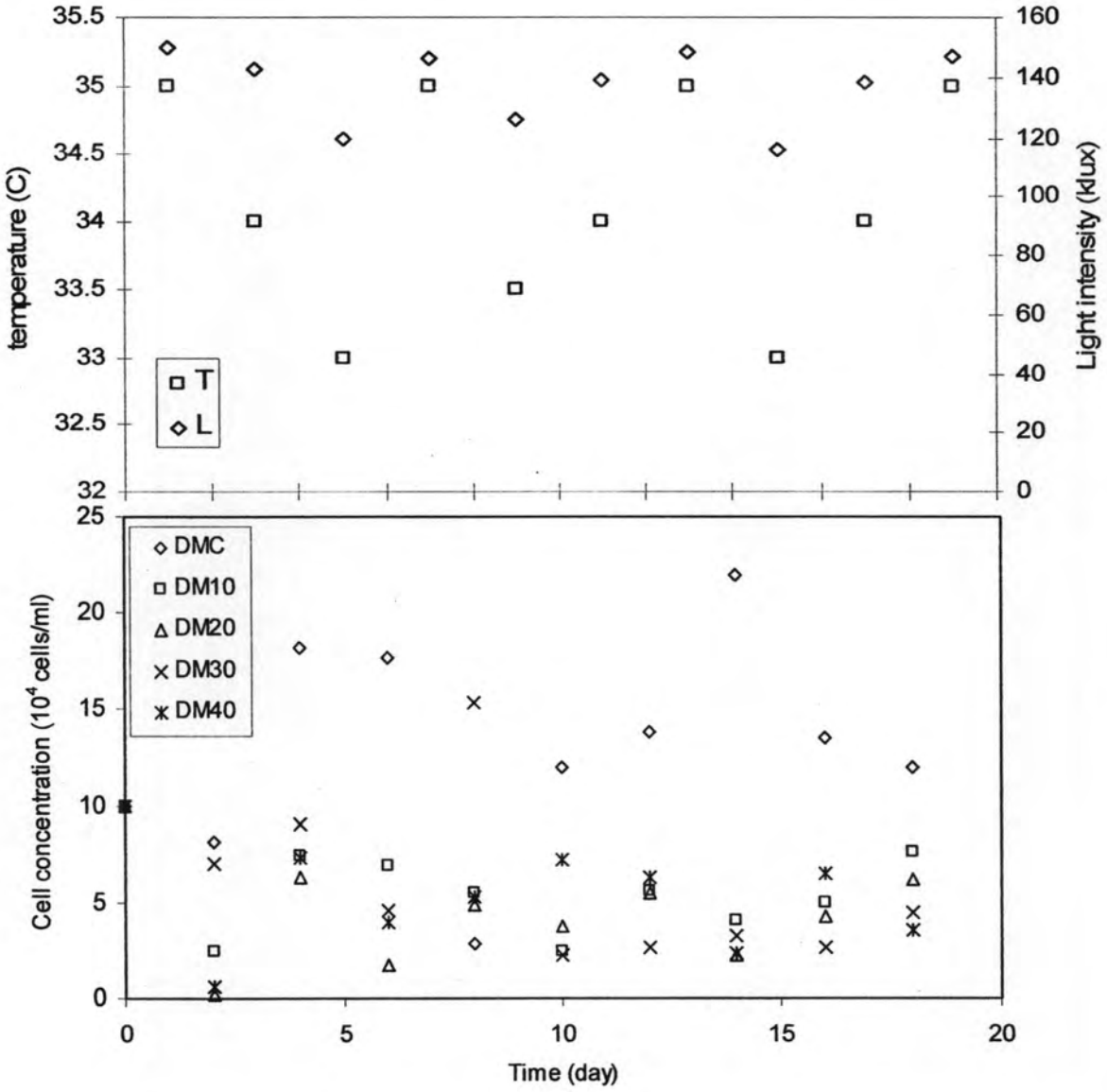

Figure 4.11 Cell density from outdoor experiments with different medium concentrations: DMC is control medium concentration; DM5 is 5X dilution of the final spent medium concentration; DM10 is 10X dilution of the final spent medium concentration; DM15 is 15X dilution of the final spent medium concentration.

