The Osaka Gas Foundation of International Cultural Exchange (OGFICE)
Research Grant FY 2013/2014

Final Report

Calcium Uptake of Filamentous Microalgae in Different Levels of Light Intensity

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Submitted to:
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Bogor, October 2014

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Executive Summary

Nowadays, supply of high quality live feed in hatchery and nursery is a necessary need. In molting phase Crustacean needs more calcium in their live feed to reach high survival rate. Enrichment in live feed is the rapid way to absorb mineral for aquatic organism. The possibility of live feed enriched with those mineral is microalgae. Filamentous microalgae could grow in a condition when calcium concentration is in maximum level. This phenomenon is match with the need of enrich live feed for crustacean in molting phase. The objective of this project is to study calcium uptake by filamentous microalgae in different level of light intensity in laboratory scale.

Filamentous microalgae are selected because the presence in waters usually becomes blooming and useless, short period of growth phase, and fast to harvest. Supply of high calcium microalgae as live feed for commercial aquatic biota could be achieved, especially in hatchery and nursery activity. The selected filamentous algae is *Spirogyra* sp. Cultivation of selected genus filamentous microalgae is succeeded under laboratory condition.

This study consist of three experiments in laboratory, under controlled environment. The first experiment is treatment of selected filamentous microalgae (*Spirogyra* sp.) cultivation in various kind of calcium ($CaCl_2$, $CaSO_4$, and $CaCO_3$). The second experiment is treatment of *Spirogyra* sp. cultivation with calcium ($CaCl_2$) in various kind of concentration and different levels of light intensity, and the last eperiment is treatment of *Spirogyra* sp. cultivation in absorbing calcium ($CaCl_2$) with different irradiation time.

The results of present study showed that $CaCl_2$ is the most effective type of calcium that absorbed by *Spirogyra* sp. The best $CaCl_2$ concentration added in *Spirogyra* cultivation was 0.5 mL/L medium at the optimum light intensity (500-750 ft cd). The concentration of Ca *Spirogyra sp.* increased 23.4% and 21,8% in treatment 12 hours/day and 24 hours/day, respectively, but final weights in treatment 24 hours/day greater than 12 hours/day. Suspected that Ca is an element that is not needed in large amounts by *Spirogyra* sp. in their growth process.

Spirogyra sp. which is contain high calcium could apply as live feed in hatchery and nursery, especially for molting phase and increase of survival rate of aquatic biota. Value added for filamentous microalgae, utilization of filamentous microalgae as valuable product not as a weed. Utilization of filamentous microalgae as natural resources is a chain of renewable energy exploration.

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I. INTRODUCTION

I.1. Background

Supply of live feed in hatchery and nursery is a necessary need. Live feed which means microalgae is a basic feed in crustacean molting phase. Molting phase is a crucial period for crustacean. In that phase, Crustacean needs more calcium in their feed to reach high survival rate. Enrichment in live feed is the rapid way to absorb mineral for aquatic organism. The possibility of live feed enriched with those mineral is microalgae.

In other side, there are microalgae that could grow well with excess calcium condition. In natural waters, filamentous microalgae could grow in a condition when calcium concentration is in maximum level. This phenomenon is a key to answer the need of enrich live feed for crustacean in molting phase. Especially, filamentous - microalgae is useless aquatic microorganism, just a few of genus that already useful. Utilization of filamentous microalgae as natural resources is a chain of renewable energy exploration.

I.2. Objectives

This research aim to cultivate of filamentous microalgae with different level light intensity in pure culture, which is related with calcium uptake in order to supply high calcium microalgae as live feed for commercial aquatic biota.

II. LITERATURE REVIEW

II.1. Filamentous Algae

Filamentous algae are a group of green algae (chlorophyta) with special features thallus such as filament-shaped, branched and unbranched. Filamentous algae commonly found in fresh waters in large numbers. These organisms typically attached, but may detach to become planktonic usually live floated on the surface of the water in lentic ecosystem, or in the lotic ecosystem with relatively small flow. Bellinger and Sigee (2010) explain that in smaller ponds, filamentous *Spirogyra* sp. and colonial *Hydrodictyon* frequently form surface blooms or scums

Reproduction of filamentous algae generally by vegetative (fragmentation of filament) and sexsual reproduction by conjugation (Kumar and Singh 1979). Sexual and asexual reproduction such as occurs in *Spirogyra* sp. (Bellinger and Sigee 2010). Filamentous algae capable of photosynthesis through the utilization of existing nutrients and act as primary producers in the waters. Filamentous algae generally be natural feed for fish.

Spirogyra sp. is one of filamentous algae, that live in the freshwater habitats. According to Hujaya (2008), *Spirogyra* sp. have a size cell between 10-1000 micrometers, which are connected to each other from end to end without branching so that it appears as a filament. Cell wall consists of two layers, the outer layer is formed of cellulose while the inner wall is formed from pectin. The size of these filaments can reach several centimeters in length (Figure 1).

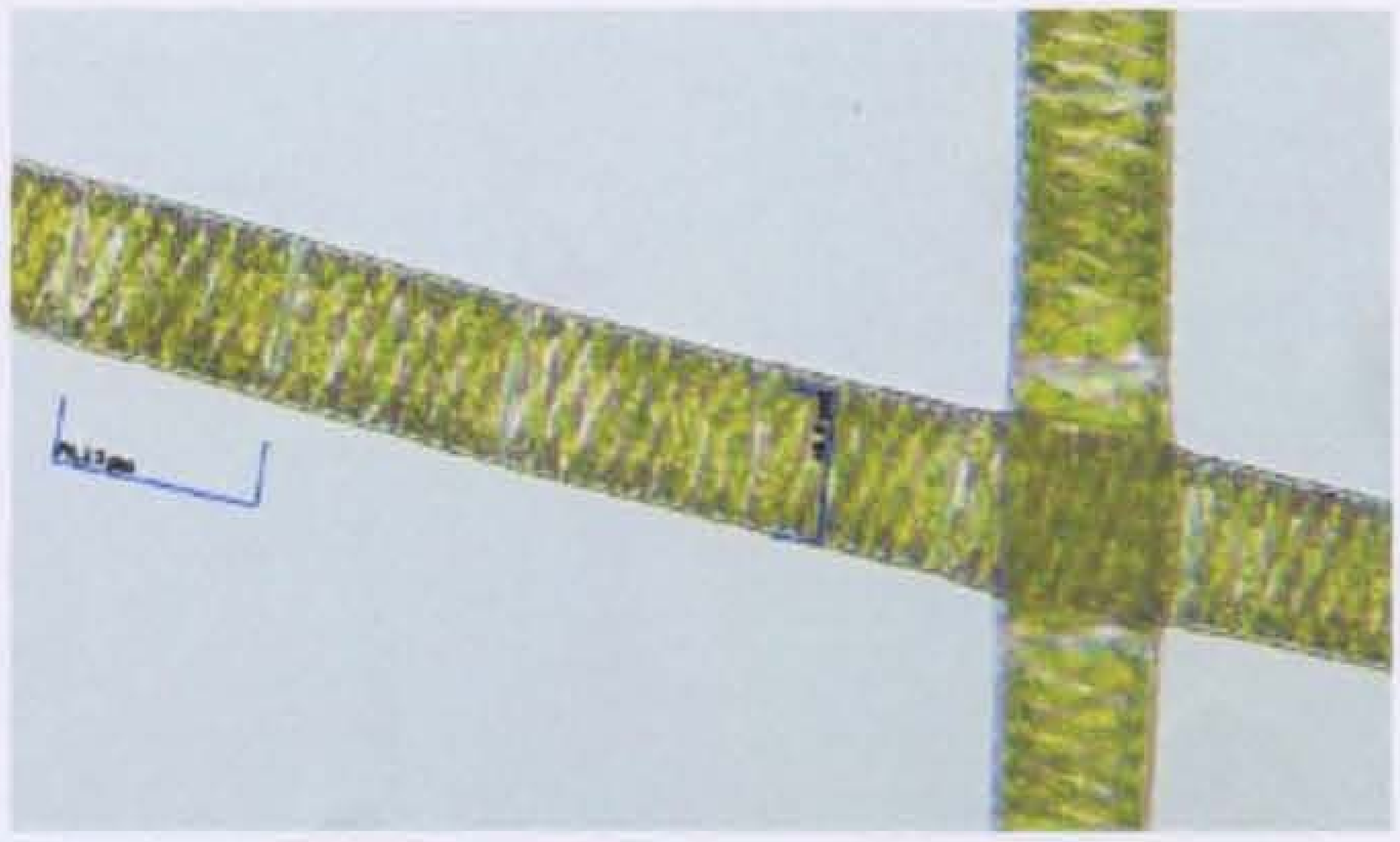


Figure 1. Filamentous algae: *Spirogyra* sp.

Spirogyra sp. in one study were found have a chlorophyll content about 0.53% by weight of the dry sample (Hujaya 2008). This varies depending on the availability of nutrients, climate and weather conditions on the ecosystem.

II.2. Calcium

The mineral composition of water is the most important environmental factor that affects the productivity, development, growth, stability, and physiological and biochemical processes of aquatic organism (Martem´yanov & Mavrin 2012).

Calcium plays a pivotal role in the signal transduction of many responses in plant cells (Hepler and Wayne 1985). Jacobshagen *et al.* (1986) explain that the calcium ion has been implicated in light-stimulated chloroplast movement responses in *Mesotaenium* and filamentous algae *Mougeotia*. Extracellular calcium is necessary for chloroplast rotation and reduction of intracellular calcium inhibits light-stimulated chloroplast reorientation in the filamentous alga *Mougeotia* (Wagner and Klein 1978).

II.3. Light Intensity

Light is an energy source that plays an important role in the primary productivity. Through the primary productivity of this energy will be fixed by autotroph organism through photosynthesis. Based on the physiology of phytoplankton spectrum of light that plays an important role in photosynthesis only the spectrum with a wavelength of 380-700 nm, known as photosynthetic active radiation (PAR).

Generally, algae showed different responses to changes in light intensity. Pigment chlorophyll absorbs blue and red light, absorb carotene absorbs blue and green light, phycoeritrin absorb green and yellow light absorbing phycocyanin. Optimum light intensity for growth of Chlorophyta is 500-750 foot candella (Ryther 1956).

III. RESEARCH METHOD

III.1. Sampling and isolation of microalgae

1. Sampling of filamentous microalgae
   - Sampling of filamentous microalgae from inland water around Bogor, West Java. Filamentous microalgae were collected from natural pond in Cikampak village, Ciampea, Bogor and also from collection pond in Bogor Botanical Garden, West Java, Indonesia.
   - Physical observation of filamentous microalgae in natural waters and match the characteristic of filamentous microalgae with literature.

Figure 2. Filamentous algae in pond

2. Cultivation of filamentous microalgae
   - Identification of filamentous microalgae which can possibly culture
   - Isolation of selected genus filamentous microalgae

Figure 3. Isolation microalgae in laboratory

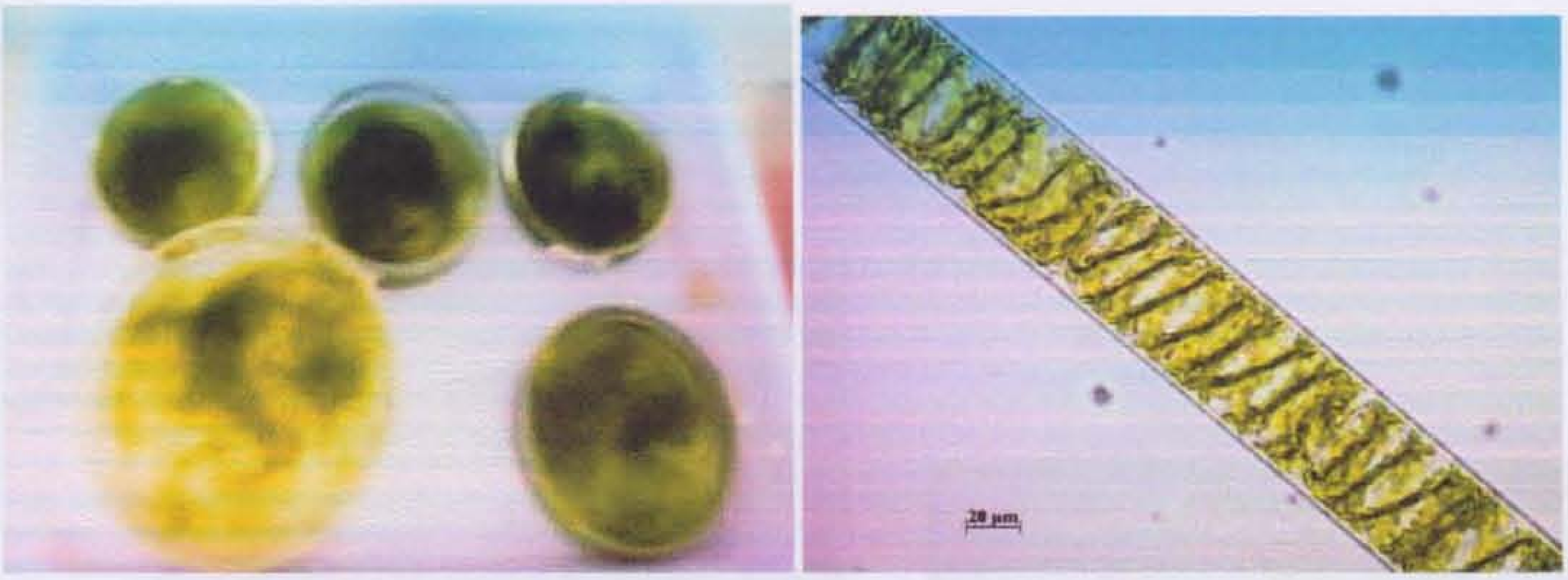


Figure 4. Isolate of microalgae, *Spriogyra* sp.

