CHAPTER IV

RESULTS AND DISCUSSIONS

4.1 Effect of low cost media on growth of *Spirulina spp.* in outdoor culture

4.1.1 The first preliminary test for the growth of *Spirulina spp.* in low cost media

This preliminary test was conducted to select the optimal low cost media for the growth of *Spirulina spp.* From the studies of Costa et al. in year 2001 and Subun in year 2003, it was found that *Spirulina spp.* can be growth well in urea fertilizer that instead of Sodium Nitrate ($NaNO_3$) in Zarrouk's media. To find the optimal concentration on the growth, this experiment was designed 4 different concentration of urea fertilizer (0, 0.3, 0.6 and 1.2 g/L). Compare the growth of *Spirulina spp.* when cultivating at 4 different concentration of urea fertilizer with standard media (Zarrouk's media). The result was shown in table 4.1 and figure 4.1, respectively.

From this experiment, it was found that in day 6, algae growth in urea at 0.3 g/L had highest growth rate and followed by 0.6 g/L. In contrast, algae cannot growth in urea at 1.2 g/L because of urea concentration is too high and it is toxic to algae. As well as, algae cannot growth at 0 g/L. This may due to nitrogen is an essential nutrient for algae growth. The results of this test were as follows;

Table 4.1 pH and optical density (560 nm) in various concentration of Urea.

Day	Zarrouk's media		Concentrations of urea (g/L)							
			0		0.3		0.6		1.2	
	pH	OD	pH	OD	pH	OD	pH	OD	pH	OD
0	9.69	0.117	9.73	0.115	9.79	0.116	9.82	0.115	9.71	0.116
3	9.70	0.164	9.79	0.114	9.92	0.132	9.86	0.125	9.84	0.106
6	9.71	0.197	9.89	0.104	9.98	0.162	9.89	0.149	9.96	0.093
9	9.75	0.283	9.97	0.089	10.01	0.141	9.97	0.132	10.98	0.082
12	9.84	0.323	10.04	0.079	10.07	0.107	10.01	0.103	10.02	0.078

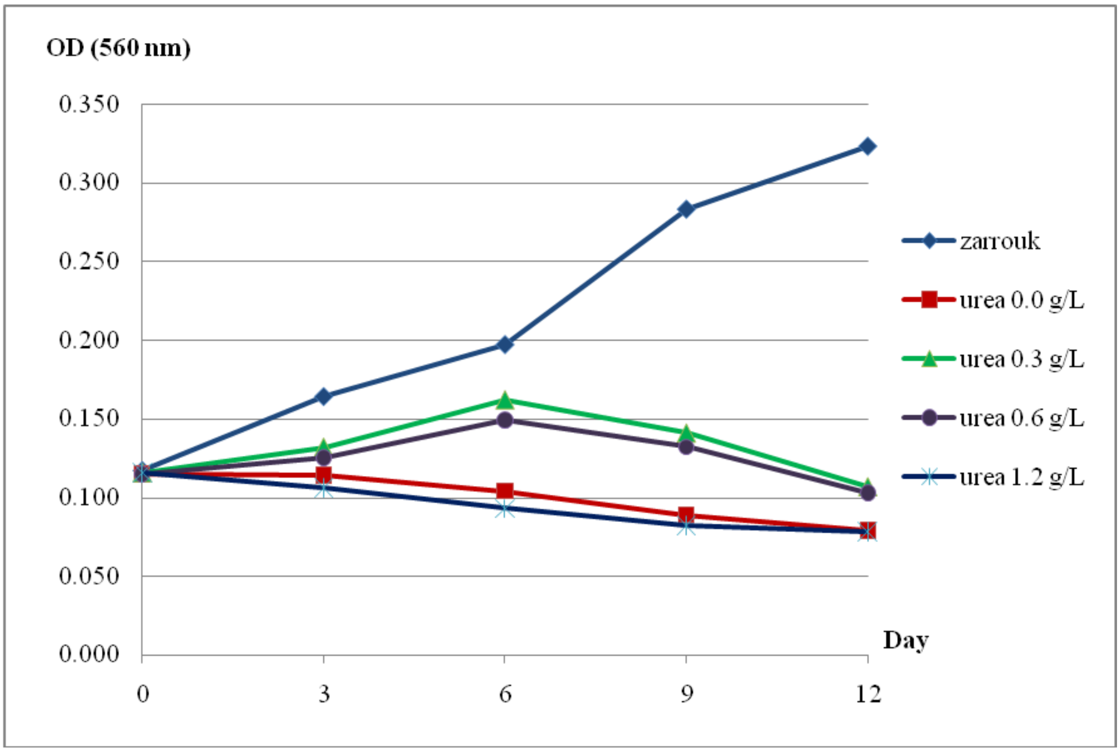

Figure 4.1 Relative between optical density (OD) and the growth of *Spirulina spp.*

From the table 4.1 and figure 4.1 show relative between optical density (OD) and cultivation time, it can be seen that algae can be growth well at 0.3 g/L and 0.6 g/L but concentration of algae is decreased significantly after day 6 when compare with standard media (Zarrouk's media). Researcher believes that it may due to the unexpected circumstance during the cultivation and perform another experiment with same concentration but the results were still the same.

In third experiment, researcher increased inoculum concentration from 1 to 1.5 gram of wet weight per liter, it was found that algae had greater growth but concentration was still decreased significantly after 6 day.

From this preliminary test, hypothesis that can be derived from these experiments was carbon source ($NaHCO_3$) and nitrogen source (urea) may not enough for algae growth. Therefore, researcher made a change in media components for further experiment in second preliminary test.

4.1.2 The second preliminary test for the growth of *Spirulina spp.* in low cost media

From the first preliminary test, researcher conducted experiment by using experiments from different studies as reference, such as the study of Tri-Panji et al. in year 2001 and Raoof et al. in year 2006 as well as as well as published papers on the cultivation of *Spirulina spp.* (Phetmani,2003). The result was shown in table 4.2 and figure 4.2, respectively.

Table 4.2 pH and optical density (560 nm) in different media.

Day	**Zarrouk**		**Phetmani's media (Formular 3)**		**Tri-Panji's media**		**Raoof's media**	
	pH	**OD**	**pH**	**OD**	**pH**	**OD**	**pH**	**OD**
0	9.81	0.187	9.76	0.188	9.84	0.186	9.89	0.186
3	9.81	0.351	9.77	0.242	9.87	0.233	9.85	0.325
6	9.94	0.578	9.93	0.287	9.99	0.271	9.90	0.512
9	9.88	0.960	9.97	0.418	9.83	0.371	10.00	0.781
12	9.86	1.080	9.89	0.486	9.94	0.403	9.95	0.879
15	9.88	1.082	9.91	0.561	9.93	0.483	9.92	0.914
18	9.92	1.079	9.97	0.568	9.88	0.465	9.91	0.909

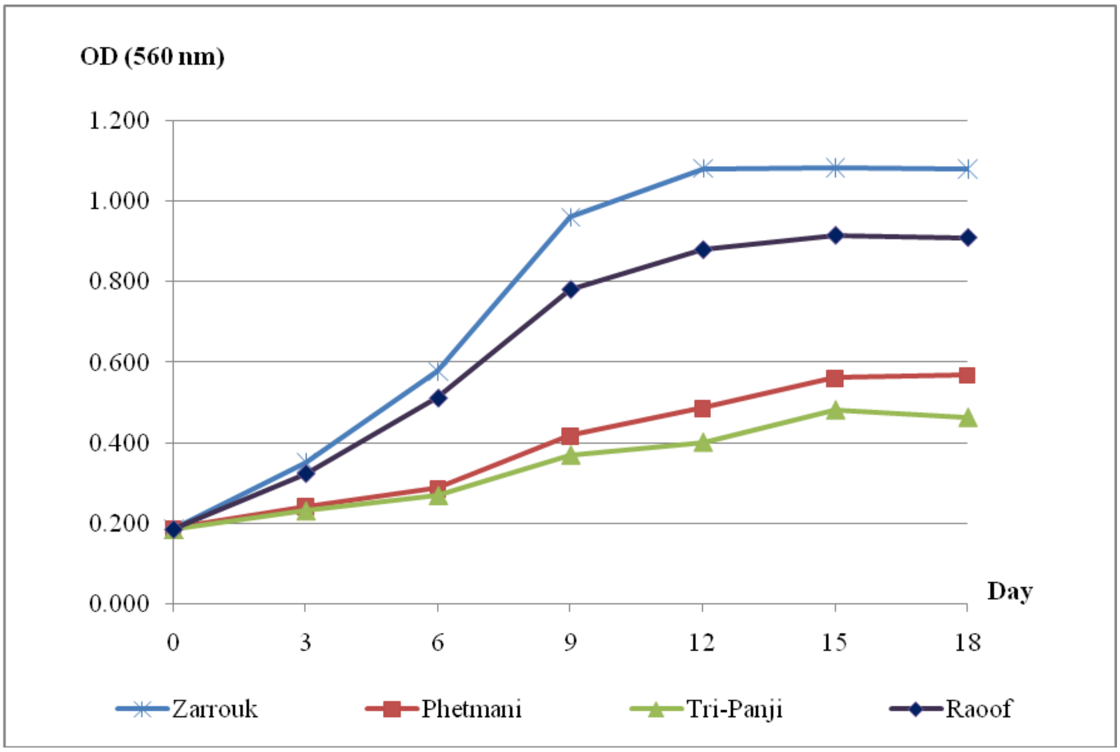

Figure 4.2 Relative between optical density (OD) and the growth of *Spirulina spp.*

It was found from the results for this experiment that algae can be growth well in media components from the study of Raoof, followed by media components (formula 3) from the cultivation of *Spirulina spp.* (Phetmani,2003) and the study of Tri-Panji, respectively. From these experiments, researcher decide to use media components according to study of Raoof et al. for experiments in the next step.