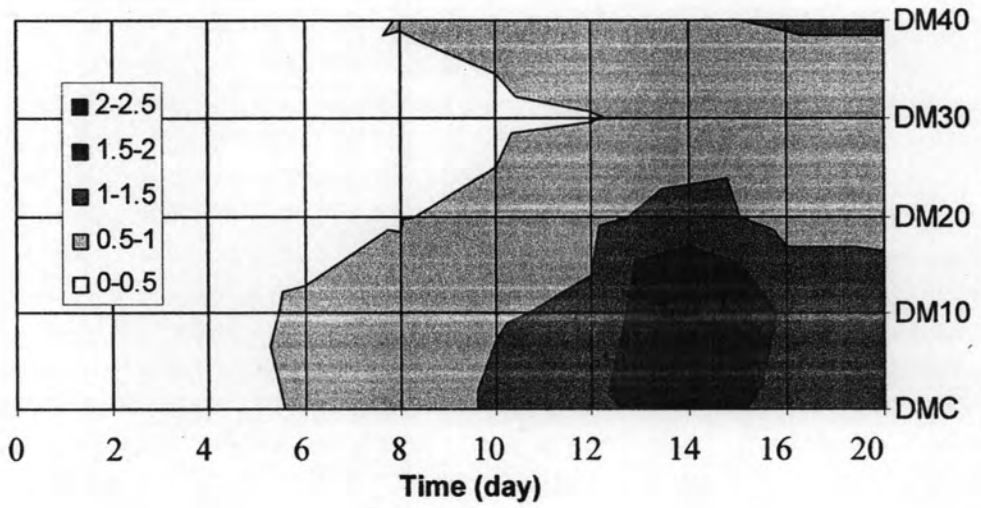

Figure 4.12 Effect of medium concentration on %dry weight in outdoor experiment: DMC is control medium concentration; DM5 is 5X dilution of the final spent medium concentration; DM10 is 10X dilution of the final spent medium concentration; DM15 is 15X dilution of the final spent medium concentration.