- Microalgae cultivation in laboratory scale with selected media

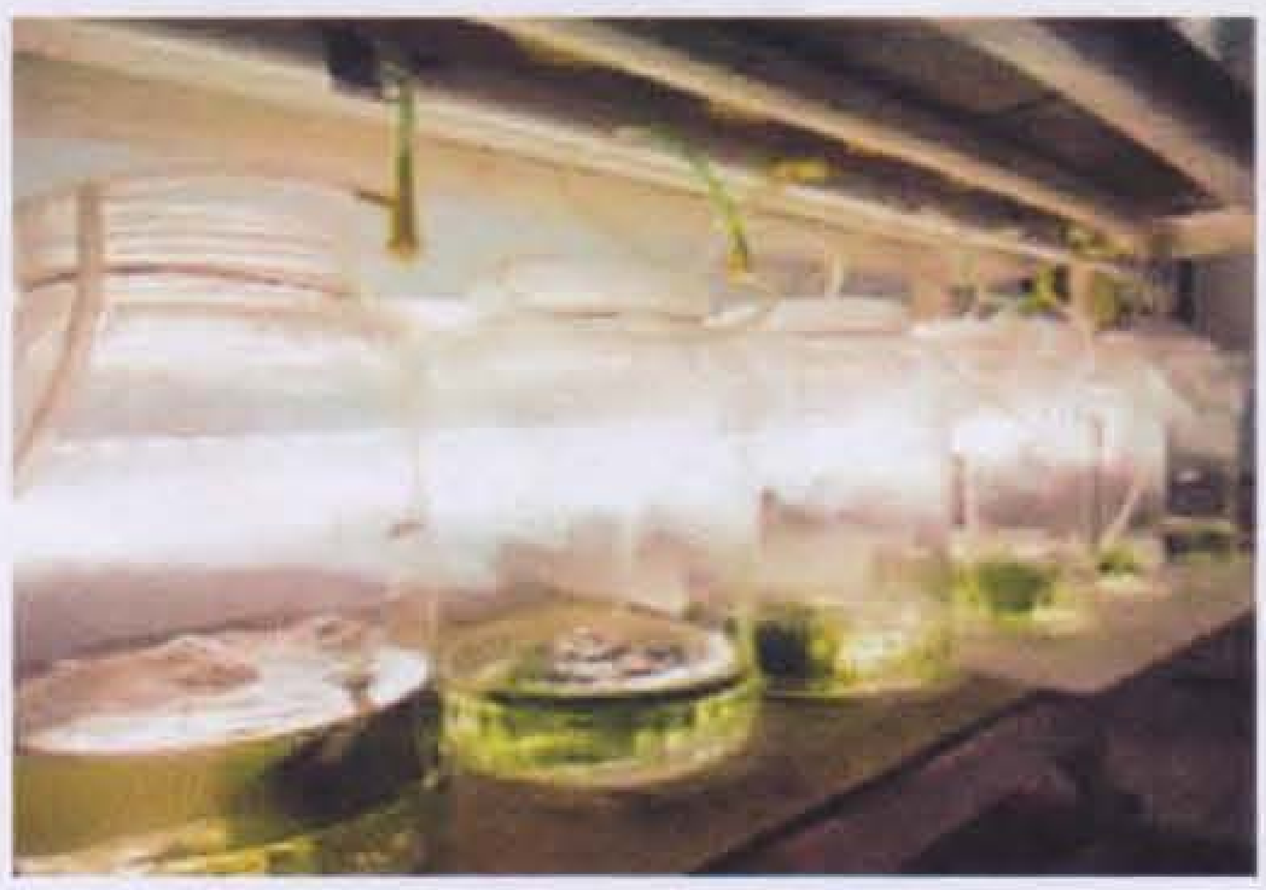


Figure 5. Aclimatitation and cultivation of Isolate of microalgae, *Spriogyra* sp.

III.2. Research in Laboratory

Experiment 1. Treatment of selected filamentous microalgae (*Spirogyra* sp.) cultivation in various kind of calcium ($CaCl_2$, $CaSO_4$, and $CaCO_3$)

The study was conducted through experiments on a laboratory scale. There were 4 treatments: control, $CaCl_2$ treatment, $CaSO_4$ treatment, and $CaCO_3$ treatment. Three replicates for each treatment.

This study used the glass jars that sterilized by $KmnO_4$. This study used 1 L of sterile water to the jar and added 0.1 mL of fertilizer Gandasil D® in each jar. Added calcium $CaCl_2$ (36,76 g $CaCl_2.2H_2O$ dissolved to 1 L of water, and then used 1 mL for 1 L media), $CaSO_4$ (60 g $CuSO_4.2H_2O$ dissolved to 1 L of water, and then used 1 mL for 1 L media), and 0,076 g $CaCO_3$ for 1 L media, appropriate treatment labels on jars. 1.5 g of *Spirogyra* sp. added on each jar (Brubaker *et al*. 2011; Hmaidan *et al*. 2011; Roman *et al*. 2011). The jar is placed on a cultivation shelf, equipped with aeration and lights.

During the 7 days retention, pH, temperature, and light intensity were measured daily. Fresh weight of *Spirogyra* sp., Ca concentration in the water, and the concentration of Ca in Spirogyra sp. measured at the beginning and at the end of the study.

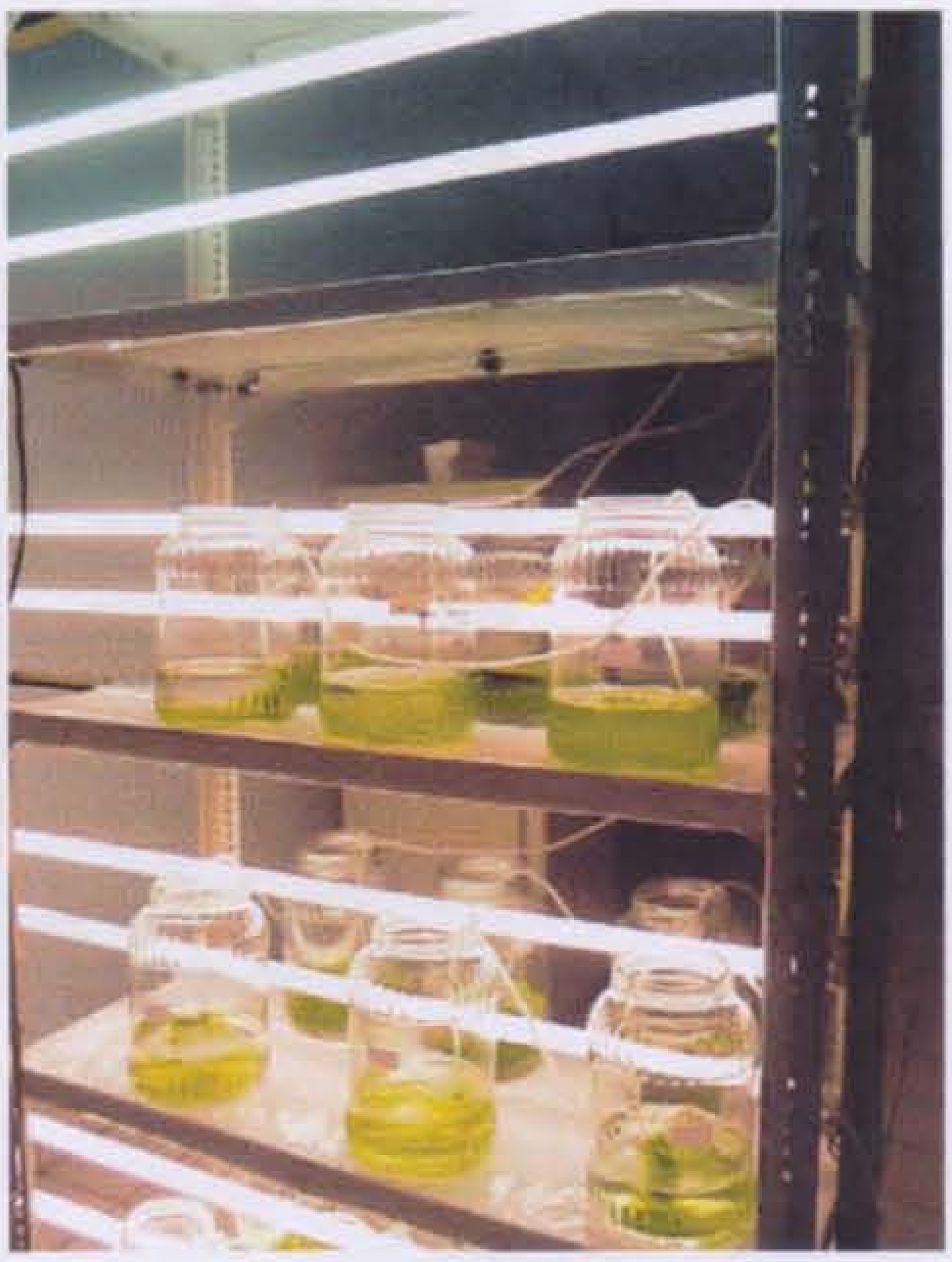
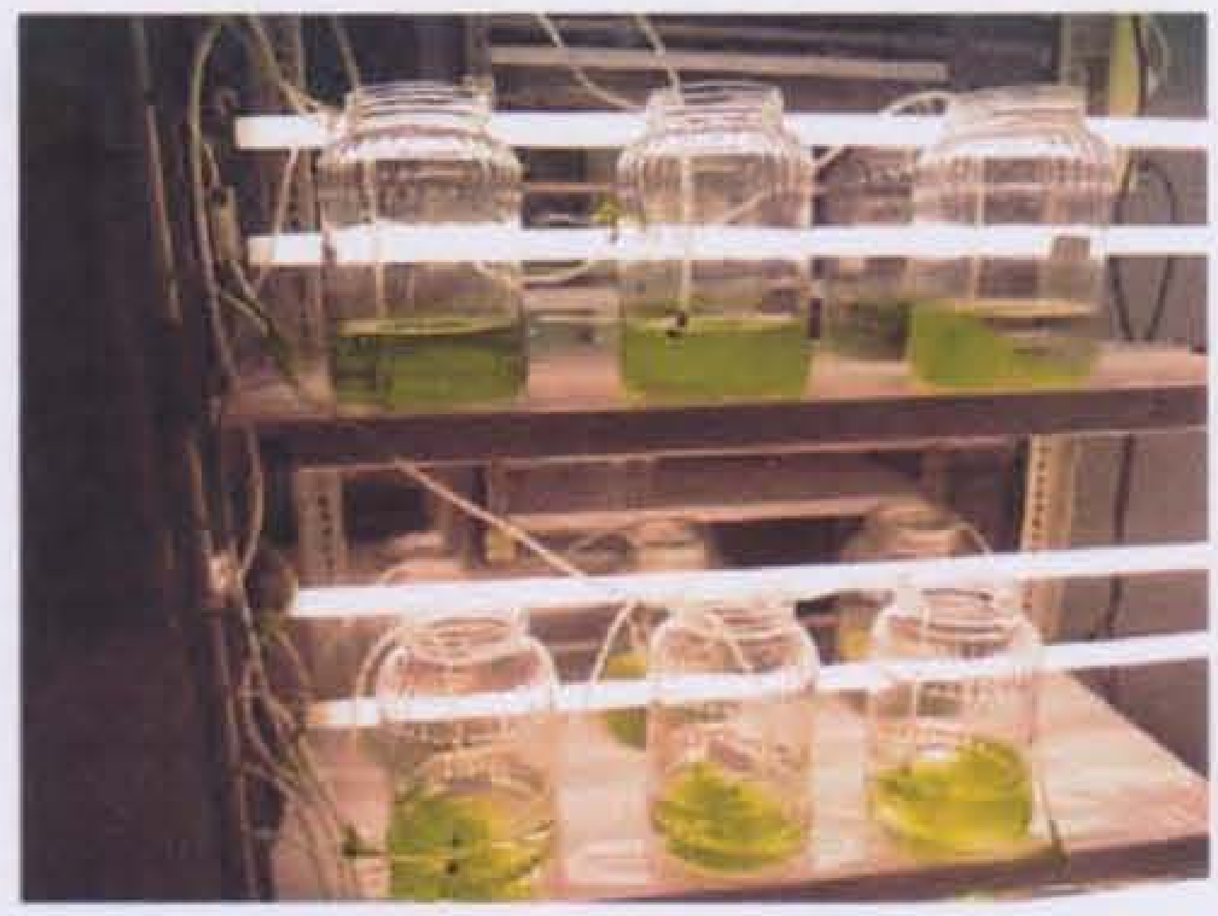



Figure 6. Various kind of calcium treatment on *Spirogyra* sp. cultivation

Experiment 2. Treatment of selected *Spirogyra* sp. cultivation with calcium ($CaCl_2$) in various kind of concentration and different levels of light intensity

Based on the results of previous studies related to the type of calcium is best absorbed by *Spirogyra* sp., calcium used in this study is $CaCl_2$. $CaCl_2$ were taken from $CaCl_2.2H_2O$ 36.76 g dissolved in 1 L of water.