4.1.3 Optimal Condition of low cost media on growth of *Spirulina spp.*

This experiment had modified media component from the study of Raoof et al. It use low cost nutrient by using fertilizer instead of chemical for cultivation. Nitrogen source, Sodium nitrate ($NaNO_3$) was substituted with Urea ($Co(NH_2)_2$), (Treatment 3–Treatment 6) and Potassium nitrate (KNO_3), (Treatment 7–Treatment 10) in different concentration of nitrogen. Use single super phosphate (SSP) as phosphorus source, potassium chloride (MOP) as potassium source and sodium bicarbonate ($NaHCO_3$) with commercial grade as carbon source

Growth rate of algae was measured in terms of dried weight (g/L). This growth rate was compared with standard media, including Zarrouk’s media (Treatment

1) and Raoof's media (Treatment 2). This experiment was conducted to find suitable media components for *Spirulina spp.* growth. This experiment had been triplicate and each experiment had 18 days growth. The results were shown as followed;

4.1.3.1 The growth of *Spirulina spp.* in different media on 3rd day of cultivation

Spirulina spp. that was cultivated in Treatment 1 had highest growth rate ($\bar{x}$=0.190±0.0052 g/L), following by cultivated *Spirulina spp.* in Treatment 8 ($\bar{x}$=0.175±0.0056 g/L) and Treatment 2 ($\bar{x}$=0.174±0.0073 g/L). The growth rate in Treatment 8 and Treatment 2 had not significant statistic differences ($p>0.05$) but when compared with Treatment 1, it had significant statistic differences ($p <0.05$). The least growth rate was found in Treatment 6 ($\bar{x}$=0.086±0.0024 g/L). When comparing the differences between these experiments, it was found that each experiment had not significant statistic growth rate ($p >0.05$). The results from 3rd day of cultivation were shown in Table 4.3 and Figure 4.3

Table 4.3 The growth of *Spirulina spp.* in different media on 3rd day of cultivation

Dry weight (g/L) of *Spirulina spp.* in different media on 3rd day of cultivation											
Experiment		**Treatment**									
		I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**
1st	1	0.189	0.175	0.159	0.102	0.091	0.087	0.168	0.173	0.169	0.144
	2	0.182	0.180	0.162	0.097	0.089	0.082	0.169	0.175	0.163	0.149
	3	0.181	0.181	0.158	0.101	0.087	0.089	0.166	0.179	0.162	0.143
2nd	4	0.195	0.164	0.149	0.111	0.091	0.088	0.153	0.182	0.171	0.145
	5	0.196	0.167	0.156	0.107	0.089	0.086	0.159	0.179	0.174	0.141
	6	0.193	0.163	0.153	0.108	0.095	0.089	0.158	0.180	0.171	0.144
3rd	7	0.192	0.177	0.163	0.103	0.093	0.084	0.173	0.170	0.173	0.153
	8	0.189	0.179	0.161	0.099	0.093	0.085	0.174	0.168	0.170	0.155
	9	0.190	0.180	0.158	0.104	0.091	0.088	0.169	0.167	0.171	0.149
Average		0.190	0.174	0.158	0.104	0.091	0.086	0.165	0.175	0.169	0.147
SD		0.0052	0.0073	0.0045	0.0045	0.0024	0.0024	0.0072	0.0056	0.0042	0.0048

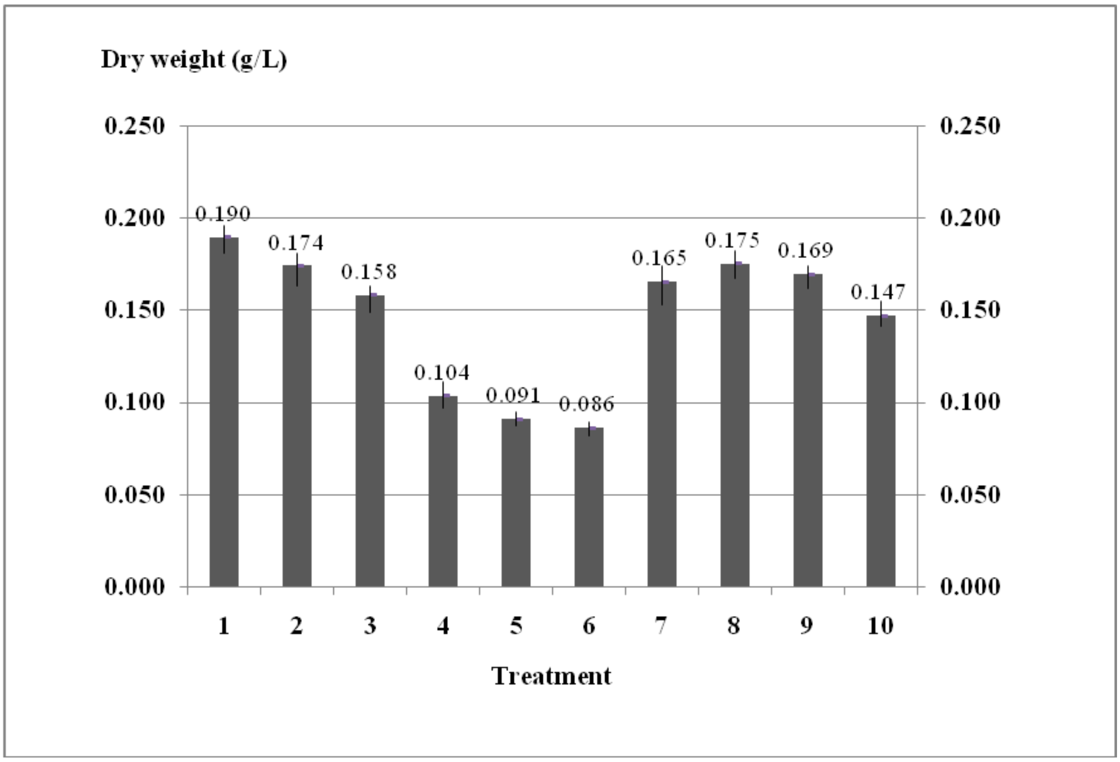

Figure 4.3 The growth of *Spirulina spp.* in different media on 3rd day of cultivation

4.1.3.2 The growth of *Spirulina spp.* in different media on 6th day of cultivation

Spirulina spp. that was cultivated in Treatment 8 had highest growth rate ($\bar{x}$=0.327±0.0116 g/L), following by cultivated *Spirulina spp.* in Treatment 2 ($\bar{x}$=0.325±0.0030 g/L) and Treatment 9 ($\bar{x}$=0.322±0.0054 g/L). The growth rate in Treatment 8 and Treatment 9 had not significant statistic differences with Treatment 2 ($p>0.05$) but when compare with Treatment 1, it had significant statistic differences ($p<0.05$). The least growth rate was found in Treatment 6 ($\bar{x}$=0.069±0.0094 g/L). When comparing the differences between these experiments, it was found that each experiment had not significant statistic growth rate ($p>0.05$). The results from 6th day of cultivation were shown in Table 4.4 and Figure 4.4

Table 4.4 The growth of *Spirulina spp.* in different media on 6th day of cultivation

Dry weight (g/L) of *Spirulina spp.* in different media on 6th day of cultivation											
Experiment		**Treatment**									
		I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**
1st	1	0.314	0.325	0.231	0.111	0.078	0.064	0.286	0.329	0.321	0.298
	2	0.315	0.327	0.233	0.114	0.086	0.071	0.284	0.334	0.325	0.301
	3	0.322	0.329	0.235	0.115	0.087	0.079	0.283	0.333	0.326	0.298
2nd	4	0.300	0.320	0.280	0.120	0.080	0.069	0.320	0.297	0.313	0.289
	5	0.301	0.326	0.291	0.135	0.086	0.050	0.319	0.331	0.318	0.290
	6	0.307	0.326	0.289	0.128	0.081	0.080	0.329	0.335	0.317	0.314
3rd	7	0.311	0.326	0.232	0.109	0.077	0.062	0.299	0.327	0.330	0.279
	8	0.323	0.321	0.221	0.101	0.074	0.068	0.291	0.327	0.326	0.287
	9	0.315	0.328	0.221	0.097	0.075	0.076	0.296	0.329	0.325	0.294
Average		0.312	0.325	0.248	0.114	0.080	0.069	0.301	0.327	0.322	0.294
SD		0.0082	0.0030	0.0295	0.0121	0.0049	0.0094	0.0174	0.0116	0.0054	0.0100

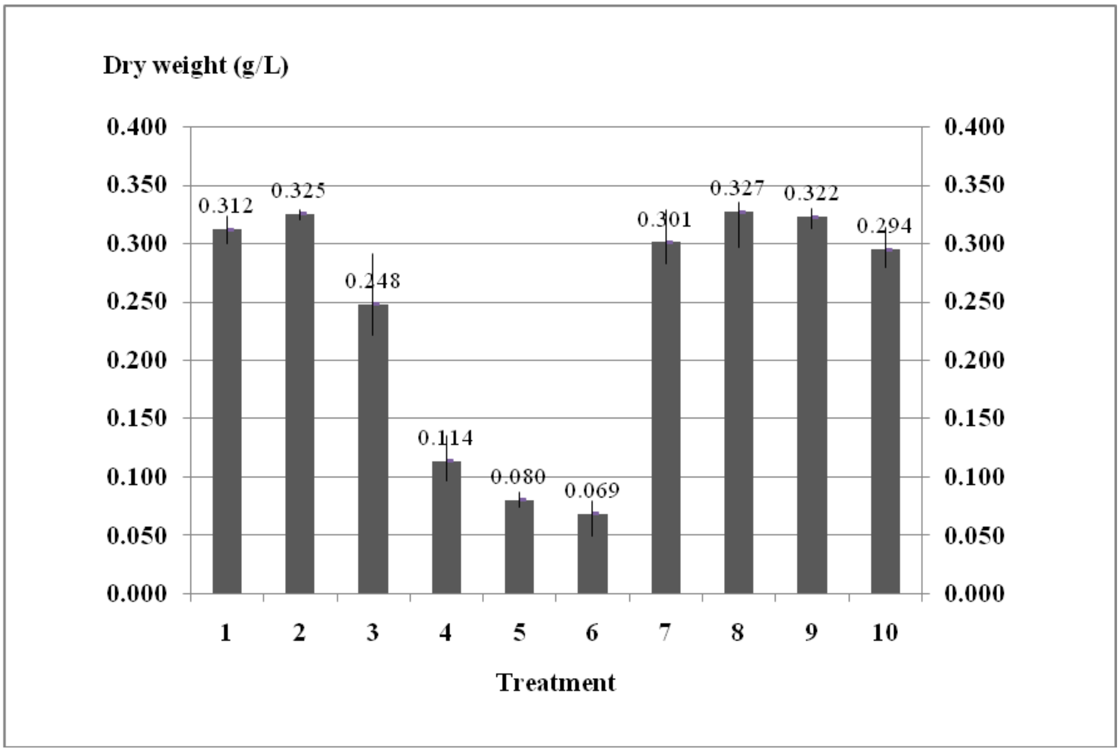

Figure 4.4 The growth of *Spirulina spp.* in different media on 6th day of cultivation

4.1.3.3 The growth of *Spirulina spp.* in different media on 9th day of cultivation

Spirulina spp. that was cultivated in Treatment 8 had highest growth rate ($\bar{x}$=0.534±0.0104 g/L), following by cultivated *Spirulina spp.* in Treatment 1($\bar{x}$=0.520±0.0119 g/L) and Treatment 9($\bar{x}$=0.485±0.0090 g/L). The growth rate in Treatment 8 had higher growth rate than Treatment 1 and Treatment 2 statistically ($p<0.05$). The least growth rate was found in Treatment 6 ($\bar{x}$=0.058±0.0051 g/L). When comparing the differences between these experiments, it was found that each experiment had not significant statistic growth rate ($p>0.05$). The results from 9th day of cultivation were shown in Table 4.5 and Figure 4.5

Table 4.5 The growth of *Spirulina spp.* in different media on 9th day of cultivation