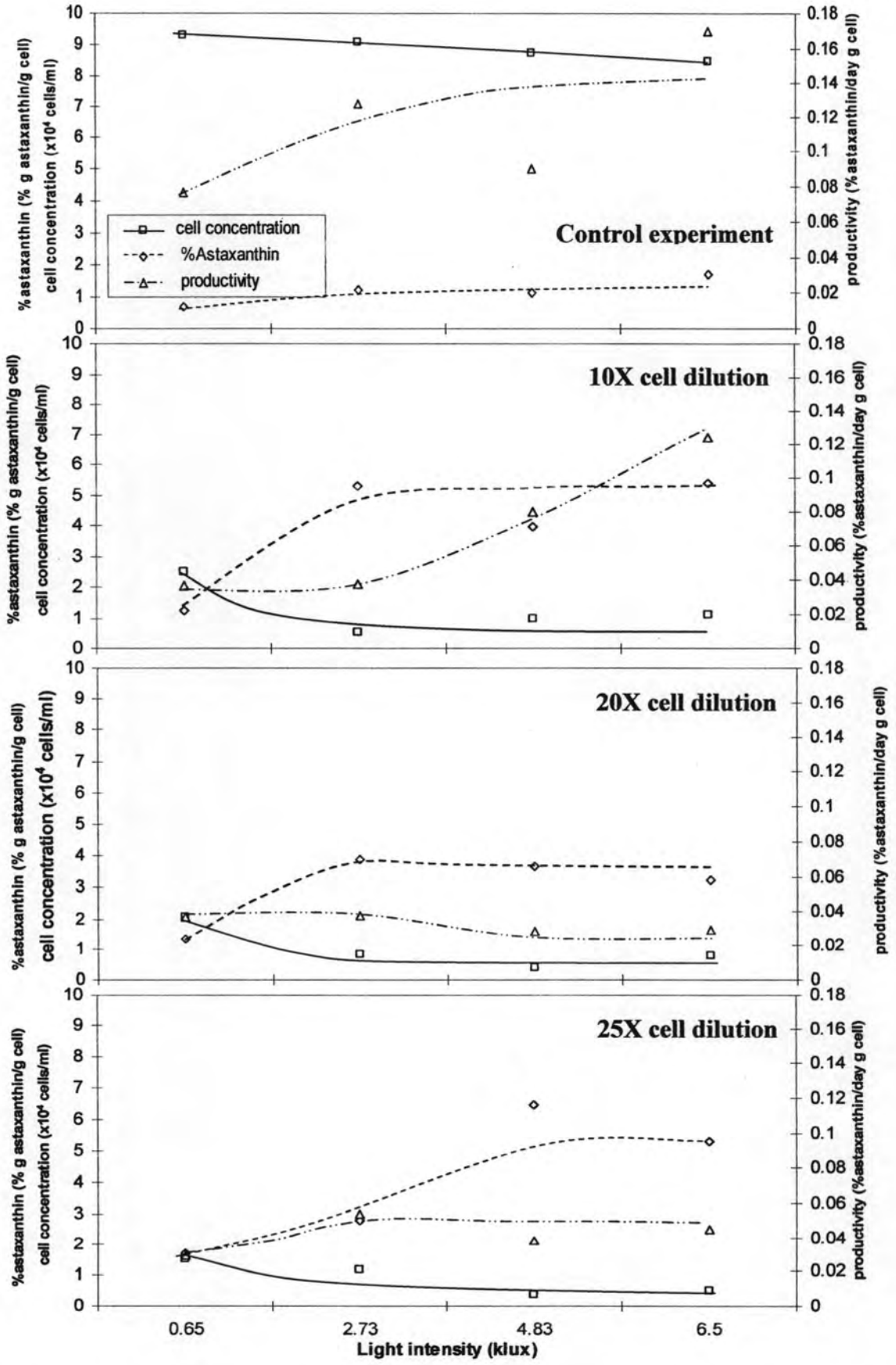

Figure 4.13 Cell concentration and astaxanthin concentration at various cell dilution rates in 1.5L bubble column photobioreactor