The study was conducted through experiments on a laboratory scale. Treatment differences were tested, namely the concentration of calcium is added (0 ml/L, 0.5 mL/L, 1 mL/L, and 2 mL/L) as well as differences of light intensity (minimum light intensity <500 ft cd, the maximum light intensity> 750 ft cd, as well as the optimum light intensity for growth Chlorophyta 500-750 ft cd (Ryther 1956). Do three replicates for each treatment.

Once the container jars ready, inserted 2 L of sterile water and added 0.2 mL of fertilizer Gandasil D® on each jar (0.1 ml/L). Added calcium (0 ml/L, 0.5 mL/L, 1 mL/L, and 2 mL/L) appropriate treatment label on the jar. Furthermore, as many as 3 g Spirogyra sp. included on each jar (1.5 g / L), a jar placed on a shelf next to the light intensity of different cultures and aerated.

During the 7 day retention, measured pH, temperature, and light intensity daily. Weight of *Spirogyra* sp., Ca concentration in the water, and the concentration of Ca in *Spirogyra* sp. measured at the beginning and at the end of the study.

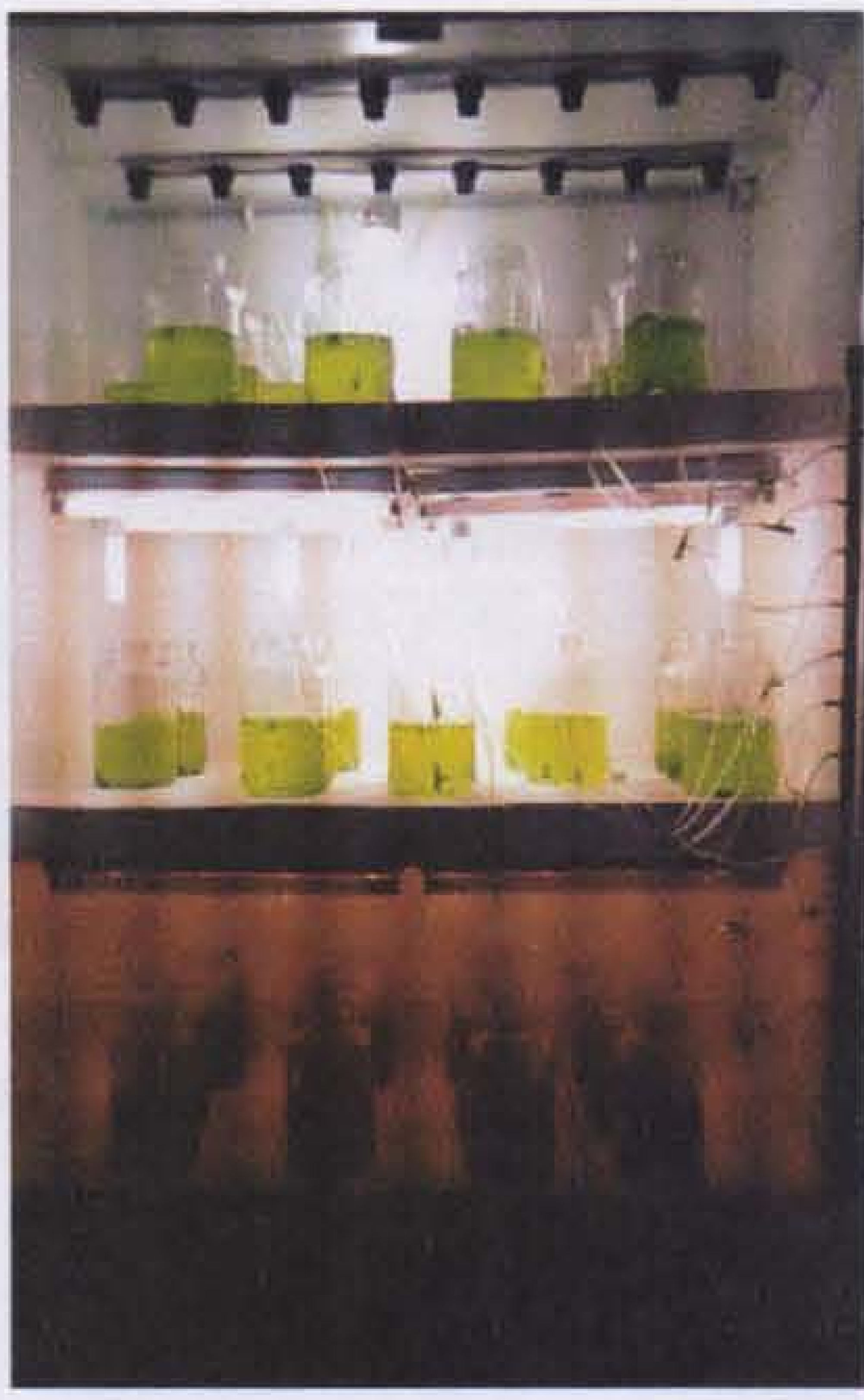


Figure 7. Various kind of light intensity treatment on *Spirogyra* sp. cultivation

Experiment 3. Treatment of selected *Spirogyra* sp. cultivation in absorbing calcium ($CaCl_2$) with different irradiation time

Based on result of Experiment 2, the best treatment in Ca uptake by *Spirogyra* sp. is 0.5 mL$CaCl_2$/ L media in optimum light intensity (500-750 ft cd). So, in third experiment will be done by using different irradiation time.

The study was conducted through experiments on a laboratory scale. A total of 1.5 g of *Spirogyra* sp. cultured on growth media were prepared. Calcium enrichment is done by adding 0.5 mL of $CaCl_2$ solution (36.76 g $CaCl_2$/L distilled water) at each 1 L of growth media *Spirogyra* sp. (Andersen *et al.* 2005).

Container jar experiments put on a shelf equipped aeration. The light source of the light given by the average light intensity 705.67 ft cd. This refers to the range of optimum light for growth at 500-750 ft cd for Chlorophyta (Ryther 1965). Treatment in this study was difference while irradiation on cultured *Spirogyra* sp. There are two treatments, namely irradiation time 12 hours/day and duration of exposure 24 hours/day. Each treatment performed three replications. Measurement of pH, temperature, and light intensity were done daily for seven days of culture. Weight of *Spirogyra* sp. Ca concentration in water (culture medium), and the concentration of Ca in *Spirogyra* sp. measured at the beginning and at the end of the study. Microscopic observation of the cells *Spirogyra* sp. also performed at the beginning and at the end of the study.

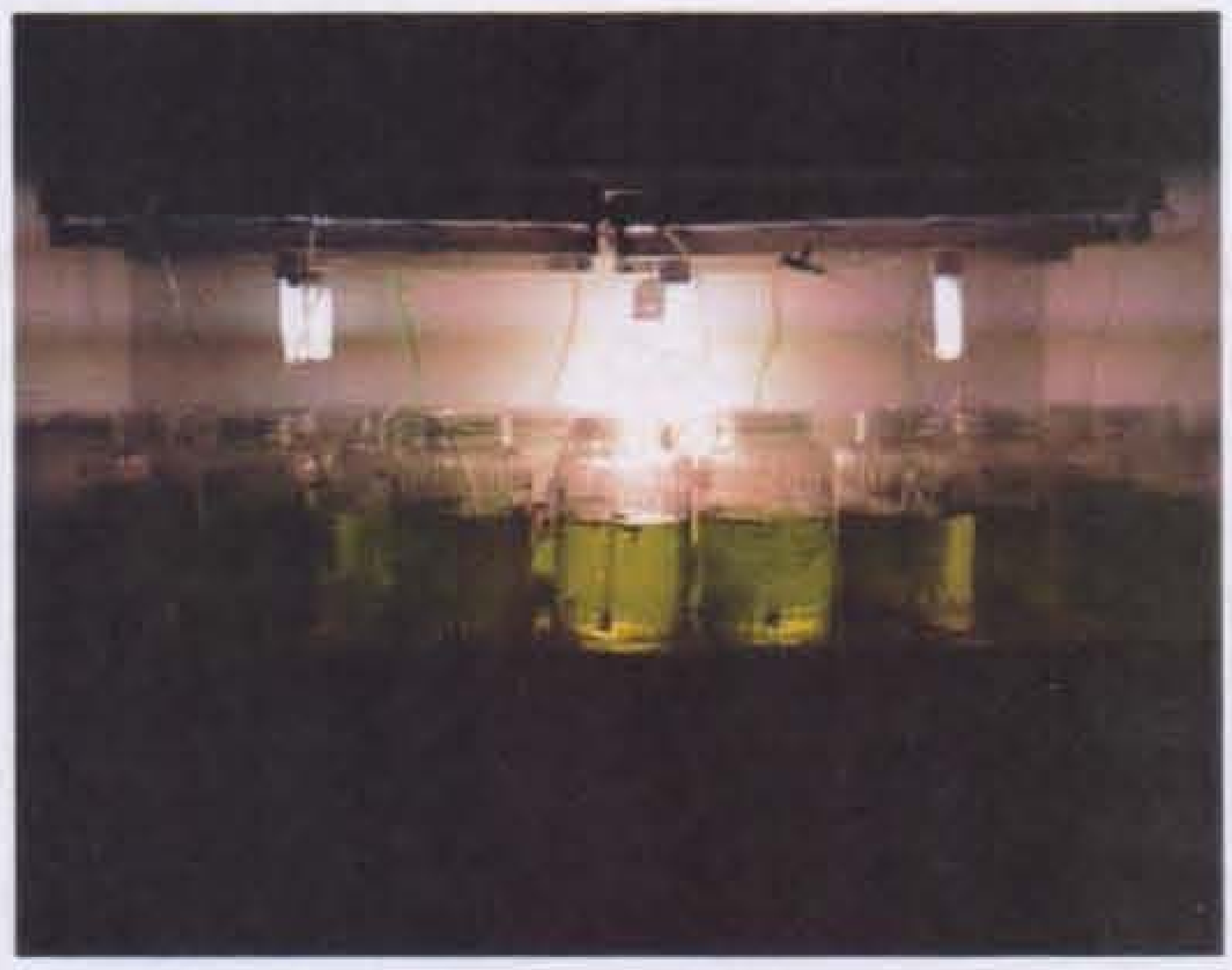


Figure 8. Different irradiation time treatment on *Spirogyra* sp. 24 hours/day (left) and 12 hours/day (right)

III.3. Data Analysis

Percentage change in weight and the concentration of Ca is calculated by comparing the magnitude of the changes that occurred after seven days of culture with the initial value. T-test is done to see the difference between treatments.

IV. RESULT AND DISCUSSION

IV.1. Result of Experiment 1 Treatment of selected filamentous microalgae (*Spirogyra* sp.) cultivation in various kind of calcium ($CaCl_2$, $CaSO_4$, and $CaCO_3$)

Calcium concentration in water

Calcium (Ca) concentration in water increased at the end of observation in all treatments (Figure 9). Calcium concentration was increase 49% in control, 33% in $CaCO_3$ treatment (33%), 16% in $CaCl_2$, and 3% in $CaSO_4$ treatment. The existence of Ca in water media trial of suspected from the addition of calcium, can also be derived from the breakdown of Ca in existing water media.