Dry weight (g/L) of *Spirulina spp.* in different media on 9th day of cultivation											
Experiment		**Treatment**									
		I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**
1st	1	0.529	0.485	0.374	0.113	0.063	0.057	0.388	0.543	0.484	0.444
	2	0.534	0.487	0.379	0.104	0.072	0.055	0.383	0.548	0.489	0.449
	3	0.526	0.490	0.381	0.111	0.078	0.066	0.385	0.545	0.492	0.444
2nd	4	0.515	0.427	0.332	0.100	0.072	0.066	0.395	0.521	0.477	0.422
	5	0.517	0.439	0.351	0.103	0.070	0.060	0.394	0.526	0.465	0.423
	6	0.493	0.450	0.359	0.101	0.065	0.054	0.390	0.519	0.492	0.417
3rd	7	0.522	0.476	0.370	0.107	0.066	0.054	0.389	0.535	0.482	0.433
	8	0.527	0.473	0.374	0.115	0.056	0.053	0.392	0.532	0.492	0.444
	9	0.521	0.482	0.379	0.112	0.061	0.061	0.394	0.537	0.488	0.435
Average		0.520	0.468	0.367	0.107	0.067	0.058	0.390	0.534	0.485	0.435
SD		0.0119	0.0231	0.0163	0.0056	0.0067	0.0051	0.0042	0.0104	0.0090	0.0116

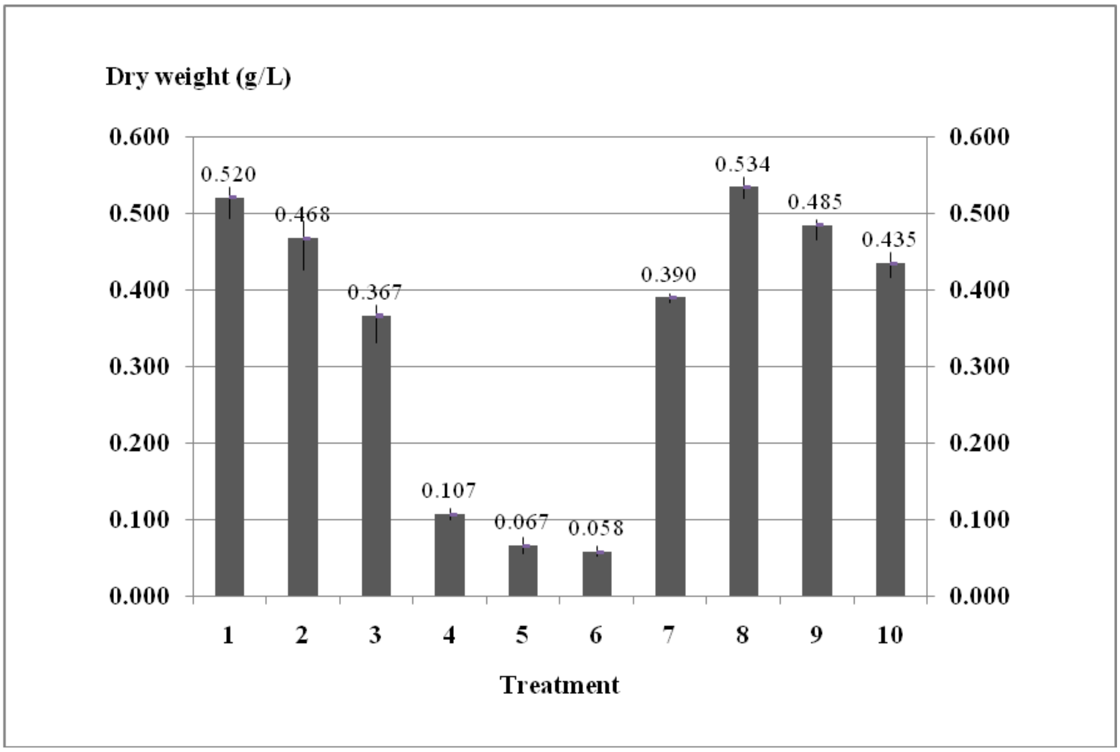

Figure 4.5 The growth of *Spirulina spp.* in different media on 9th day of cultivation

4.1.3.4 The growth of *Spirulina spp.* in different media on 12th day of cultivation

Spirulina spp. that was cultivated in Treatment 1 had highest growth rate ($\bar{x}$ =0.602±0.0044 g/L), following by cultivated *Spirulina spp.* in Treatment 8 ($\bar{x}$ =0.555±0.1050 g/L) and Treatment 9($\bar{x}$ =0.521±0.0436 g/L). The growth rate in Treatment 8 and Treatment 9 had less growth rate than Treatment 1 statistically ($p<0.05$). The least growth rate was found in Treatment 6 ($\bar{x}$ =0.045±0.0038 g/L). When comparing the differences between these experiments, it was found that each experiment had not significant statistic growth rate ($p>0.05$). The results from 12th day of cultivation were shown in Table 4.6 and Figure 4.6

Table 4.6 The growth of *Spirulina spp.* in different media on 12th day of cultivation

Dry weight (g/L) of *Spirulina spp.* in different media on 12th day of cultivation											
Experiment		**Treatment**									
		I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**
1st	1	0.609	0.481	0.489	0.085	0.054	0.048	0.448	0.685	0.572	0.543
	2	0.597	0.483	0.494	0.079	0.055	0.042	0.455	0.693	0.584	0.558
	3	0.599	0.481	0.486	0.083	0.050	0.047	0.460	0.689	0.580	0.553
2nd	4	0.606	0.501	0.452	0.081	0.048	0.046	0.489	0.442	0.497	0.466
	5	0.598	0.501	0.451	0.092	0.052	0.039	0.471	0.444	0.490	0.472
	6	0.607	0.512	0.447	0.088	0.052	0.041	0.474	0.478	0.497	0.470
3rd	7	0.602	0.511	0.474	0.084	0.059	0.050	0.443	0.526	0.491	0.460
	8	0.599	0.503	0.475	0.077	0.051	0.049	0.449	0.519	0.487	0.458
	9	0.605	0.510	0.445	0.081	0.055	0.045	0.445	0.522	0.490	0.461
Average		0.602	0.498	0.468	0.083	0.053	0.045	0.459	0.555	0.521	0.493
SD		0.0044	0.0130	0.0195	0.0046	0.0033	0.0038	0.0156	0.1050	0.0436	0.0438

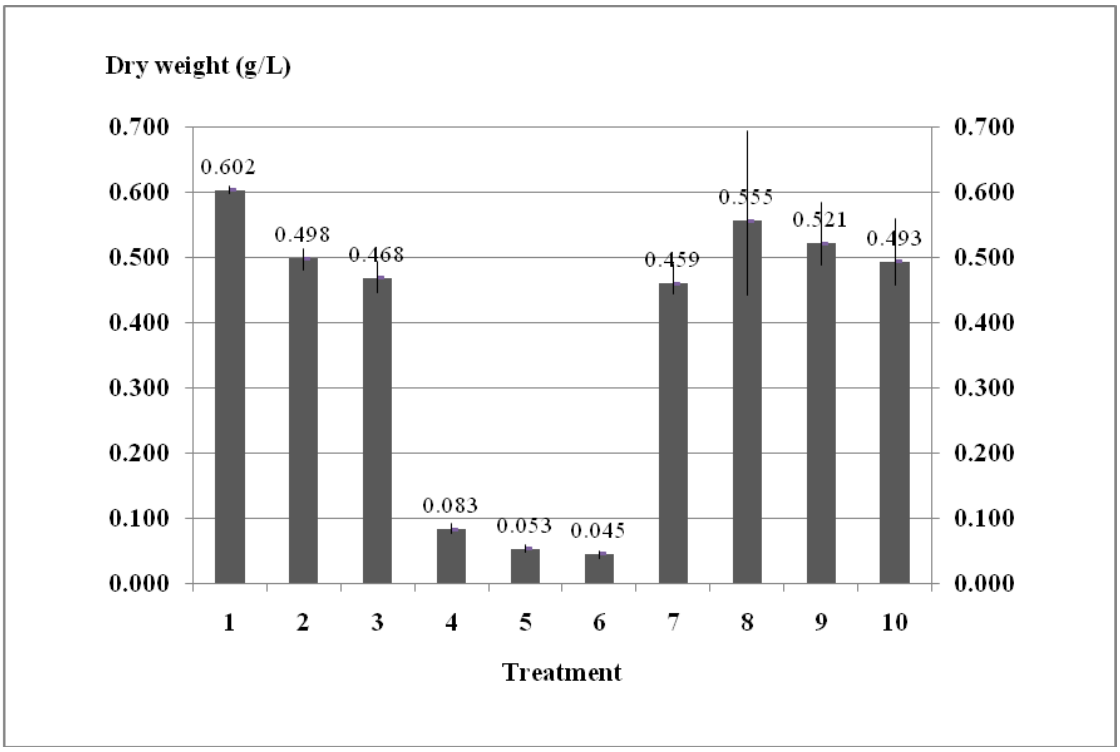

Figure 4.6 The growth of *Spirulina spp.* in different media on 12th day of cultivation

4.1.3.5 The growth of *Spirulina spp.* in different media on 15th day of cultivation

Spirulina spp. that was cultivated in Treatment 1 had highest growth rate ($\bar{x}$ =0.585±0.0049 g/L), following by cultivated *Spirulina spp.* in Treatment 8 ($\bar{x}$ =0.572±0.0034 g/L) and Treatment 2 ($\bar{x}$ =0.548±0.0024 g/L). The growth rate in Treatment 8 and Treatment 2 had less growth rate than Treatment 1 statistically ($p<0.05$). The least growth rate was found in Treatment 6 ($\bar{x}$ =0.039±0.0018 g/L). When comparing the differences between these experiments, it was found that each experiment had not significant statistic growth rate ($p>0.05$). The results from 12th day of cultivation were shown in Table 4.7 and Figure 4.7

Table 4.7 The growth of *Spirulina spp.* in different media on 15th day of cultivation

Dry weight (g/L) of *Spirulina spp.* in different media on 15th day of cultivation											
Experiment		**Treatment**									
		I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**
1st	1	0.587	0.548	0.458	0.067	0.042	0.041	0.451	0.573	0.504	0.474
	2	0.582	0.545	0.460	0.065	0.040	0.038	0.452	0.575	0.499	0.473
	3	0.586	0.549	0.461	0.064	0.043	0.037	0.449	0.577	0.501	0.477
2nd	4	0.579	0.551	0.454	0.070	0.039	0.038	0.446	0.569	0.503	0.469
	5	0.580	0.549	0.456	0.069	0.041	0.039	0.449	0.567	0.501	0.470
	6	0.581	0.552	0.453	0.065	0.041	0.042	0.452	0.571	0.500	0.474
3rd	7	0.590	0.547	0.459	0.068	0.037	0.041	0.451	0.568	0.504	0.467
	8	0.589	0.549	0.453	0.069	0.041	0.037	0.450	0.575	0.498	0.469
	9	0.593	0.545	0.454	0.071	0.043	0.039	0.447	0.572	0.500	0.470
Average		0.585	0.548	0.456	0.068	0.041	0.039	0.450	0.572	0.501	0.471
SD		0.0049	0.0024	0.0031	0.0025	0.0019	0.0018	0.0021	0.0034	0.0021	0.0032