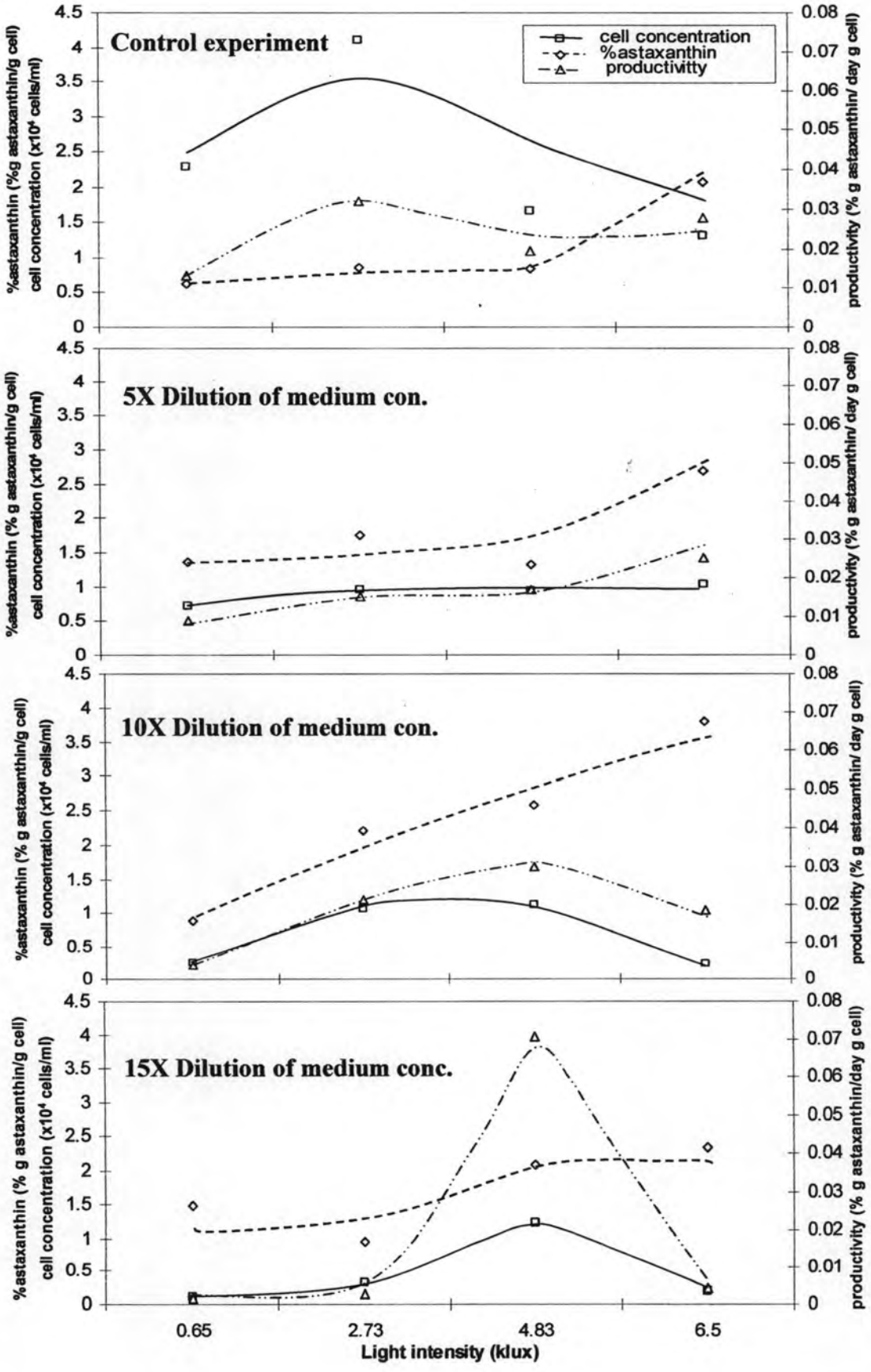

Figure 4.14 Cell concentration and astaxanthin concentration at various medium dilution rates in 1.5L bubble column photobioreactor

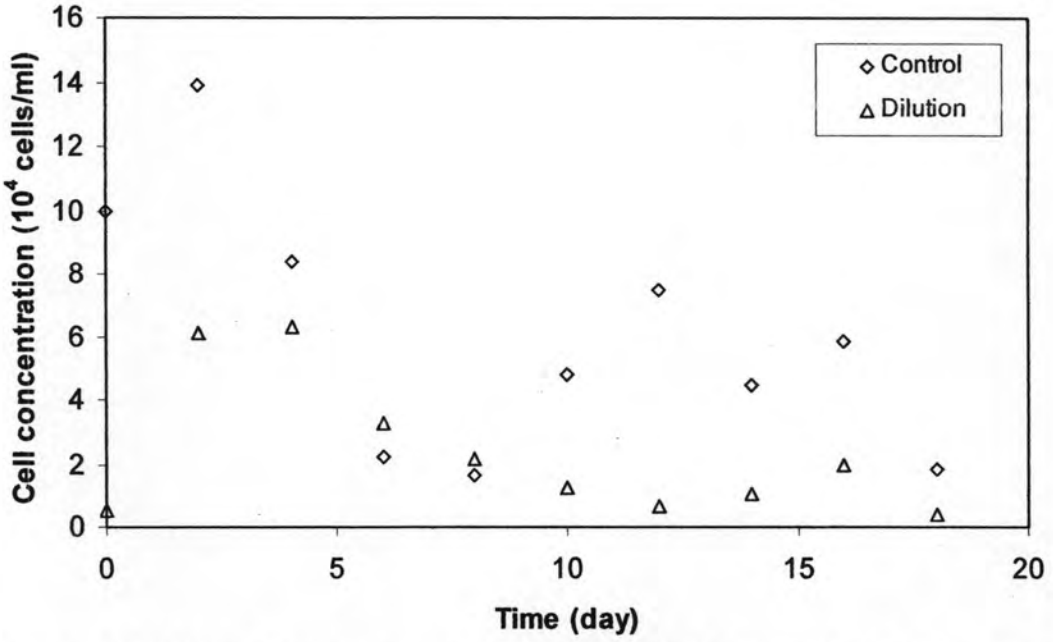

Figure 4.15 Cell concentration in 2.7L airlift photobioreactor at 6.5 klux : "Control" is the condition without dilution, "Dilution" is at 10X dilution of final spent medium concentration and 10X initial cell concentration (10,000 cells/ml)