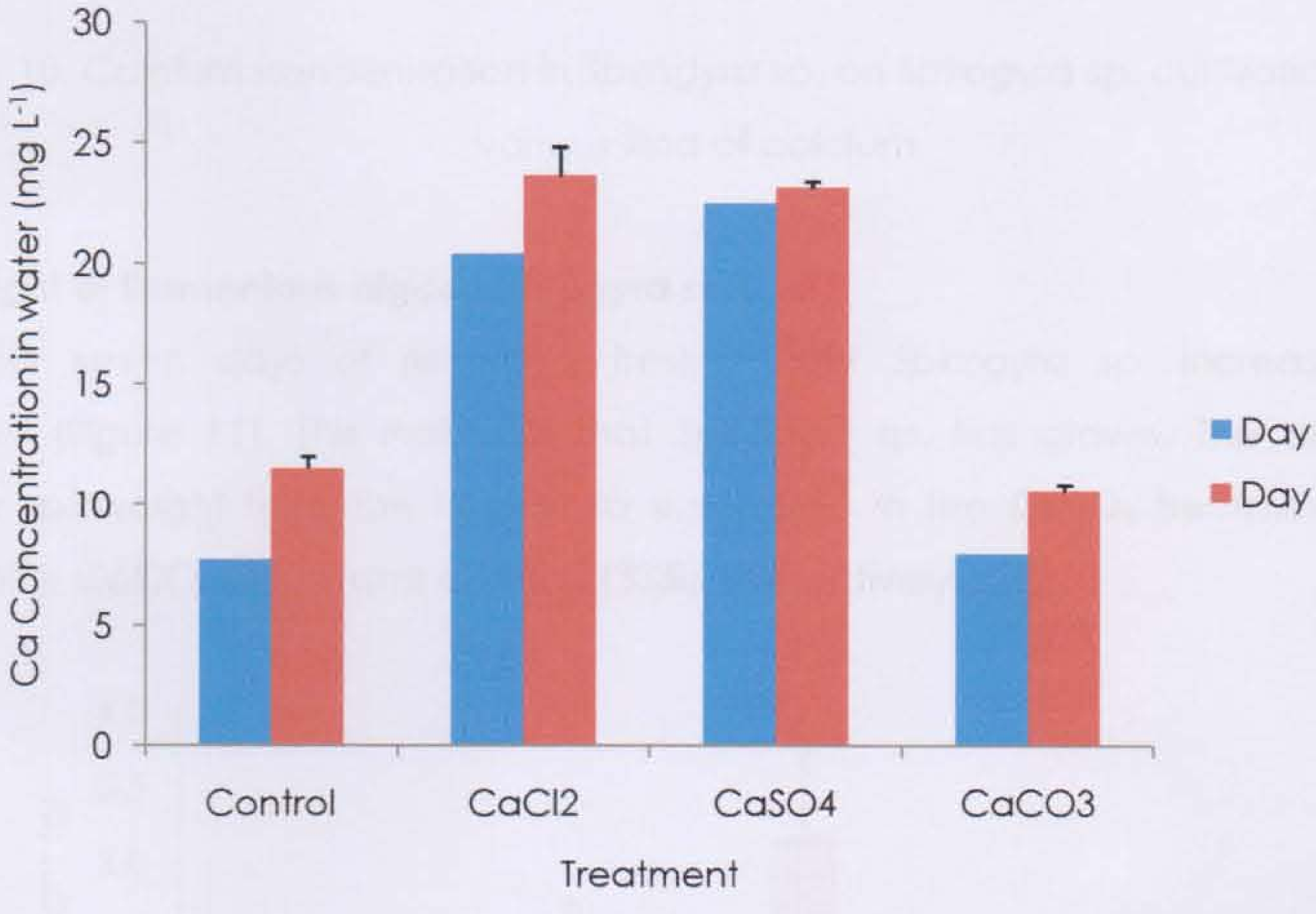


Figure 9. Calcium concentration in water on *Spirogyra* sp. cultivation with various kind of calcium

Calcium concentration in filamentous algae (*Spirogyra* sp.)

Ca values in *Spirogyra* sp. also increased at the end of the observation in all treatments (Figure 10). The percentage increase in the Ca value of the highest to the lowest in the control (40%), $CaCl_2$ (38%), $CaCO_3$ (25%), and $CaSO_4$ (6%), respectively.

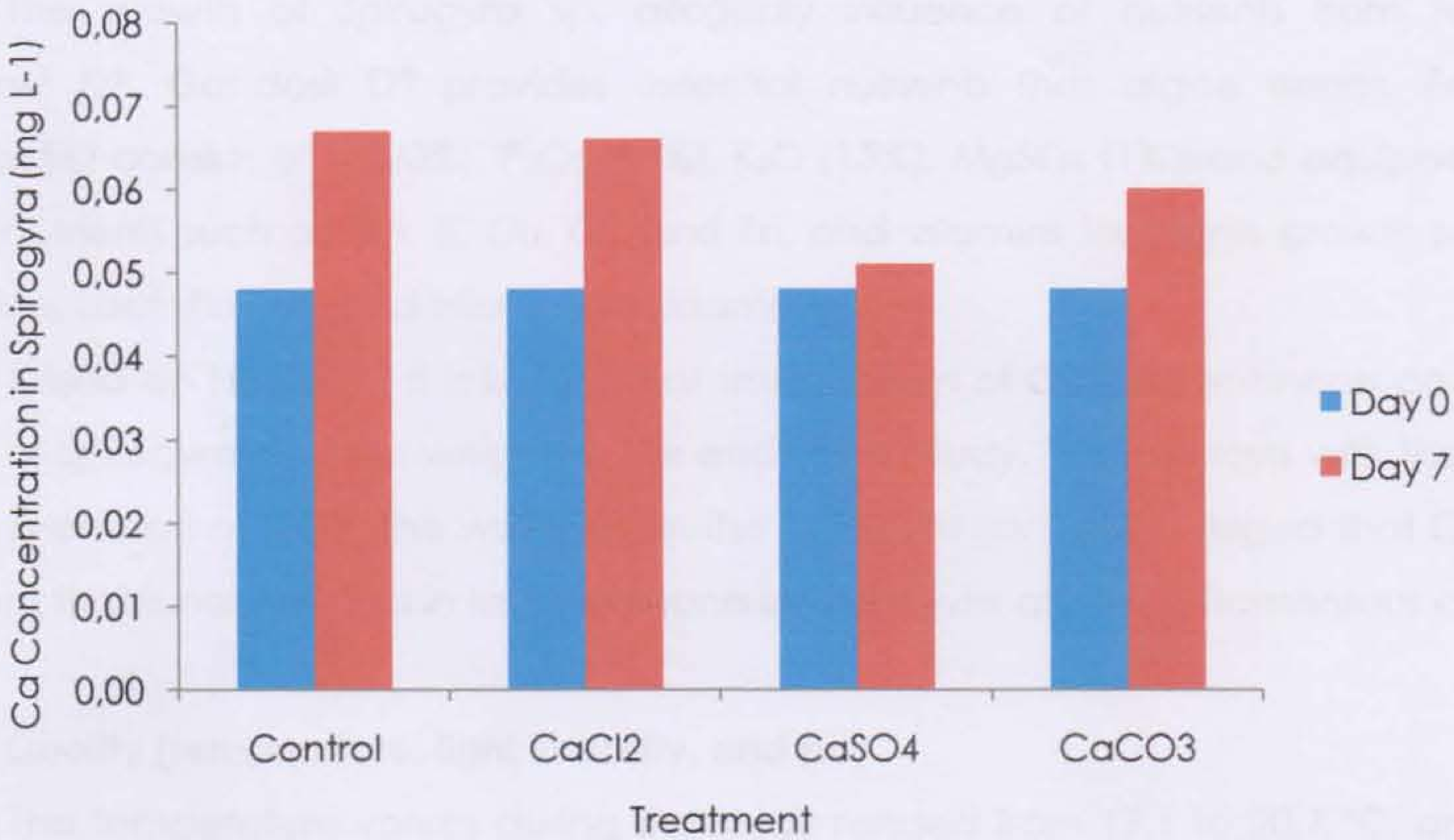


Figure 10. Calcium concentration in *Spirogyra* sp. on *Spirogyra* sp. cultivation with various kind of calcium

Fresh weight of filamentous algae (*Spirogyra* sp.)

After seven days of retention, fresh weight *Spirogyra* sp. increased in all treatments (Figure 11). This indicates that *Spirogyra* sp. has grown. The addition of *Spirogyra* sp. weight from the highest to the lowest in the $CaSO_4$ treatment (110%), $CaCl_2$ (66%), $CaCO_3$ (46%), and controls (33%), respectively.

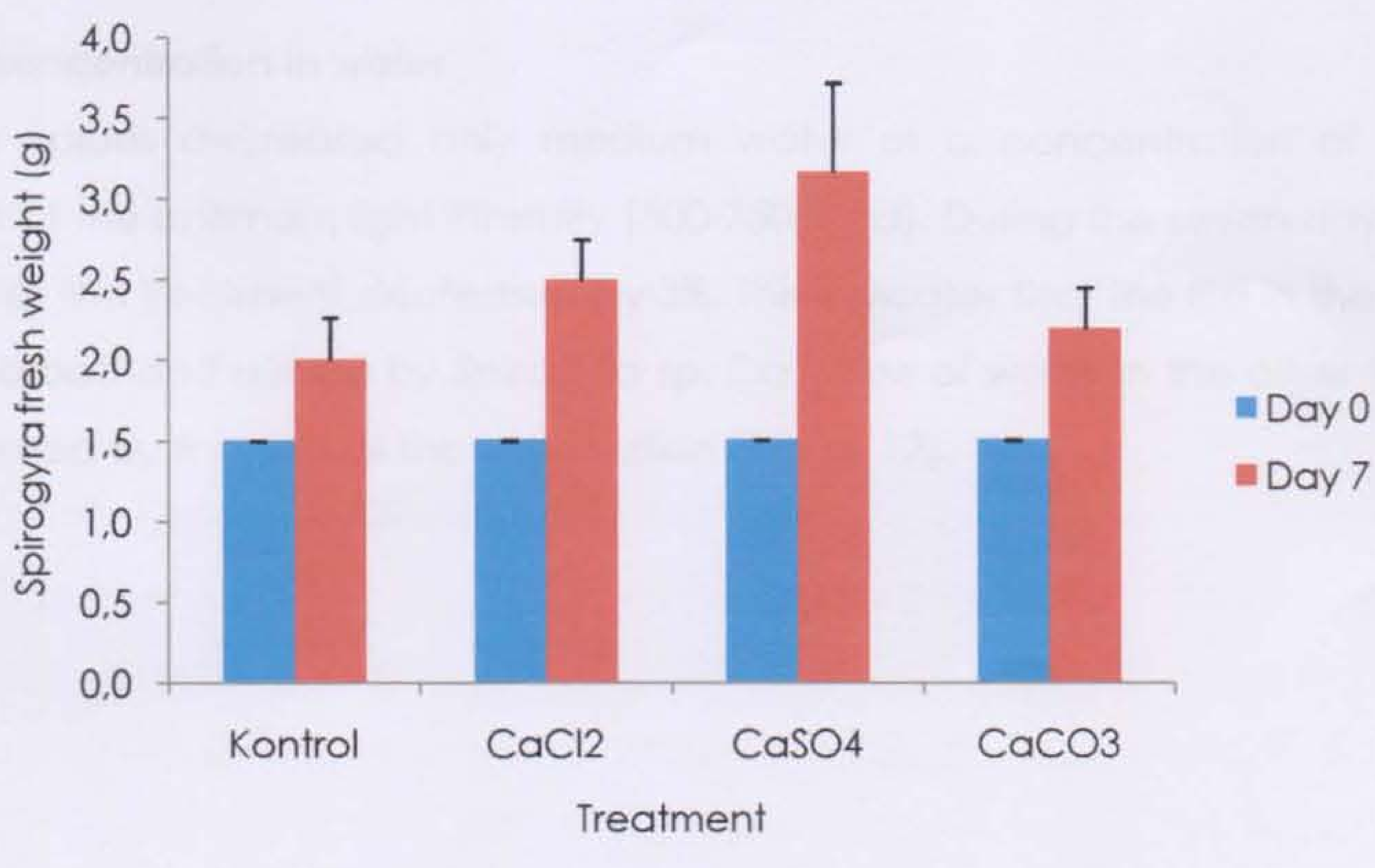


Figure 11. Fresh weight of *Spirogyra* sp. on *Spirogyra* sp. cultivation with various kind of calcium

The growth of *Spirogyra* sp. allegedly influence of nutrients from fertilizers Gandasil D®. Gandasil D® provides essential nutrients that algae needs. Fertilizers Gandasil D consists of N (20%), P_2O_5 (15%), K_2O (15%), $MgSO_4$ (1%), and equipped with micro-nutrients such as Mn, B, Cu, Co and Zn, and vitamins for plants growth such as: Aneurine, Lactoflavine, and Nicotinic acidamide.

Based on Figure 11, it is known that the addition of $CaSO_4$ treatments cause the higest of *Spirogyra* sp. fresh weight at the end of the study. This contrasts with the results of the presence of Ca in the water or on the *Spirogyra* sp. It was alleged that Ca is an element that is not needed in large amounts by *Spirogyra* sp. in this filamentous algae.

Water Quality (temperature, light intensity, and pH)

The temperature values during the study ranged from 19.1 to 20.7 °C, and light intensity ranged from 2120-4650 lux. The average pH values during the study ranged from 7.51 to 9.13. The pH values fluctuated during the observation. Generally, the pH value in the treatment of $CaSO_4$ has a higher pH range than other treatments. Changes in pH values during the study relates to the activity of ion transport in and out of cells. Allegedly this is also affecting the uptake of Ca by *Spirogyra* sp.

IV.2. Result of Experiment 2. Treatment of selected *Spirogyra* sp. cultivation with calcium ($CaCl_2$) in various kind of concentration and different levels of light intensity

Calcium concentration in water

Ca values decreased only medium water at a concentration of 0.5 $CaCl_2$ treatment at the optimum light intensity (500-750 ft cd). During the seven-day retention, Ca water at the treatment decreased by 3%. This indicates that the Ca in the water has been absorbed and utilized by *Spirogyra* sp. Ca value of water in the other treatments had increased by the end of the observation (Figure 12).