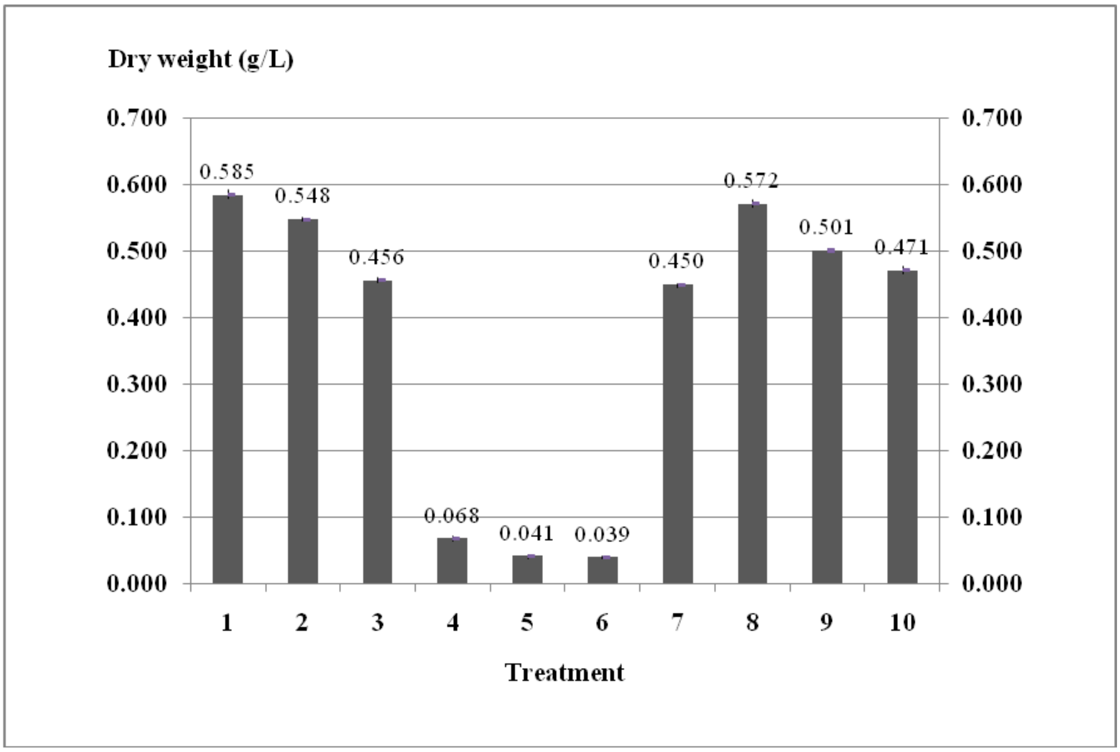

Figure 4.7 The growth of *Spirulina spp.* in different media on 15th day of cultivation

4.1.3.6 The growth of *Spirulina spp.* in different media on 18th day of cultivation

Spirulina spp. that was cultivated in Treatment 1 had highest growth rate ($\bar{x}$ =0.581±0.0014 g/L), following by cultivated *Spirulina spp.* in Treatment 2 ($\bar{x}$ =0.534±0.0016 g/L) and Treatment 8 ($\bar{x}$ =0.504±0.0564 g/L). The growth rate in Treatment 2 and Treatment 8 had less growth rate than Treatment 1 statistically ($p<0.05$). The least growth rate was found in Treatment 6 ($\bar{x}$ =0.036±0.0027 g/L). When comparing the differences between these experiments, it was found that each experiment had not significant statistic growth rate ($p>0.05$). The results from 18th day of cultivation were shown in Table 4.8 and Figure 4.8

Table 4.8 The growth of *Spirulina spp.* in different media on 18th day of cultivation

Dry weight (g/L) of *Spirulina spp.* in different media on 18th day of cultivation											
Experiment		**Treatment**									
		I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**
1st	1	0.582	0.535	0.449	0.057	0.038	0.037	0.457	0.562	0.483	0.469
	2	0.581	0.533	0.445	0.058	0.040	0.032	0.458	0.560	0.480	0.466
	3	0.579	0.536	0.447	0.060	0.039	0.033	0.460	0.459	0.484	0.467
2nd	4	0.583	0.537	0.451	0.056	0.037	0.040	0.459	0.457	0.484	0.467
	5	0.581	0.534	0.450	0.056	0.035	0.039	0.461	0.455	0.485	0.469
	6	0.581	0.535	0.448	0.059	0.036	0.037	0.463	0.452	0.487	0.471
3rd	7	0.580	0.533	0.447	0.058	0.033	0.036	0.460	0.567	0.478	0.472
	8	0.579	0.532	0.449	0.059	0.038	0.034	0.458	0.565	0.482	0.469
	9	0.582	0.534	0.450	0.060	0.035	0.037	0.457	0.460	0.479	0.468
Average		0.581	0.534	0.448	0.058	0.037	0.036	0.459	0.504	0.482	0.469
SD		0.0014	0.0016	0.0019	0.0015	0.0022	0.0027	0.0020	0.0564	0.0030	0.0019

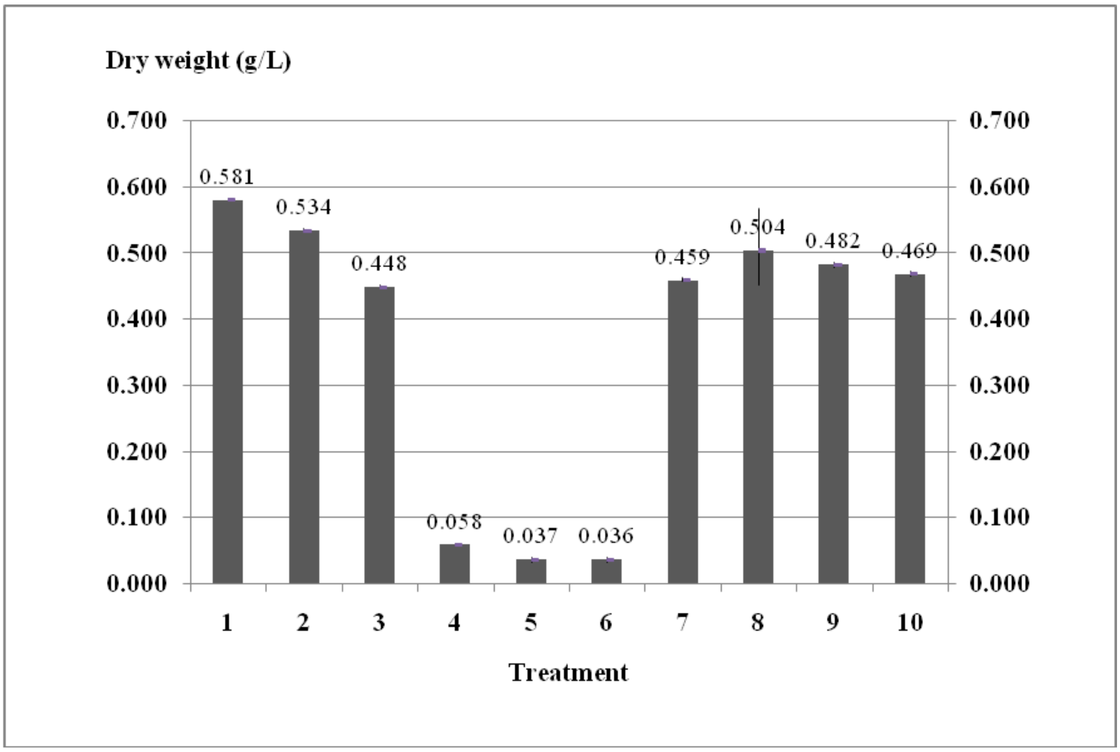

Figure 4.8 The growth of *Spirulina spp.* in different media on 18th day of cultivation

4.1.3.7 The growth of *Spirulina spp.* in different media with total 18 days of cultivation

Spirulina spp. can be growth well in partial formula which similar to standard media (Treatment 1) and reference media (Treatment 2). It was found from these experiments that types of fertilizers and difference nitrogen concentration influenced growth rate of *Spirulina spp.* statistically ($p < 0.05$). *Spirulina spp.* that was cultivated in media that contains with potassium nitrate as nitrogen source (Treatment 7-10) had higher growth rate than *Spirulina spp.* that growth in urea media as nitrogen source (Treatment 3-6). The results from these experiments were shown in Table 4.9, Figure 4.9 and Figure 4.10

Table 4.9 The growth of *Spirulina spp.* in different media with total 18 days of cultivation

					Dry Weight (g/L)						
Treatment		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Incubation (days)	0	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100
	3	0.190	0.174	0.158	0.104	0.091	0.086	0.165	0.175	0.169	0.147
	6	0.312	0.325	0.248	0.105	0.080	0.069	0.301	0.327	0.322	0.294
	9	0.520	0.468	0.367	0.107	0.067	0.058	0.390	0.534	0.485	0.435
	12	0.602	0.498	0.468	0.083	0.053	0.045	0.459	0.555	0.521	0.493
	15	0.585	0.548	0.456	0.068	0.041	0.039	0.450	0.572	0.501	0.471
	18	0.581	0.534	0.448	0.058	0.037	0.036	0.459	0.504	0.482	0.469

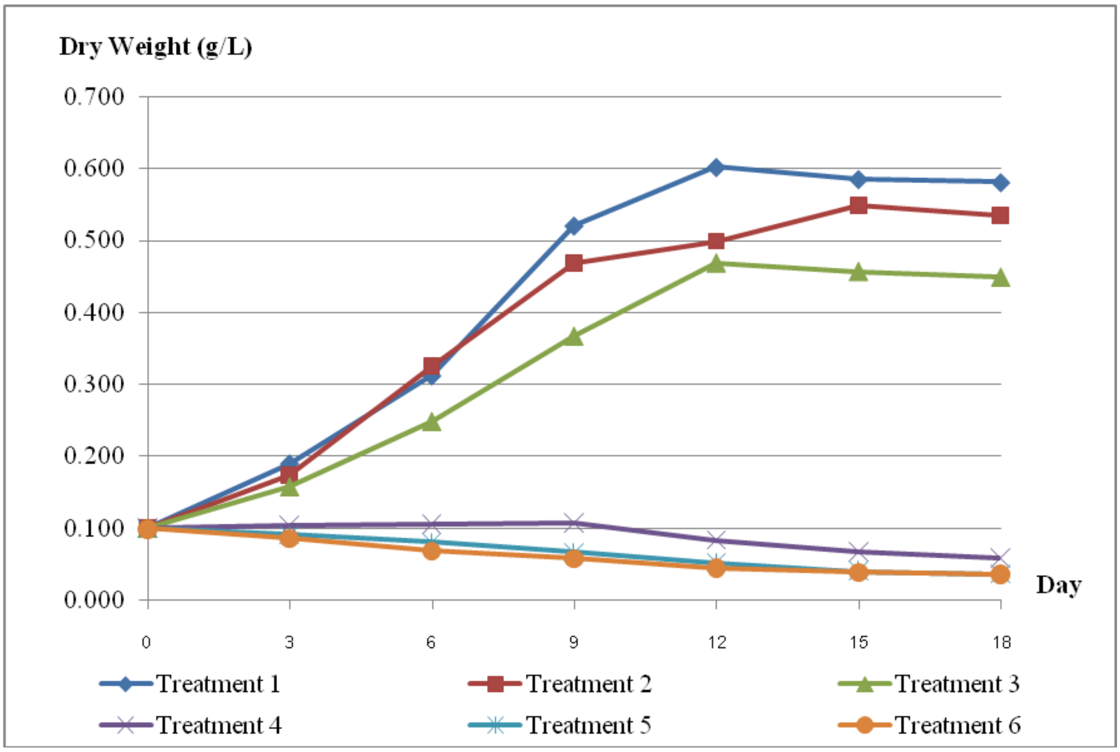

Figure 4.9 The growth of *Spirulina spp.* in urea as nitrogen source with total 18 days of cultivation

From Figure 4.9 which is media that contains with urea as nitrogen source, *Spirulina spp.* can be growth well in this media that had concentration of nitrogen at about 10% (Treatment 3) and reached the peak at day 12 then it will decrease. In addition, *Spirulina spp.* had least growth at concentration 20% (Treatment 4) and cannot growth at concentration 30% (Treatment 5) and 40% (Treatment 6). In this concentration, *Spirulina spp.* was decreased from day 3 of cultivation.