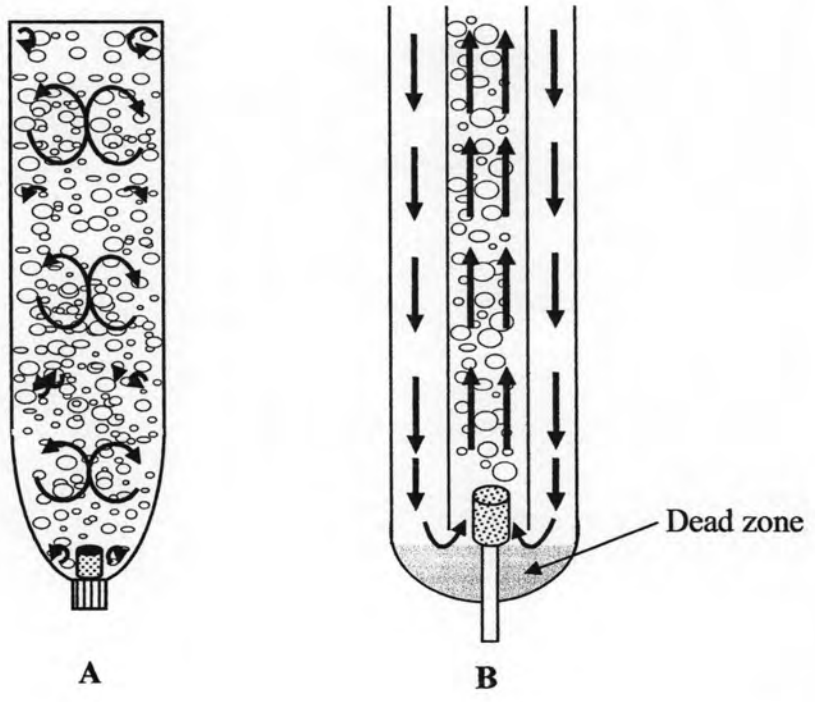

Figure 4.16 Dead zone in airlift: A is bubble column photobioreactor; B is airlift photobioreactor.

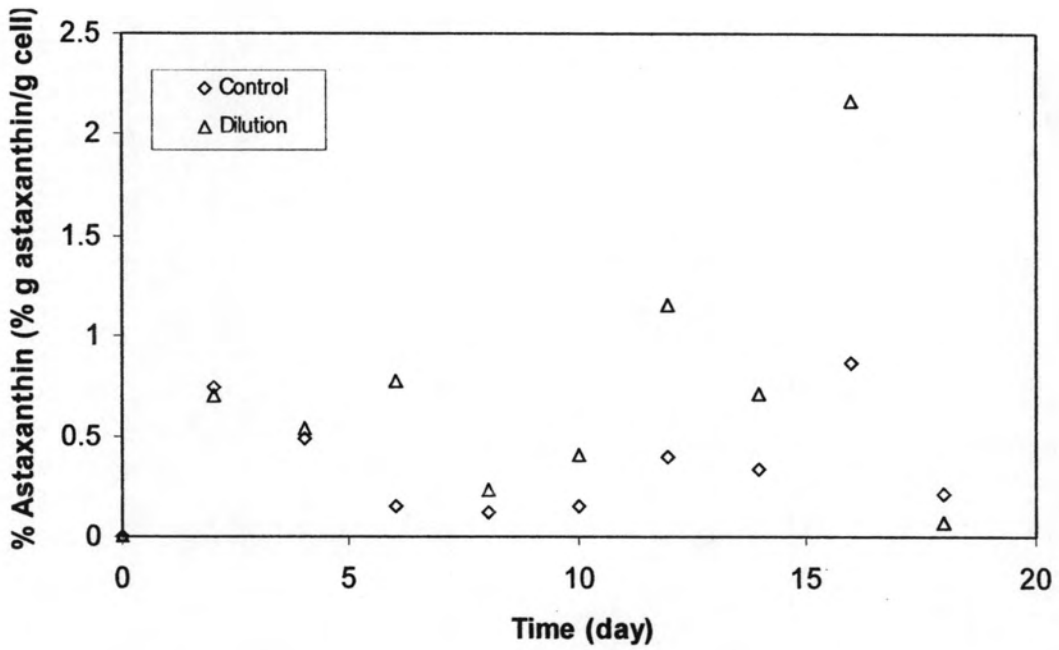

Figure 4.17 Astaxanthin concentration in 2.7L airlift photobioreactor at 6.5 klux : "Control" is the condition with no dilution; "Dilution" is 10X dilution of final spent medium concentration and 10X initial cell concentration (10,000 cells/ml)