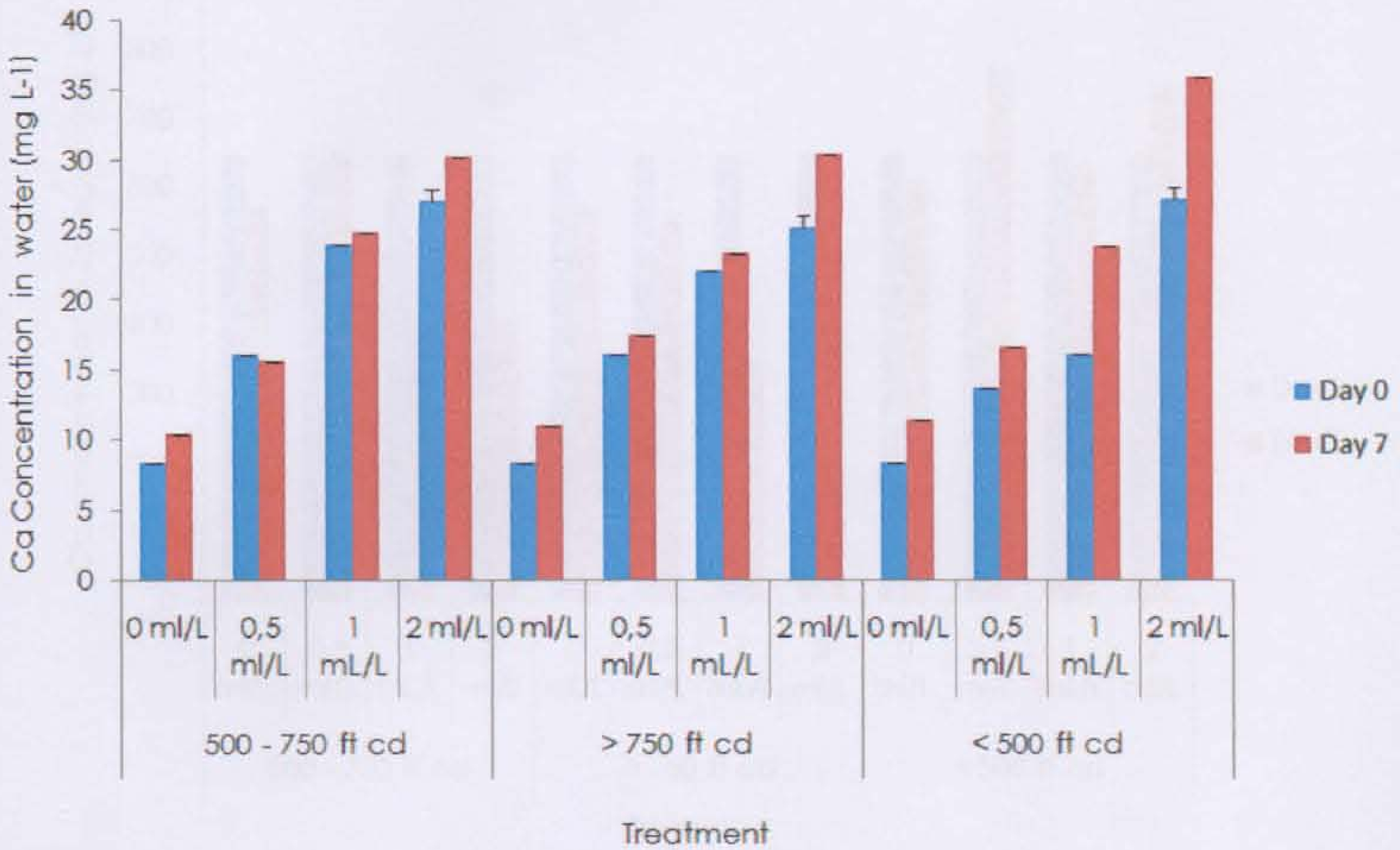


Figure 12. Calcium concentration in water on *Spirogyra* sp. cultivation with various kind of concentration and different levels of light intensity

Based on the concentration of Ca is added, it can be seen that the $CaCl_2$ concentration and 1 mL/L and 2 mL/L Ca concentration increased value for all levels of light intensity. The highest increase in Ca concentration at the end of the observations found in the treatment concentration of 2 mL/L with low light intensity <500 ft cd. It is suspected that the added Ca Ca exceeds the amount required by *Spirogyra* sp. so no further use. Low light intensity is also thought to reduce the ability of *Spirogyra* sp. in the process of photosynthesis and uptake of Ca. An increase in Ca in water treatment studies in other media besides allegedly derived from the addition of calcium, can also be derived from the breakdown of existing water Ca media research. This causes Ca are in excess amount.

Calcium concentration in filamentous algae (*Spirogyra* sp.)

Ca values in *Spirogyra* sp. at the end of the observation has decreased almost in all treatments. Increased value addition of Ca found in the treatment of 0.5 ml $CaCl_2$/L at optimum light (500-750 ft cd) and minimum light (500 ft cd) as well as the addition of 2 ml $CaCl_2$/L at minimum light, with the percentage increase in the value of Ca 1%, 15%, and 19%, respectively.

Figure 13. Calcium concentration in *Spirogyra* sp. on *Spirogyra* sp. cultivation with various kind of concentration and different levels of light intensity

Fresh weight of filamentous algae (*Spirogyra* sp.)

After seven days of retention, weight Spirogyra sp. increased in almost all treatments (Figure 14). This indicates that there is a growth in Spirogyra sp. were tested in the study. The highest addition of weight *Spirogyra* sp. was found in the treatment of the addition of 0.5 ml/L at the optimum light intensity (500-750 ft cd) and followed by the maximum light intensity (> 750 ft cd), with the addition of weights of the both treatments 46.3 and 44.3%, respectively.

Based on Figure 14 also obtained information that the weight of *Spirogyra* sp. an increase in the optimum light intensity (500-750 ft cd) and maximum (750 ft cd), whereas the minimum light intensity (<500 ft cd) tend to decrease the weight of the algae. This is allegedly due to differences in the availability of light required by *Spirogyra* sp. in the process of photosynthesis, which in turn affect the growth of filaments in *Spirogyra* sp.

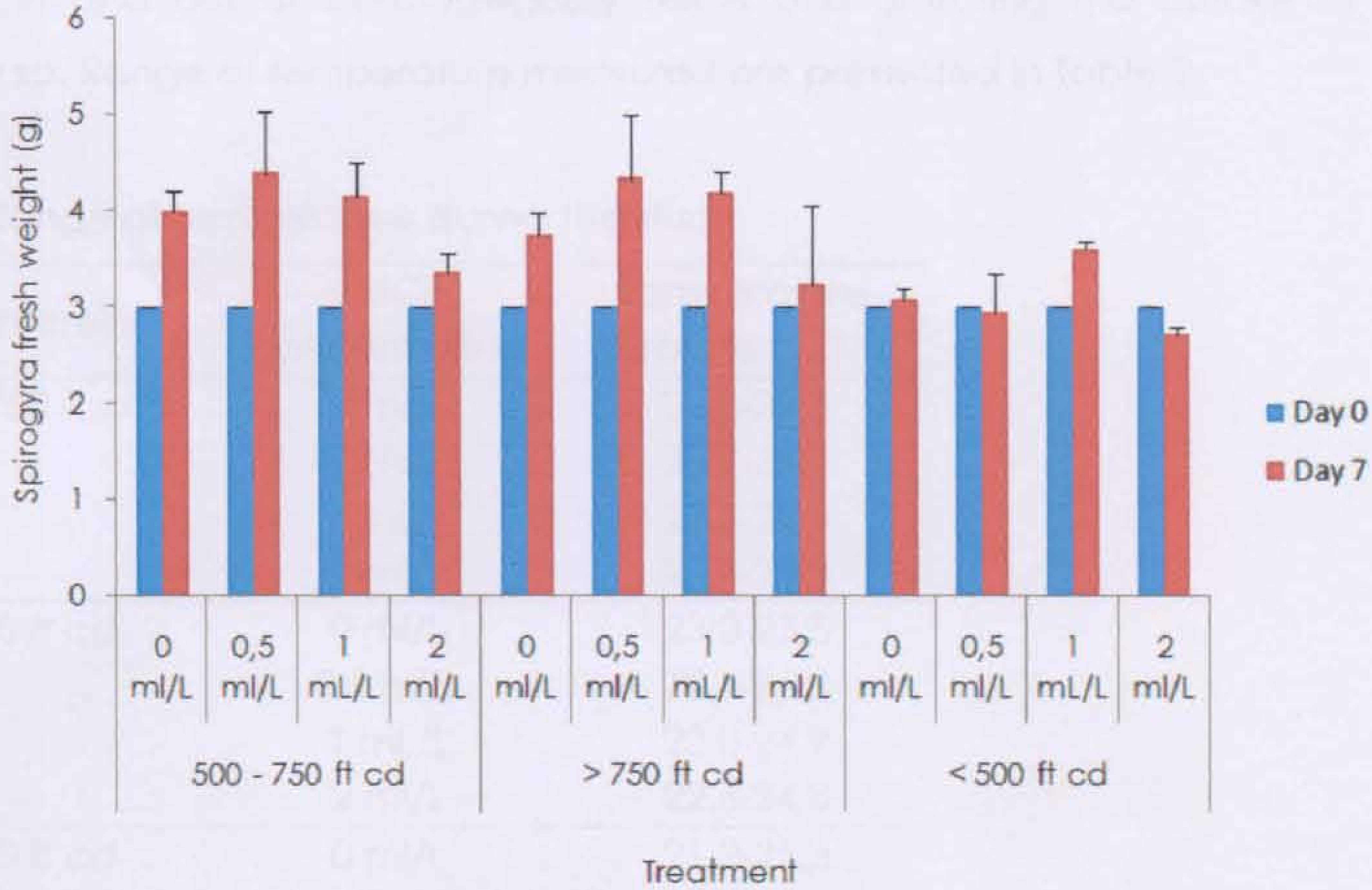


Figure 14. Fresh weight of *Spirogyra* sp. on *Spirogyra* sp. cultivation with various kind of concentration and different levels of light intensity

Water Quality (temperature, light intensity, and pH)

Range of pH values measured during the study are presented in Table 1.

Table 1. Range of pH values during the study

Light intensity	$CaCl_2$ Concentration	pH range
500 - 750 ft cd	0 ml/L	7,21-8,61
	0,5 ml/L	7,09-8,98
	1 mL/L	7,13-8,55
	2 ml/L	7,16-8,98
> 750 ft cd	0 ml/L	7,25-8,64
	0,5 ml/L	7,36-8,85
	1 mL/L	7,32-8,73
	2 ml/L	7,28-8,56
< 500 ft cd	0 ml/L	7,03-8,27
	0,5 ml/L	7,17-7,97
	1 mL/L	7,24-8,40
	2 ml/L	7,22-7,86

The pH values fluctuated during the observation. Results of research conducted by Sulfahri and Wulanmanuhara (2013) provide information that the pH value of the culture of Spirogyra sp. with the addition of fertilizer Gandasil D® changes during 40 days of retention. Changes in pH values during the study relates to the activity of ion transport in and out of cells. Allegedly this is also affecting the uptake of Ca by *Spirogyra* sp. Range of temperature measured are presented in Table 2.

Table 2. Range of temperature during the study

Light intensity	$CaCl_2$ concentration	Temperature range (°C)
500 - 750 ft cd	0 ml/L	23,3-25,3
	0,5 ml/L	23,2-25,5
	1 mL/L	22,7-25,6
	2 ml/L	23,4-25,0
> 750 ft cd	0 ml/L	23,0-25,5
	0,5 ml/L	22,4-24,8
	1 mL/L	23,0-24,9
	2 ml/L	22,6-24,8
< 500 ft cd	0 ml/L	21,2-25,3
	0,5 ml/L	22,4-25,7
	1 mL/L	22,1-25,8
	2 ml/L	22,3-26,3

The average value of the range of light intensity at the optimum light treatment (500-750 ft cd) of 616-674 ft cd, maximum light treatment (> 750 ft cd) of 852-990 ft cd, and at minimum light treatment (<500 ft cd) at 39-73 ft cd. The average value of the intensity of light is presented in Figure 15.