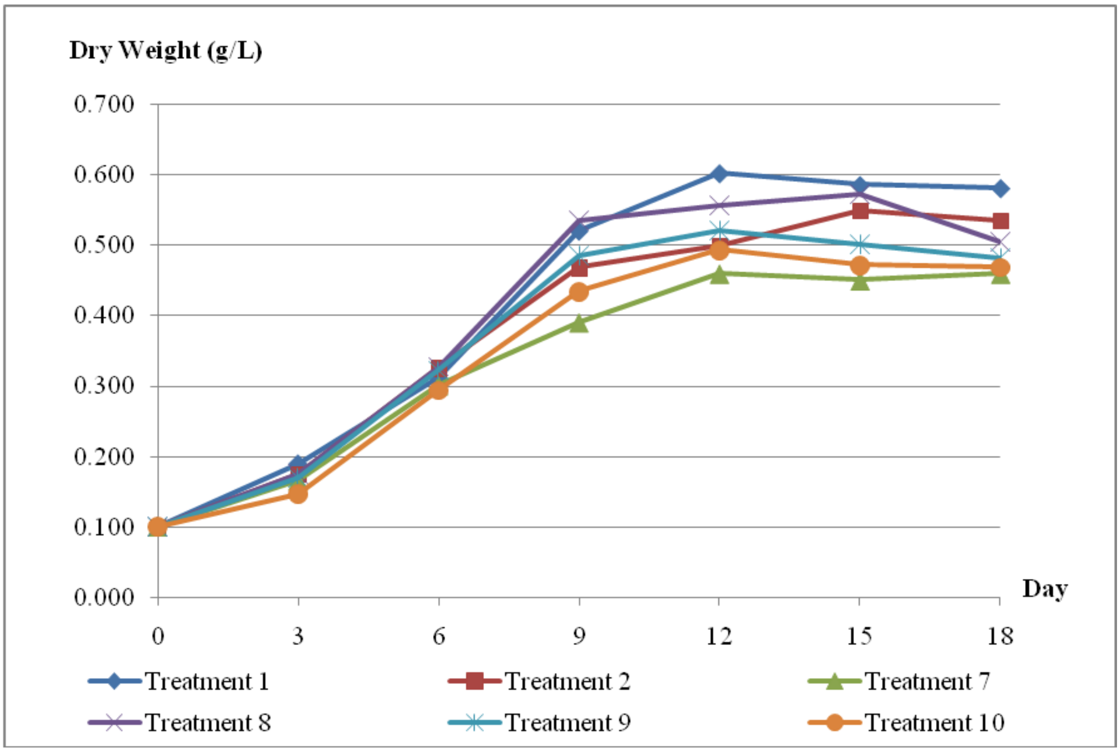

Figure 4.10 The growth of *Spirulina spp.* in potassium nitrate as nitrogen source with total 18 days of cultivation

From Figure 4.10 which was media that contains with potassium nitrate as nitrogen source, *Spirulina spp.* cans growth respectively in Treatment 8 that had nitrogen concentration 20%. It reached the peak at day 15 and then starting to decrease, followed by nitrogen concentration 30% (Treatment 9), nitrogen concentration 40% (Treatment 10) and nitrogen concentration 10% (Treatment 7), respectively.

4.1.3 Effect of low cost media on growth of *Spirulina spp.* in outdoor culture

Nutrient composition was key factor for *Spirulina spp.* production yield and growth rate (Vonshak and Richmond, 1988). It also plays a role in determining the biochemical composition of algae (Mostert and Grobbelaar, 1987).

The new media was easy to find and cheap by substituted chemicals in Zarrouk’s media with available fertilizers were way to reduce *Spirulina spp.* production cost. Among the necessary nutrients for algae growth, phosphorus was an essential element that plays a key role, allowing higher production rate of algae (Mostert and Grobbelaar, 1987). By the year 1994, Becker reported that the best concentration of required phosphate for the growth of microorganisms had a wide range (between 0.05 to 20 mg / L), and did not depend on the concentration of other nutrients. However, the concentration of phosphate in this research and concentration in Zarouk's media were above the concentration in Becker's study.

Commercial fertilizer, Single Super Phosphate (SSP), was selected as a source of phosphate (16% P_2O_5) and it also had the suitable amount for calcium (19-25%) and sulfur (8-12%) (Madkour, 2012). Other expensive chemical which was K_2SO_4 was replaced by commercial fertilizer, Potassium Chloride (MOP). MOP fertilizer had potassium equivalent to K_2SO_4 in Zarrouk's media. Raoof et al. (2006) was successful in cultured *Spirulina spp.* in Zarrouk's media that K_2HPO_4 was replaced by SSP and K_2SO_4 was replaced by MOP.

The changes in nitrogen sources and composition of the media affect growth rate, pigment and biochemical composition of algae (Mostert and Grobbelaar, 1987). Cyanobacteria use inorganic nitrogen for growth, particularly nitrate and ammonium. However, some of them had the ability to grow by using organic nitrogen (Fogg et al., 1973). As a result, the concentration of two different nitrogen sources was tested to check the *Spirulina spp.* production efficiency. Potassium nitrate and urea were chosen because they were cheaper than others (such as ammonium nitrate, sodium nitrate or ammonium chloride). Potassium nitrate and urea had nitrogen content 35% and 46%, respectively, while other nitrogen sources had only 14-16%.

When cultivated *Spirulina spp.* in Zarrouk's media and low cost media, the results revealed that the growth of *Spirulina spp.* was significantly difference at different nitrogen sources. The final biomass yield in Potassium nitrate was comparable with Zarrouk's media. The growth of *Spirulina spp.* that cultivated in Potassium nitrate as the nitrogen source, biomass yield were similar to biomass yield in Zarrouk's media. On the other hand, *Spirulina spp.* growth that cultivated in Urea as the nitrogen source, biomass yield were significantly lower ($p < 0.05$) than biomass yield of Zarrouk’s media or Potassium nitrate. Although urea had been recognized as a respectable source of nitrogen and succeeded in metabolism by the algae (Baldia et al., 1991). However, the results showed that *Spirulina spp.* can use Potassium nitrate more effectively compare with Urea. These results corresponded with the Richmond's study (1988) that nitrate and ammonium were primarily nitrogen sources for *Spirulina spp.*, Higher growth rate of *Spirulina spp.* in Potassium nitrate source may be due to a greater efficiency of ammonia accumulation in cells from the conversion of photo-synthetic that generated power and turned into net growth as mentioned by Rhee and Lederman (1983).

The growth of *Spirulina spp.* was enhanced by the increasing concentration of Potassium nitrate. It caused highest biomass in Treatment 8, which was comparable with the result in Zarrouk's media. However, further increasing of Potassium nitrate concentration, growth rate will be limited at certain point. On the other hand, the growth rate of *Spirulina spp.* in urea showed a significant decrease. The *Spirulina spp.* growth rate was associated with urea concentration. Highest biomass in urea was occurred when urea concentration was lowest (Treatment 3). This may be due to the inhibitory effect of urea on *Spirulina spp.* production at concentration more than 0.3 g/L as mentioned by Richmond (1990). This also corresponded with Filali et al. (1997), reported that between inorganic and organic nitrogen sources, nitrate was the more applicable because it can feed into the culture at high concentrations, while ammonium and urea were toxic at concentrations higher than 2 mM.

Due to these reasons, it may be concluded that in order to get the maximum biomass of *Spirulina spp.*, the concentration of potassium nitrate or urea should not exceed 8.83 or 2.94 mM of Nitrogen content, respectively. This new media can be used by local people for *Spirulina spp.* production because it was low cost, high efficiency and rich in protein similar to Zarrouk's media.

4.1.4 Effect of climates on growth of *Spirulina spp.* in outdoor culture

Climatic conditions can heavily affect in *Spirulina spp.* cultivation. In outdoor conditions, light intensity and temperature were varied throughout the year depends on seasons. Both temperature and light intensity value increases in the morning and it will reach the peak around 11.00 AM to 14.00 PM then it will decrease in the afternoon until light was gone. These variations in the environment affect growth rate, synthesis of biochemical within the cell as well as biomass yield, which also varied according to the climate in each day.

In the tropical area with short rainy season, long daytime and solar abundance, this was favorable natural conditions for the *Spirulina spp.* production. Normally, the production period was possibility around ten months for this area.

The growth will only occur in the light presence (Photosynthesis), but the lighting (illumination) throughout the day (24 hour) was not recommended. *Spirulina spp.* can be destroyed if it had been exposed under strong illumination for a long period. Therefore, it was necessary to reduce the time that they will be exposed to full sunlight. During the absence of light, the chemical reactions that occur within *Spirulina spp.* were protein synthesis and respiration.

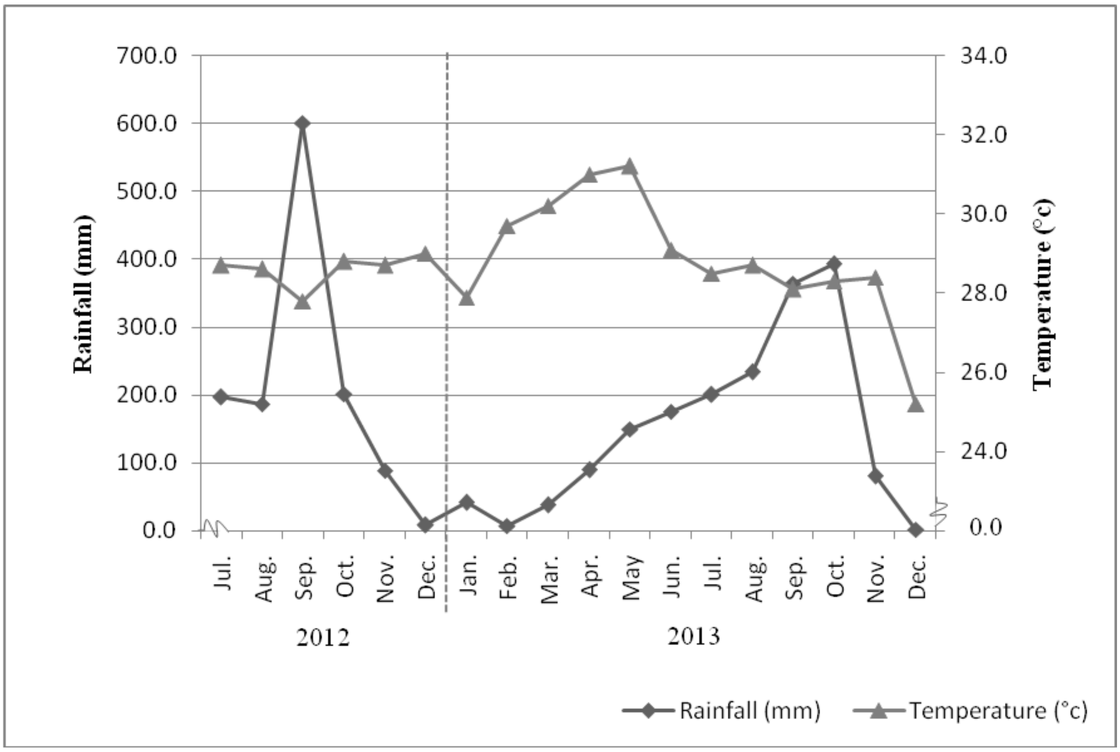

Figure 4.11 Average rainfall (mm) and Average Temperature (°C) since July 2012-December 2013 at station Bangkok Metropolis, Thailand

Source: Adapted from "Monthly Current Report" (Meteorological Department, 2014).