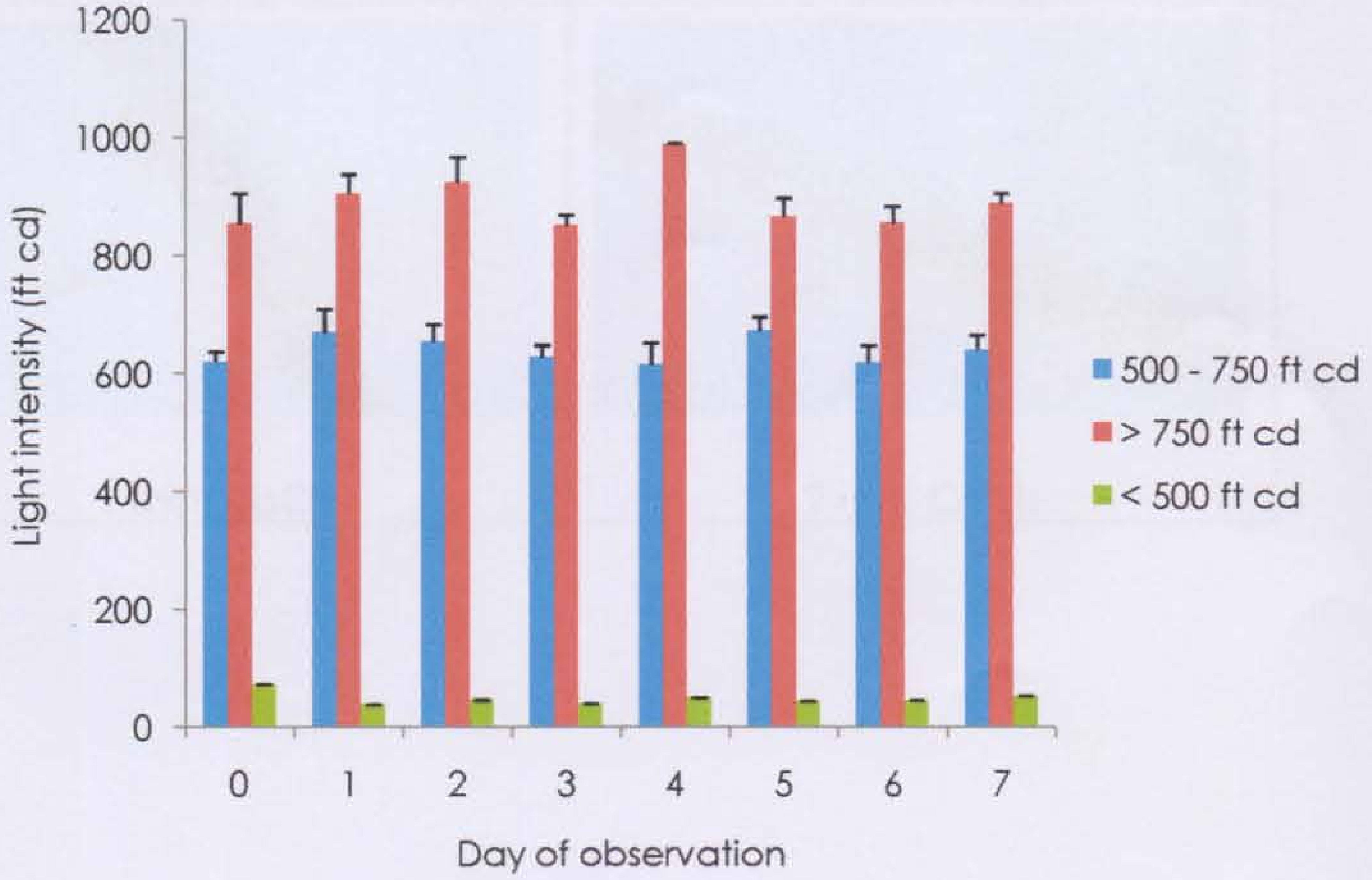


Figure 15. Value of light intensity during the study

***Spirogyra* sp. cells Conditions During the Study**

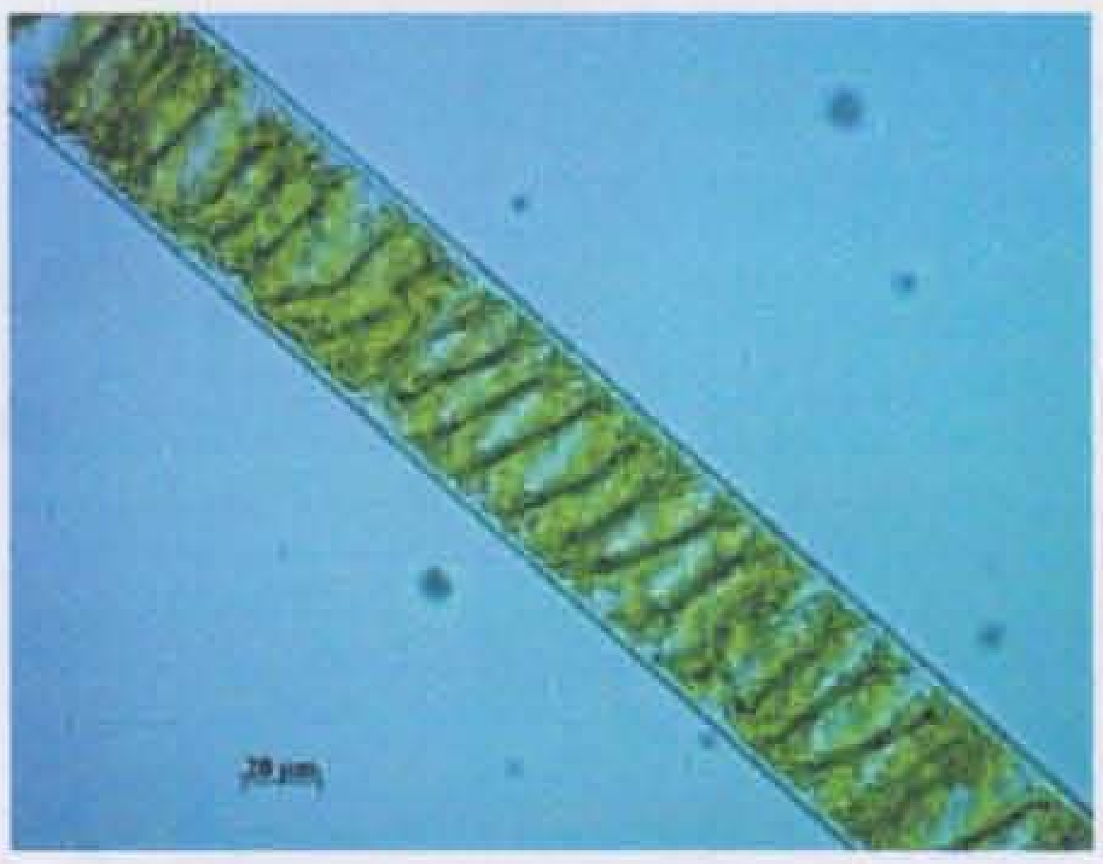
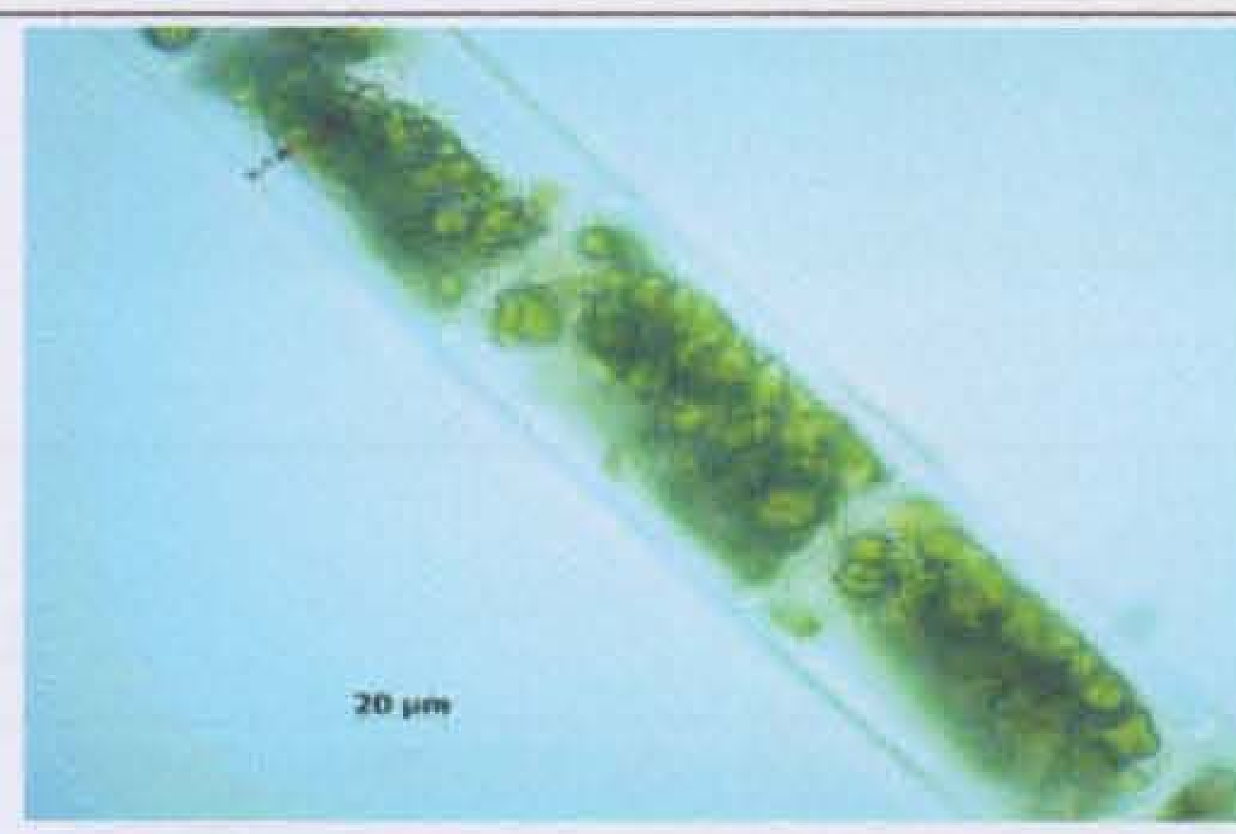
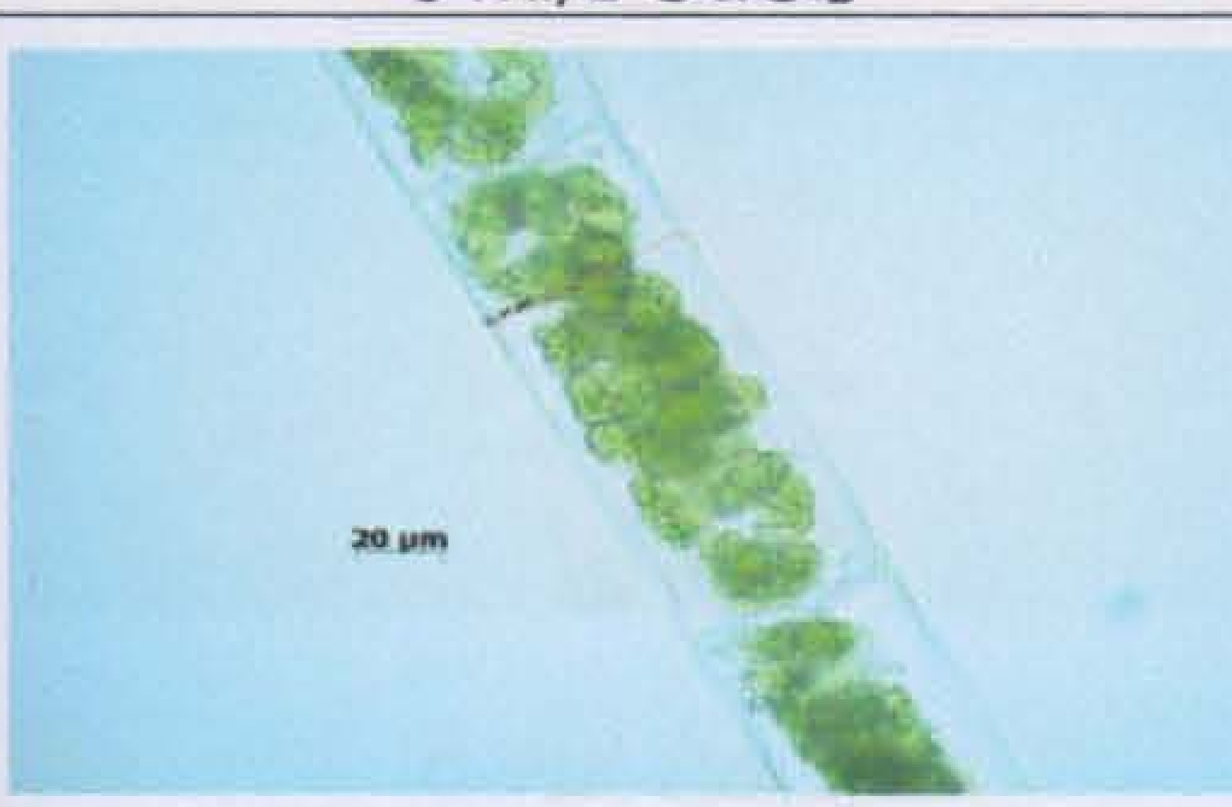
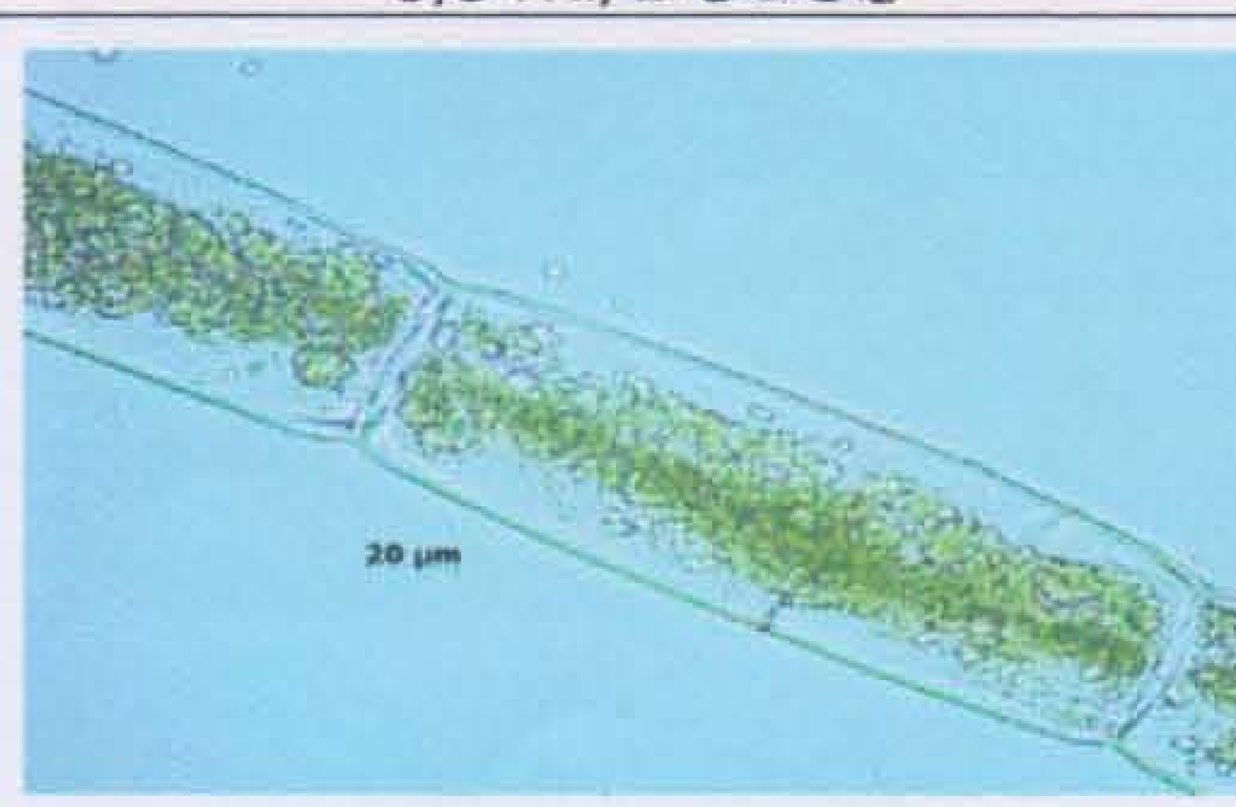


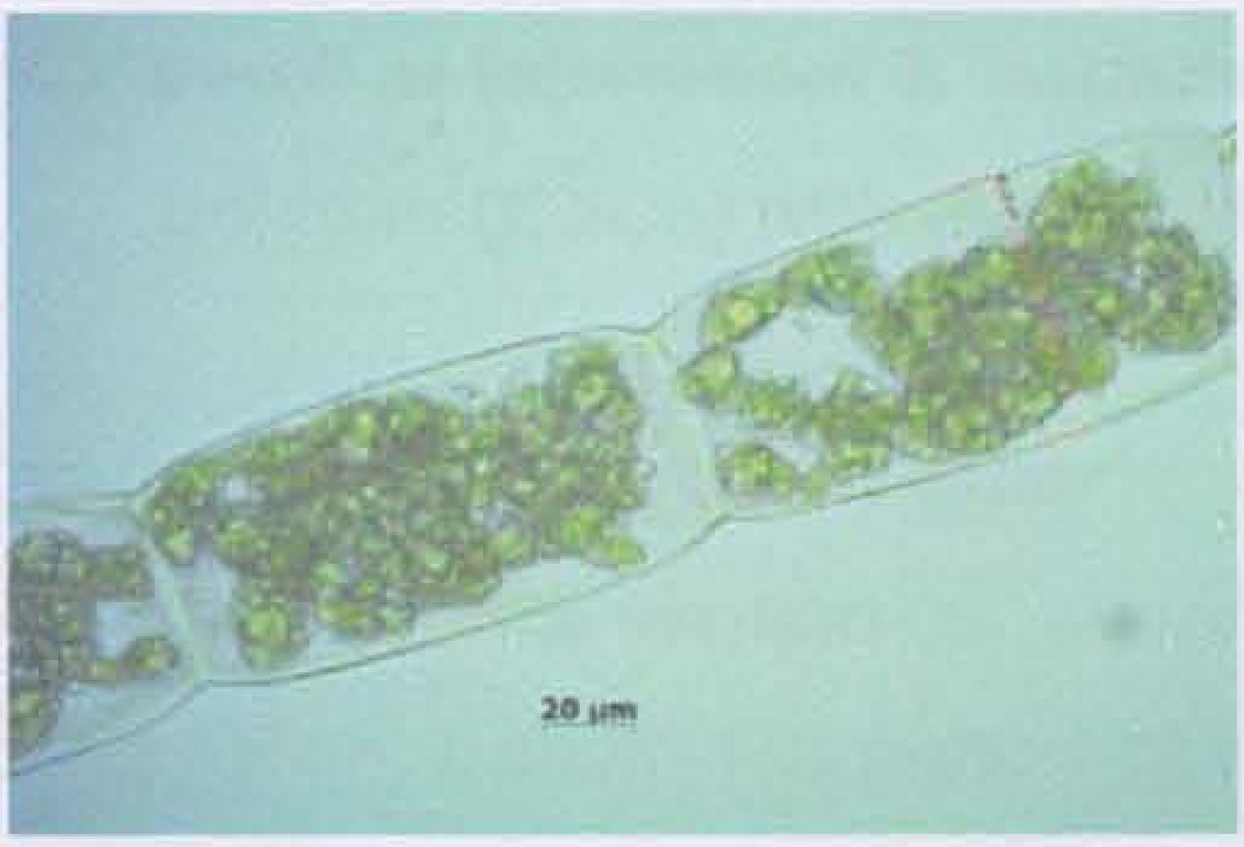
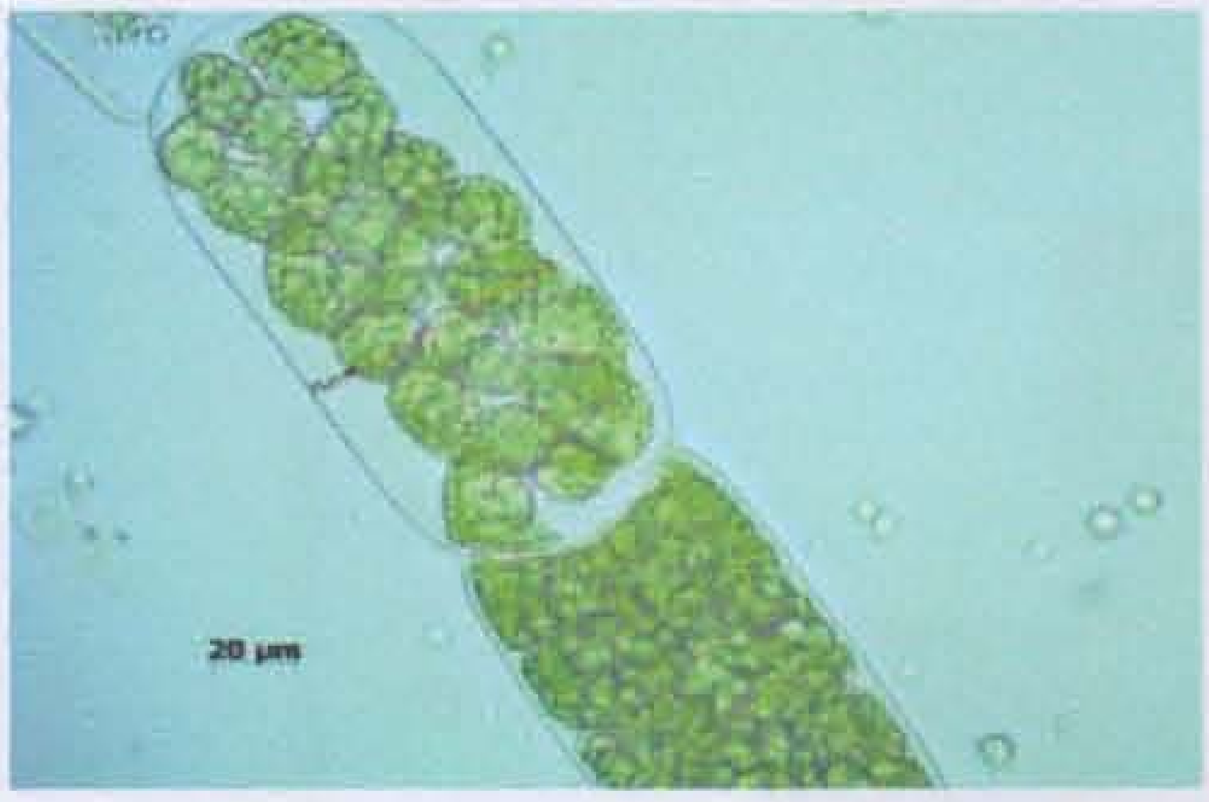
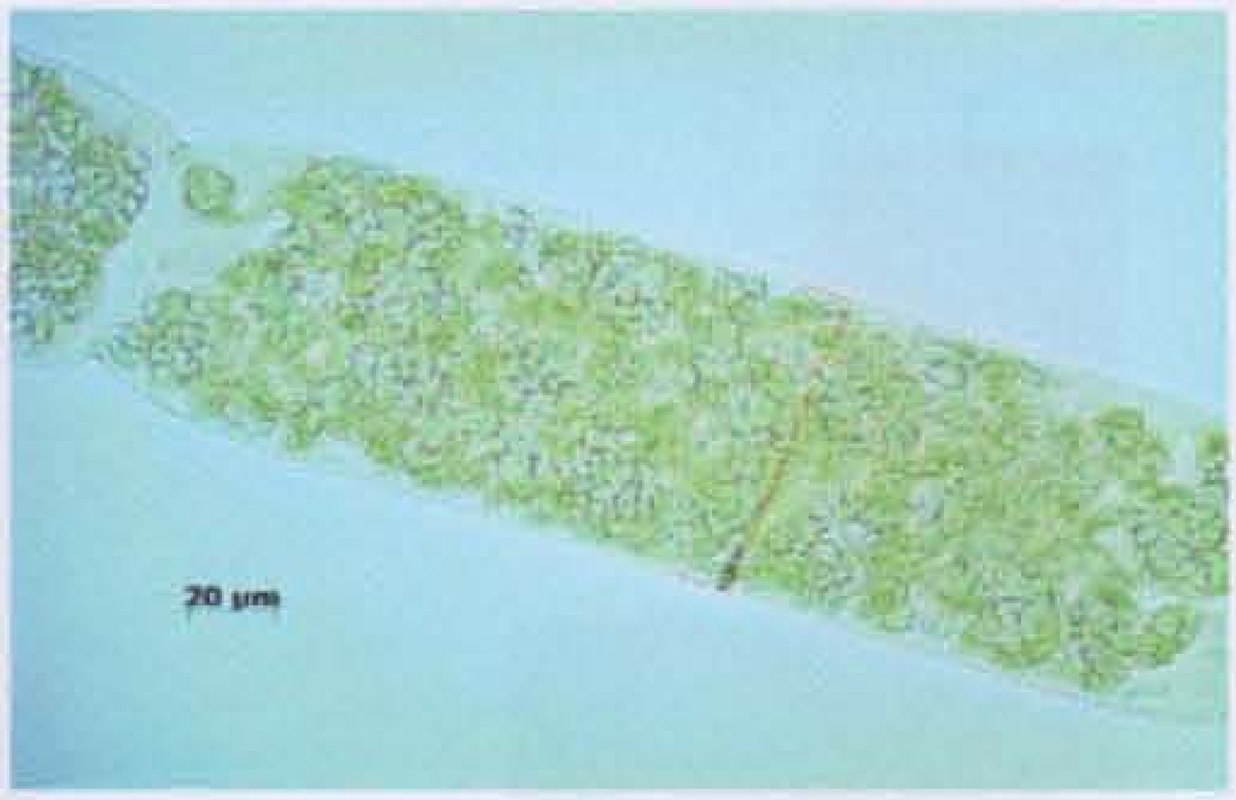
Figure 16. Conditions of cells at the start of treatment (Day 0)

Conditions of cells at the end of treatment (magnification 10 x 40)

a. Optimum light treatment (500-750 ft cd)

 0 ml/L $CaCl_2$	 0,5 ml/L $CaCl_2$
 1 ml/L $CaCl_2$	 2 ml/L $CaCl_2$

b. Maximum light treatment (> 750 ft cd)

 0 ml/L $CaCl_2$	 0,5 ml/L $CaCl_2$
 1 ml/L $CaCl_2$	 2 ml/L $CaCl_2$

c. Minimum light treatment (<500 ft cd)