From figure 4.11, during summer (February-April), weather in Thailand was commonly hot and drought from low rainfall. The peak will be around April which the average temperature was 28-32 °C. From January to March was the end of winter and start of summer season which was affected by the high pressure from China. During these 3 months, average temperature was higher than normal value in every region. Particularly February, temperature will rise during daytime and this will be continued throughout the month due to inter tropical convergence zone. From April to June were summer and the beginning of raining season. Climate in this period was hot in general throughout April.

Raining season was from May to October, From July to October, Thailand was affected by Southeast monsoon, tropical cyclone and depression, as well as typhoon. This caused higher average rainfall than normal value around 23%. And winter season was from November to January. Due to the high pressure from China, temperature was started to decrease at the end of October to December.

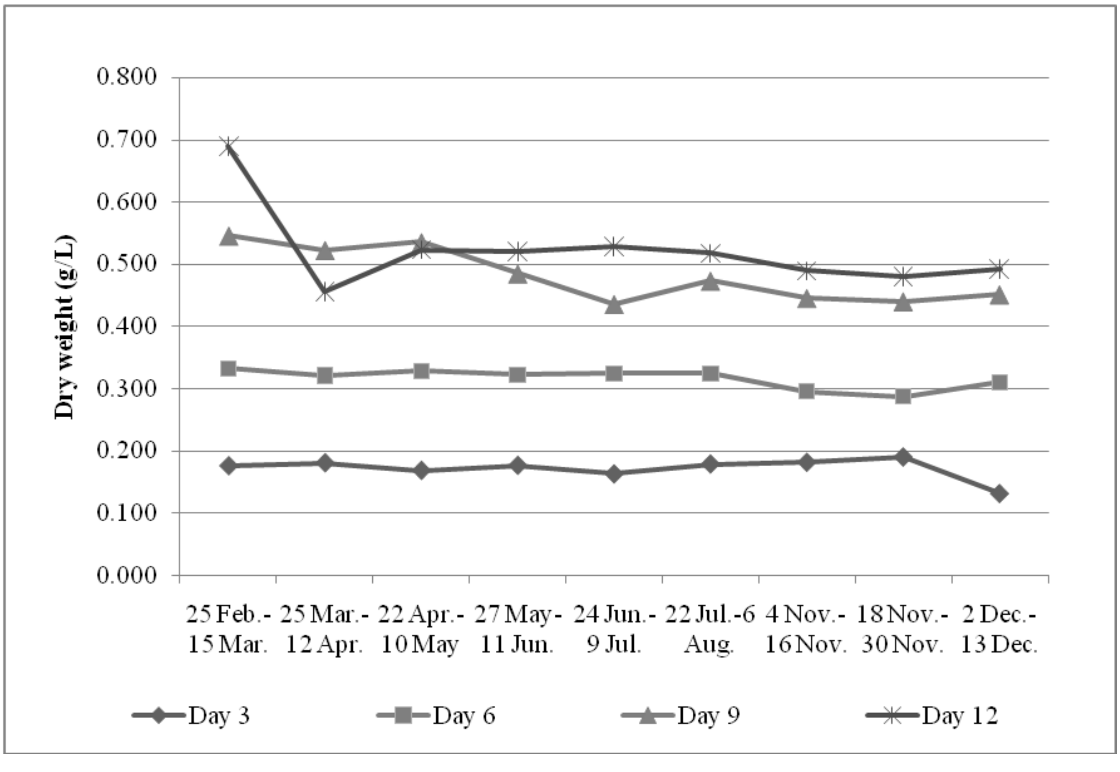

Figure 4.12 Growth of *Spirulina spp.* throughout the experiment

From figure 4.12, it can be seen that *Spirulina spp.* cultivation was heavily depends on the climate. Light intensity was important factors for growth rate. In general, light intensity during raining season which was cloudy weather was lower than summer season. As a result, production yield was low during this season. But this research was experiment in the balcony of building. *Spirulina spp.* did not receive light directly during summer, raining and winter season. Therefore, light intensity was not the main factor for *Spirulina spp.* cultivation.

Biomass yield of *Spirulina spp.* from batch method in day 3 and day 6 of cultivation was not difference in every treatment. In day 9 of cultivation, the 4th experiment (27 May-11 June 2013) and the 5th experiment (24 June-9 July 2013) which was in raining season, the amount of algae was significantly decreased. The reason of this result maybe at the beginning of cultivation algae received enough food. When food was depleted, light become important factor for algae growth. As a result, dried weight was significantly decreased in this period. In day 12, dried weight was slightly increased from day 9 because algae was became stationary phase. In 2nd experiment (25 March-12 April 2013), average dried weight was lower than the results of day 9 in the former experiments. This may be due to error during experiment.

Temperature was the most important climatic factors that influence the growth rate of *Spirulina spp.* The optimum temperature range was 30 °C. – 35 °C. The suitable area for *Spirulina spp.* growing was mostly in the tropical area between the Tropic of Cancer (23.5 degrees north) and the Tropic of Capricorn (23.5 degrees south). In lower temperate areas, the use of a greenhouse had proven to be successful method for *Spirulina spp.* growth by increasing temperature inside the greenhouse (Piccolo, 2012). Too high or too low temperature can affect the growth rate and production yield. It was found that at winter season, growth rate of *Spirulina spp.* was slightly lower than cultivation during summer and raining season.

Changing in global climate can cause extreme weathers more frequently and severe, such as heat wave, drought and flood. Even there was only small change; it can heavily affected global climate and cause extreme weather globally, such as increase of more severe heat wave or heavily rainfall. Climate prediction from atmosphere-ocean general circulation models (AGCM) found that many areas around center latitude and high latitude were more droughts during summer and heavy rainfall during winter. In the future, it was possible that droughts will be longer and rainfall will be heavier. Increased temperature and rainfall will surely affect open-pond cultivation. However, different area had different season which was depended on temperature, rainfall and distance from the sea. Area and cultivation method was important aspect to get high quality and controllable product.

4.2 Design a tubular photo-bioreactor for the community level

4.2.1 Effect of media flow rate in photo-bioreactor that influences *Spirulina spp.* growth rate

Study of Wichitchinda in year 1988, the most suitable recycle flow rate (N_R) was at around 5,000 which were full turbulent. Microorganisms had least growth rate at low recycle flow rate (N_R < 4,000) because flow rate in tube was not high enough. In contrast, at high recycle flow rate ($N_R \geq$ 6,000), it may increase microorganisms growth rate but it also damaged cell because of shear force from high velocity. From this study, it was found that suitable recycle flow rate caused high influence growth rate of microorganisms. Recycle media flow rate in photo-bioreactor had relation with types of flow in tube which was determined by Reynolds number (N_R). Flow in tube will be considered as turbulent flow when N_R was higher than 4,000. Higher liquid flows as turbulent; more algae were exposed to the light. This influences the growth rate of algae because algae need light as energy source and it also help the problem of algae accumulation. Reynolds number (N_R) can be finding follow;

$$N_R = \frac{vD}{V} \qquad (4.1)$$

When; v was kinematic viscosity of H_2O at 30°C which was at about 8.03 x 10^{-7} m^2/sec, Diameter of tubular photo-bioreactor for this study was 0.013 m. When determining the value of N_R was equal to 5,000, velocity will be 0.3088 m/sec. This velocity was used to calculate flow rate follow;

$$Q = AV \qquad (4.2)$$

When; A was tube inlet area which was 1.33×10^{-4} m^2. Therefore, flow rate will be at about 2.5 L/min.

This experiment was performed in tubular photo-bioreactor that was constructed for this study. Water pump was used to create flow in the tube and it was controlled to had flow rate at about 2.5 L/min (N_R=5,000) which was full turbulent. Different water pumps was tested to find the suitable pump that can created flow rate at around 2.5 L/min.

4.2.2 Study of nutrient content that used for feeding in semi-continuous cultivation system

From the experiments to find suitable low cost media for *Spirulina spp.* cultivation, it was found that *Spirulina spp.* in Treatment 8 had growth rate similar to standard media (Treatment 1) and reference media (Treatment 2). So media in Treatment 8 was used to test the efficiency of photo-bioreactor. The growth of *Spirulina spp.* was measured on a dry weight (g / L). Measure the growth of *Spirulina spp.* on a dry weight (g / L) and amount of nutrients (bicarbonate) in media every 3 days. The results from this experiment were shown in Table 4.10 and Figure 4.11

Table 4.10 The relation between growth of *Spirulina spp.* and bicarbonate content

Incubation (days)	**Dry weight (g/L)**		**Bicarbonate (g/L)**	
	Average	**SD**	**Average**	**SD**
0	0.100	0.0019	4.67	0.0612
3	0.172	0.0078	4.11	0.0808
6	0.324	0.0029	2.16	0.0635
9	0.464	0.0231	1.53	0.0870
12	0.521	0.0055	1.41	0.0750
15	0.545	0.0024	1.39	0.1054

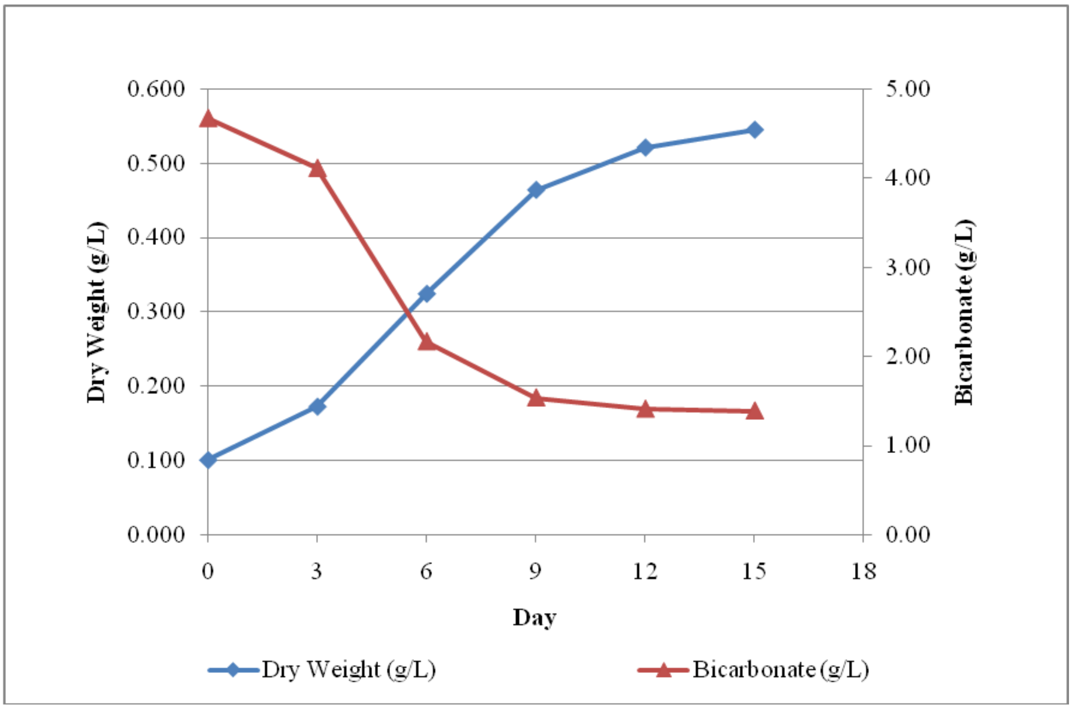

Figure 4.13 The relation between growth of *Spirulina spp.* and bicarbonate content

From Figure 4.13, the growth rate of *Spirulina spp.* was influenced by the amount of nutrients. In the beginning of log phase, nutrient was high and growth rate was also higher than latter of log phase or stationary phase which had low nutrients because it was used at the beginning. When nutrient deceased, growth rate also decreased as well. The consumption of nutrient was related to specific growth rate (μ).

To cultivate continuously, it was important to control the substrate concentration. This can be maintained by feeding nutrient at constant rate. As a result, balancing concentration was the most important aspect for this study because during experiments algae cell was removed only once. Therefore, this cultivation was fed-batch cultivation. When considering at substrate concentration, it can be shown as followed;

$$\frac{ds}{dt} = \frac{F}{V} s_i - \frac{F}{V} s - q_S x \qquad (4.3)$$

When;	x	was cell concentration in reactor (g/L)
	s	was substrate concentration in reactor (g/L)
	s_i	was substrate concentration in feeding nutrient (g/L)
	q_s	was specific substrate uptake rate
	F	was substrate feeding rate (L/hr)
	V	was tank volume (L)
	t	was time (hr)

To maintain constant substrate concentration during cultivation (ds/dt=0), substrate feeding rate was needed to be find from following;

$$F_0 = \frac{\mu}{s_i Y_{x/s}} \left(x V_0 \right) \qquad (4.4)$$

When; F_0 was initial substrate feeding rate (L/hr) and V_0 was volume of initial media (L). So feeding rate at any point of time can be finding from following;

$$F\left(t\right) = F_0 e^{\mu t} \qquad (4.5)$$

From Study of nutrient content that used for feeding in semi-continuous cultivation system (Table 4.10), $Y_{x/s}$ was at about 0.136 and μ was 0.113 (day^{-1}) when defining volume of initial media at 7 L.

To maintain continuously cultivation, feeding rate (F_0) and removing rate (F_i) must had same value ($F=F_0=F_i$) to maintain constant of nutrient in reactor (dV/dt=0). When the feeding rate was suitable, it was possible to maintain the algae growth rate in exponential growth. When the cells newly created balanced with the cells that removed from the reactor, it will cause a steady state. To control system in steady state by controlling dilution rate. The dilution rate can calculate as following;

$$D = \frac{F}{V} \qquad (4.6)$$

When; F was substrate feeding rate (L/hr)

V was reactor volume (L)

D was dilution rate (hr^{-1})

From D=F/V and steady state at constant cell concentration (dx/dt=0), it can be said $\mu x = Dx$ or $\mu = D$.

It is shown that at steady state, specific growth rate will be equal to dilution rate. When dilution rate was higher than specific growth rate, cell will be washed and removed from reactor. This circumstance was called wash out. As a result, to prevent wash out, it was necessary to find critical dilute rate (D_{crit}). At wash out state, cell was not in media and substrate was not used. Therefore, s was equal s_i and critical dilute rate can be calculated as followed;

$$D_{crit} = \frac{\mu_{\max} s_i}{K_s + s_i} \qquad (4.7)$$

When; K_s was constant rate which equal to media concentration that caused μ=1/2 μ_{max} (g/L). At normal circumstance, substrate feeding concentration will be higher than K_s (s_i>>K_s) and $D_{crit} \approx \mu_{\max}$. Therefore, to ensure the operation can be continued should avoid dilution rate close to the maximum specific growth rate. Feasibility of Substrate concentration, Substrate feeding rate and dilution rate in this experiment can be shown in table 4.11

Table 4.11 Feasibility of Substrate concentration, Substrate feeding rate and dilution rate in this experiment

Substrate concentration (g/L)	**Substrate feeding rate (L/hr)**	**Dilution rate (hr^{-1})**
0.2	0.1212	0.0173
0.4	0.0606	0.0087
0.6	0.0404	0.0058
0.8	0.0303	0.0043
1.0	0.0242	0.0035

From the table 4.10 can shown the relationship between the specific growth rate and the amount of nutrients in Figure 4.14

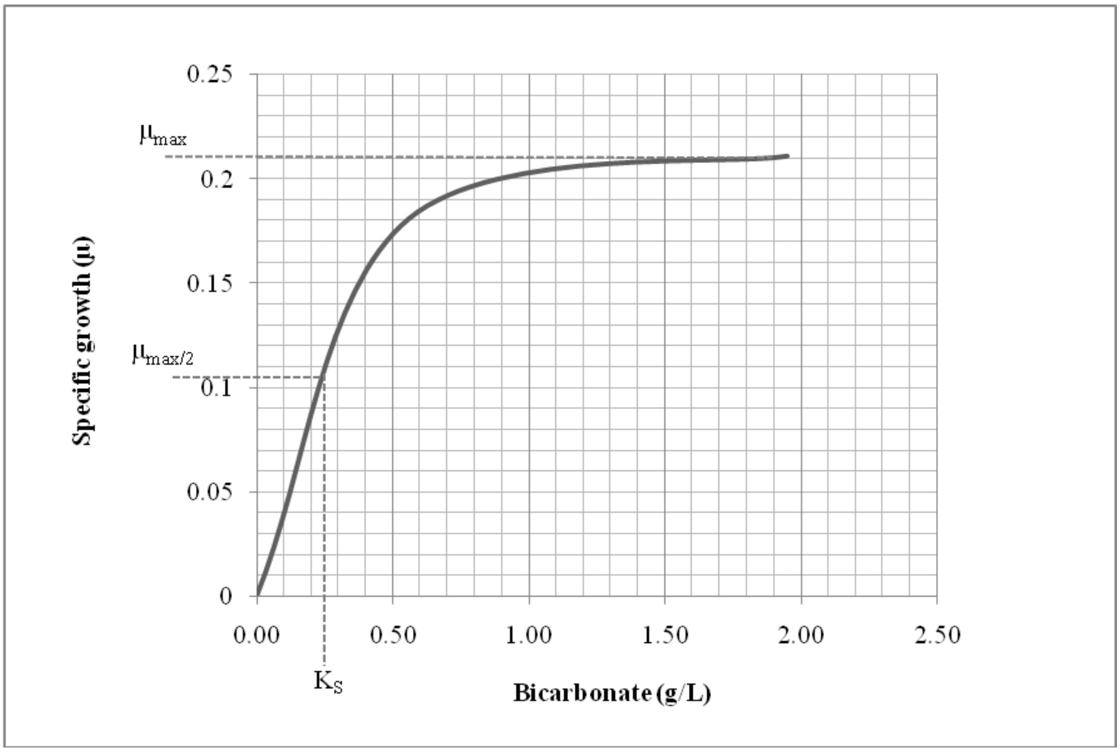

Figure 4.14 Relationships between the specific growth rate and bicarbonate content

From Figure 4.14 can be seen that the concentration of nutrients that cause specific growth rate value was half of maximum specific growth rate was equal to 0.25 g/L. Based on this data will be used to calculate the appropriate dilution rate.

So to prevent wash out the cell, researcher determined substrate concentration in feeding nutrient was 0.6 g/L, substrate feeding rate was 0.0404 L/hr and dilution rate was 0.0058 hr^{-1} for the next experiment. The cells can be harvested up to 1 L/day.

To constant substrate feeding rate can be done by drip system. The drip rate can calculate following;

$$\text{Drip Rate} = \frac{\text{I} \times \text{D}}{t} \qquad (4.8)$$

Where; I was the nutrient content per hour, in this case was 0.0404 L/hr or 40.4 mL/hr. D was the number of drops per milliliters, it was equal to 15 and t was the time (60 minutes). When substitute in the equation, the rate will be 10.1 drops/min, or about 10 drops per minute.

4.2.3 Design a tubular photo-bioreactor for the community level

Bioreactor was consists of clear plastic tank and clear tube with transparent 85% and diameter 0.013 m This tube was placed circle around the bioreactor and connected together with bending plastic (PVC). As a result, this tank was looked like a loop with total length 15 m and total volume 7 L. It had gas inlet and outlet. The cultivation for this study was semi-continuous. Every 24 hour, substrates which contains with sodium bicarbonate were feed into the reactor with velocity 0.0404 L/hr. The mixing system to mix food and microorganism was worked with flow rate 2.5 L/min. Sodium bicarbonate that feed into the system was a carbon source. It was essential for growth and it also help to control pH within the range of 9-10. This cultivation uses only light source from the sun. The temperature was varied on seasonal and time in each day.

Circulation system was an important factor to cultivate at high concentration and high productivity because it provides the complete mixing that give high light exposure and nutrient availability within the reactor. The flow rate was supported by Torzillo (1997). The cell stress was depended on the flow rate of cells. Higher flow rate can cause greater damage to cells. The suitable culture flow rate without causing damage to *Spirulina spp.* cells was estimated at 0.3 m/s. Cell concentration was reduced by 16% when it was increased to 0.8 m/s. In addition, Converti et al (2006) and Masojı´dek et al. (2003) found that the use of the photo-bioreactor configuration at flow rates about 0.21 m/s will suit to prevent excess thricome stress to cells of *Spirulina spp.* As a result, constant flow rate of 0.3088 m/s in this work proved to be suitable for the photo-bioreactor structure.

Algae growth rate in tubular reactor was depended in tube size, culture mixing rate and cell circulation system (Molina et al., 2001). There were three different cell circulation systems, namely airlift (AS), a motor driven pumping (MDPS), and a pressurized one (PS) (Ferreira et al., 2012). In general, airlift cell circulation system will be used because it can reduce cell damage from mechanical stress, especially for filamentous cyanobacteria. Due to its complex system, researcher use peristaltic pump for cell circulation in this research.

For carbon source, commercial grade sodium bicarbonate which was known as baking soda was used for this study. *Spirulina spp.* needs high bicarbonate not only for carbon source but also maintains alkalinity for their growth (pH value about 9.5). As the cells multiply, bicarbonate concentration will be reduced and pH will be increased. pH can be controlled by feed bicarbonate at suitable rate. (Binaghi et al., 2003; De Morais and Costa, 2007; Soletto et al., 2008). From the Venkataraman's experiment (1985), it was found that reducing concentration of bicarbonate below 4 g/L can reduce biomass and chlorophyll A significantly. Therefore, it was important to control the concentration of bicarbonate in the substrate at suitable rate.

Light was an important factor for *Spirulina spp.* growth. Cultivation of *Spirulina spp.* in tubular photo-bioreactor can provide completely light exposure more than open pond method because of tubular photo-bioreactor characteristic. Light can be exposed at every direction and cell was circulated thoroughly the reactor to receive continuously light. On the other hand, open pond cultivation can receive light only from the top and side of jar or other cleared containers (if algae concentration was high, inside algae will receive lack of light). Only algae at these areas can receive light. If cultivated in cement pond, algae can receive light only from the top of pond. The result was consistent with the fact that *Spirulina spp.* as photosynthetic microorganism can absorb light efficiently and photosynthesis to convert light energy to a chemical form of ATP for cell growth (Mohanty et al., 1997).

4.3 Preliminary performance tests on tubular photo-bioreactor

To estimate efficiency of constructed photo-bioreactor for *Spirulina spp.* cultivation, the growth rate from this photo-bioreactor was compared with the open pond system (glass jar). Cultivation in tubular photo-bioreactor was conducted in semi-continuous system at media flow rate about 2.5 L/min. Substrate feeding rate was defined at 0.0404 L/min. Substrate concentration for feeding was 0.6 g/L which using drip system at 10 drops per minute. At steady state, it was equal to the dilution rate at 0.0058 hr^{-1}. The results from this experiment were measured as following;

Spirulina spp. that was cultivated in tubular photo-bioreactor had higher growth more than *Spirulina spp.* that was cultivated in glass jar. The dry weight and chlorophyll a concentration of *Spirulina spp.* in tubular photo-bioreactor highest was 0.563 g/L and 21.05×10^{-3} mg/mL, respectively. When comparing the differences between these experiments, it was found it had significant statistic growth rate ($p<0.05$). The results with total 15 days cultivation were shown in Table 4.12 and Figure 4.15

Table 4.12 Dry weight and chlorophyll a concentration of *Spirulina spp.* with total 12 days of cultivation

Incubation (days)	**Batch Process**		**Semi-Continuous Process**	
	Dry weight (g/L)	**Chlorophyll a ($\times 10^{-3}$ µg/mL)**	**Dry weight (g/L)**	**Chlorophyll a ($\times 10^{-3}$ µg/mL)**
0	0.100	5.6868	0.100	5.6868
3	0.167	9.0311	0.205	11.6819
6	0.298	11.7828	0.401	17.1732
9	0.445	15.1694	0.522	19.2476
12	0.486	17.6248	0.563	21.0397

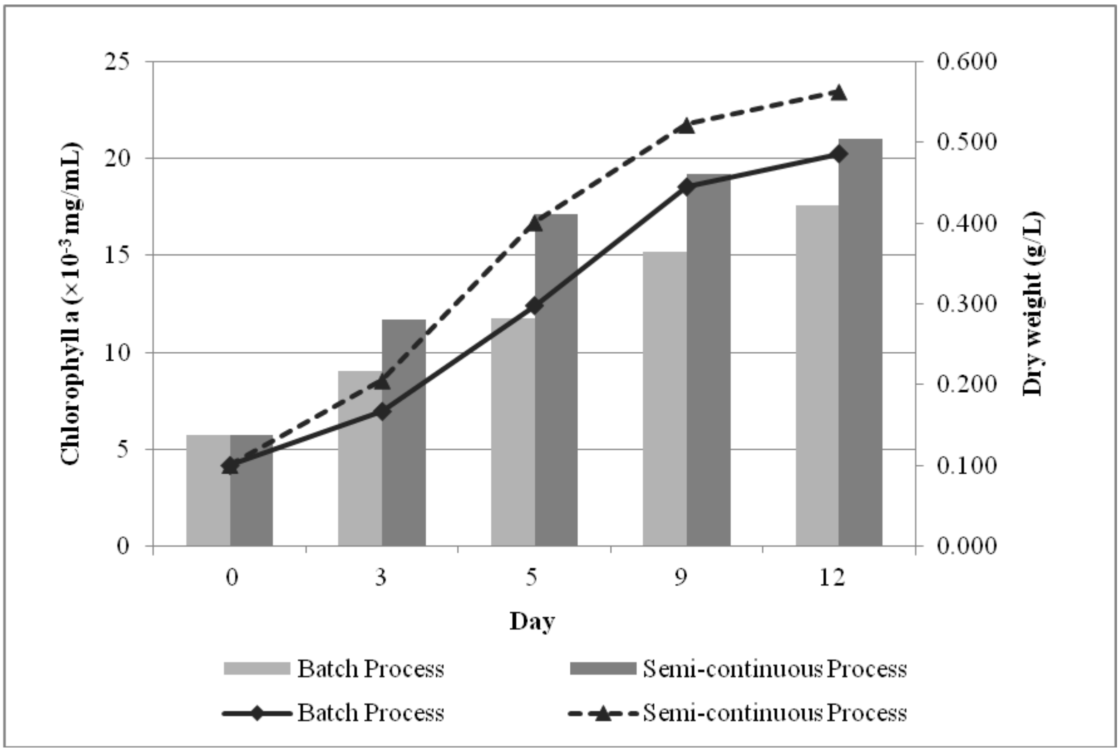

Figure 4.15 Dry weight and chlorophyll a concentration of *Spirulina spp.* with total 12 days cultivation

From the results in Table 4.12 and Figure 4.15, it was found that *Spirulina spp.* cultivation in photo-bioreactor had cell production at (X_{max}) 0.563 g/L which was higher than batch cultivation because the substrate in tubular photo-bioreactor had full turbulent flow which causes substrate and algae completely mixed. It received high light exposure rate and low accumulation rate. As a result, *Spirulina spp.* in this system had high growth rate than batch system.

Spirulina spp. production can be range from large-scale to small-scale. They were currently being implemented in Burkina Faso, Cameroon, Central African Republic, Democratic Republic of Congo, Guinea Conakry, Kenya, Madagascar, Mali, Niger, India, Vietnam and Thailand (Jourdan, 2011). The large-scale production uses a large greenhouse and a large basin (Raceway ponds). Media (water and fertilizer) will be flowed continuously with pumps or paddle wheels.

For small production, production process was relatively simple and straightforward. It requires tank or ponds. The size of these depends on the scale of production and the number of tanks. Any open tank or pond can be used in growth of *Spirulina spp.* but they had corrosion resistant and non-toxic. The shape was not important, though sharp corners should be avoid for easy mixing and cleaning. It may be as small as one square meter, but 5, 20, or 100 m^2, will save more cost. Sizes were limited for access to the agitation and cleaning. The bottom had a slight slope and breaks to facilitate sediment.

Materials for simple tank consist of about 30 cm high walls packed with earth, bricks or planks. Materials selection was depended on weather conditions and the presence of rodents or termites, etc. Cover the sides and bottom with a plastic, metal frame or bamboo can enhance the wall strength. However, tanks usually need to be replaced or repaired after 3-4 years. Other equipment also had to be replaced.

One example of successful initiative business of *Spirulina spp.* production can be found in Madurai, India. The cost of building a tank of 18 m^2 was 166 Euro or 7,387 Bath. These tanks made of wood and covered with UV-resistant plastic at the height 40 cm. Even though, tank must be stirred by manually, harvesting and fertilizing once a day, but it can produce *Spirulina spp.* approximately 150 g/day. (NOTE: 1 EUR=44.50 THB, Exchange rate in February 2014)

All the *Spirulina spp.* in western Kenya was produced in open ponds made from cement or basins lined with thick polyethylene plastic (Piccolo, 2012). These ponds had a width at about 3-4 m and length up to 100 m. Size of these ponds will depend on the quantity of products that they need. Cement ponds was more cost effective in the short term; however it had proven to be more expensive for long term due to the decay of plastic material. (Figure 4.16)

Figure 4.16 *Spirulina spp.* Cultivated in western Kenya; (A) A polyethylene cover over the pond and (B) Cement ponds in outdoor cultivation.

Source: Adapted from *Spirulina* – A Livehood and a Business Venture (Piccolo, 2012).

In Sri Somdet, Roi Ed province, Thailand, *Spirulina spp.* cultivation use simple method. Open pond was covered with plastic sheet. Each pond had size 4 m width x 20 m length x 50 cm depth with total 30 ponds. Machine or padding wheel was used for mixing and used only during daytime in order to completely mix with food and expose to sunlight. *Spirulina spp.* can be harvest around day 10 of cultivation depends on light factor. They will be harvest only ¾ part of pond. The rest part will be remained for inoculum. Water in pond was drained, filtered by cheesecloth and washed with freshwater. This process was used only 2 day. It will be dried at temperature around 40-45 °C. The final process was crushing and packing into 100 kg package for transport to Bangkok, Lampang and Chiang Mai. The cultivation time was around 10 days and one pond can make a profit up to 2,000 Baht

In Mae Sai, Chiang Rai province, Thailand, *Spirulina spp.* cultivation was consists of 22 ponds with 5 m width and 50 m length. Area for this cultivation was around 3-4 rai. The fresh production yield was around 400-500 kg/day. It can be sold at rate 40 baht per kg. After drying process, dried weight will be at about 50 kg. It can be sold at 700 Baht/kg.

From the above examples, it can be seen that *Spirulina spp.* farms is an open pond. In the rainy season, production may drops due to frequent rains and lack of sunlight. On a rainy day the yields are usually very low or the production may as well be stopped (Heierli, 2007). In fact, rain can help reduce water evaporation, however if too much rain may cause damage to cultivation ponds. For example, due to flooding *Spirulina spp.* farms in Cambodia was affected by flooding during the rainy season in 2001. They must increase the base of each tank for *Spirulina spp.* production at least 50 cm. The cost of each tank was US$850 in 12 tanks. The approximate cost for this equipment was another US$10,200 or 331,500 Bath (So-nutritious, 2014). So rain not only affects the whole cultivation process, but also cost for building a new pond. (NOTE: 1 USD=32.50 THB, Exchange rate in February 2014)

Wind was useful for aerating and agitating culture. Meanwhile, due to strong winds and storms often occur, sand and dust may be contaminated in open ponds which reduces the quality of the product. To prevent such problems, building greenhouses to cover raceway ponds and small pond was needed for reserve *Spirulina*'s inoculums when the main ponds was died or contaminated.

These factors influence the algae growth rate. In addition, it also cannot prevent contamination from other microorganisms from the air. It was difficult to control pure cultivation. Therefore, continuous cultivation in open pond did not receive much attention because it cannot control the system into steady state. Photo-bioreactor can reduce contamination from microorganisms and bugs in the air and it also can reduce evaporation effect as well.

Batch cultivation in open-pond reactor was common method because it was not complex system, easy to operate and high performance in open area. However, there also many restrictions, including cannot control evaporation effect that influence the substrate concentration, the difference in temperature and sunlight in each day and seasonal effect. So this photo-bioreactor had a several advantages for cultivated *Spirulina spp.* as followed;

- Able to cultivate although there was no land or environment was not suitable for cultivation.
- Can save time, labor and expense in preparing the soil and weeding for cultivation pond.
- Can eliminate contamination problems caused by soil and cultivated continuously throughout the year.
- System containing water use and nutrients as efficiently as possible.
- Able to controlled environments affect the growth of algae. Especially, nutrient control, pH value, temperature, etc. which generally open pond cultivation can be difficult.