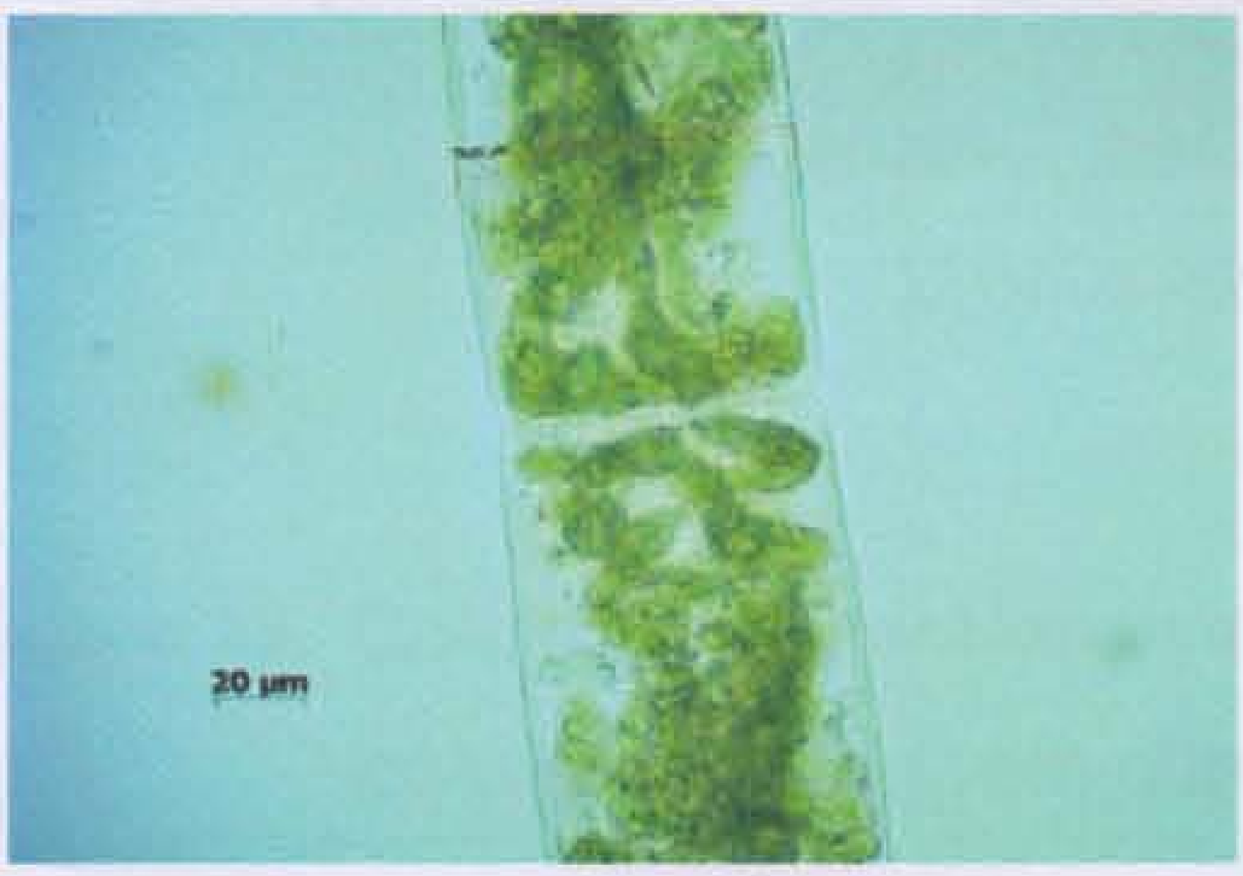
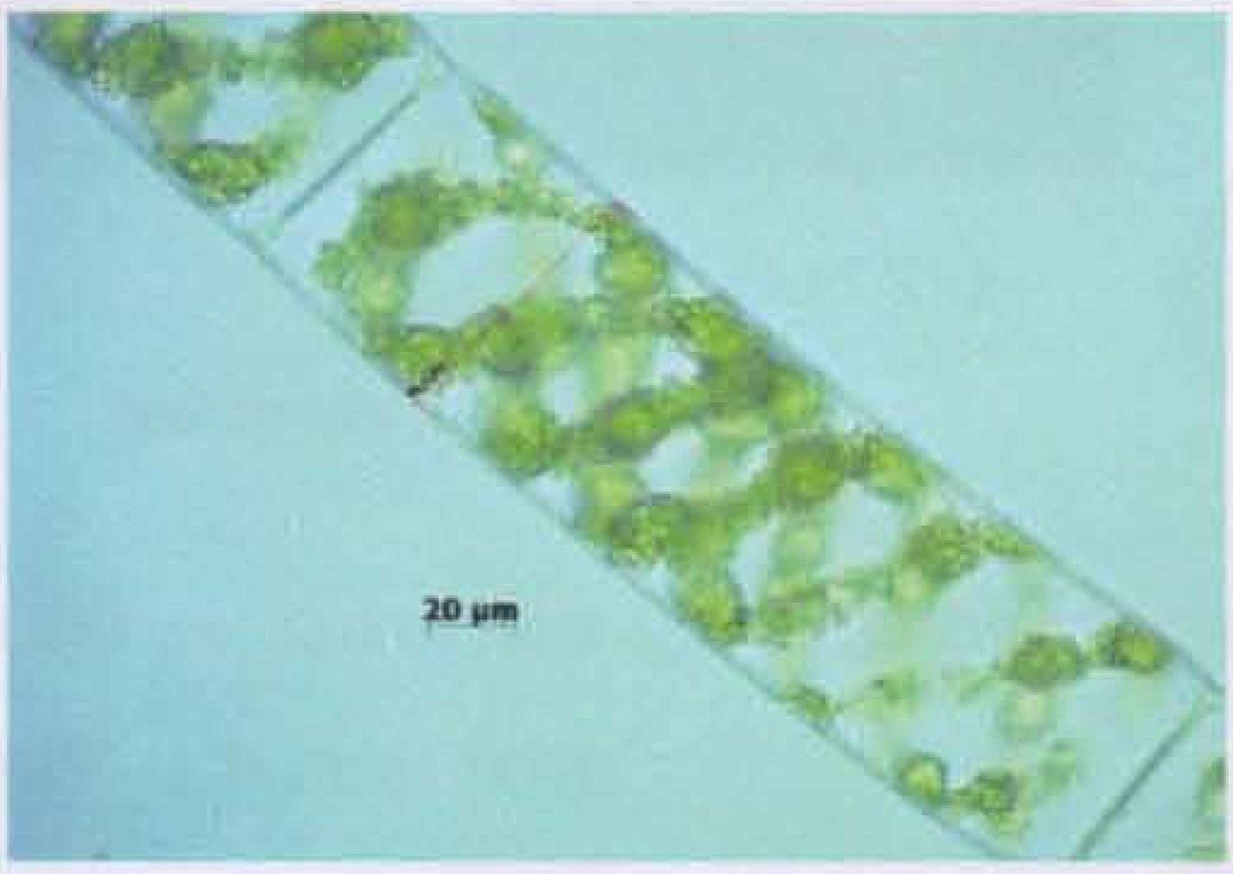
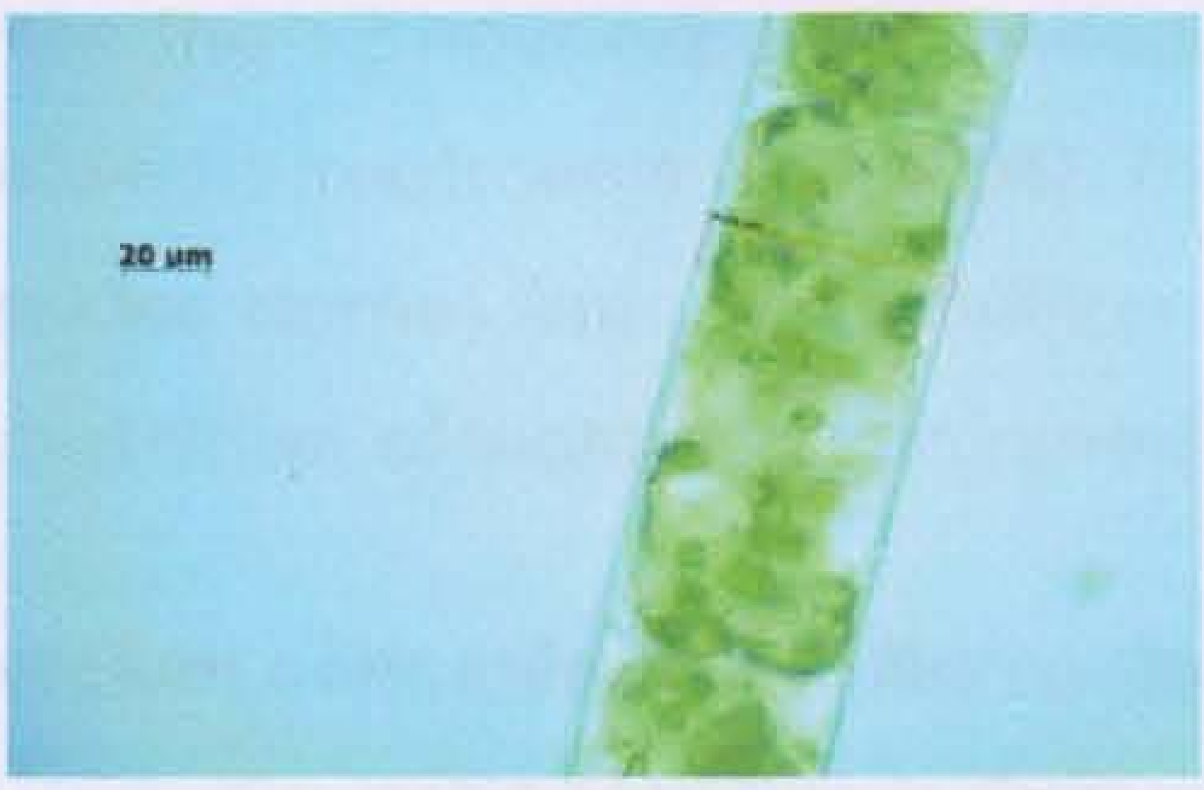
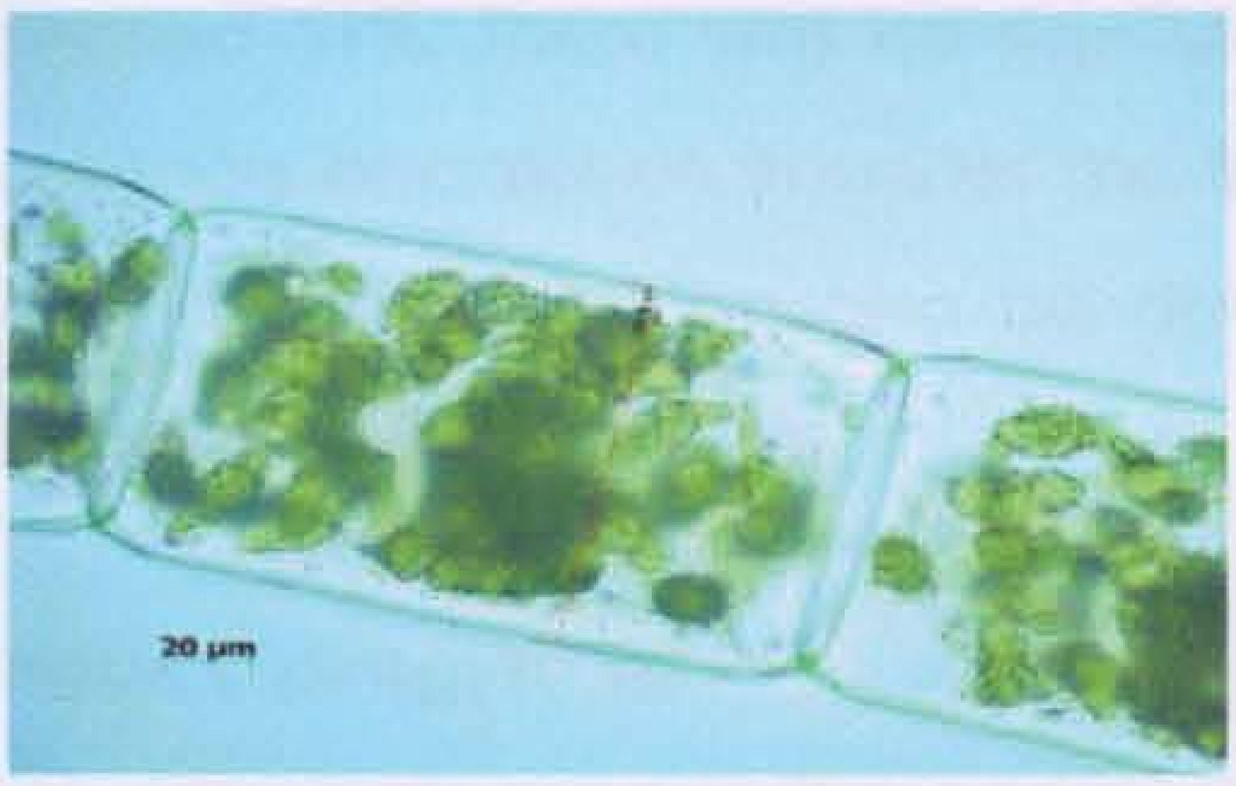
 0 ml/L $CaCl_2$	 0,5 ml/L $CaCl_2$
 1 ml/L $CaCl_2$	 2 ml/L $CaCl_2$

Figure 17. Conditions of cells at the end of treatment (Day 7)

IV.3. Result of Experiment 3. Treatment of selected *Spirogyra* sp. cultivation in absorbing calcium ($CaCl_2$) with different irradiation time

Calcium concentration in water

Ca concentration in water (culture medium) increased at the end of the observation, both in the period of irradiation treatment 12 hours/day and 24 hours/day (Figure 18).

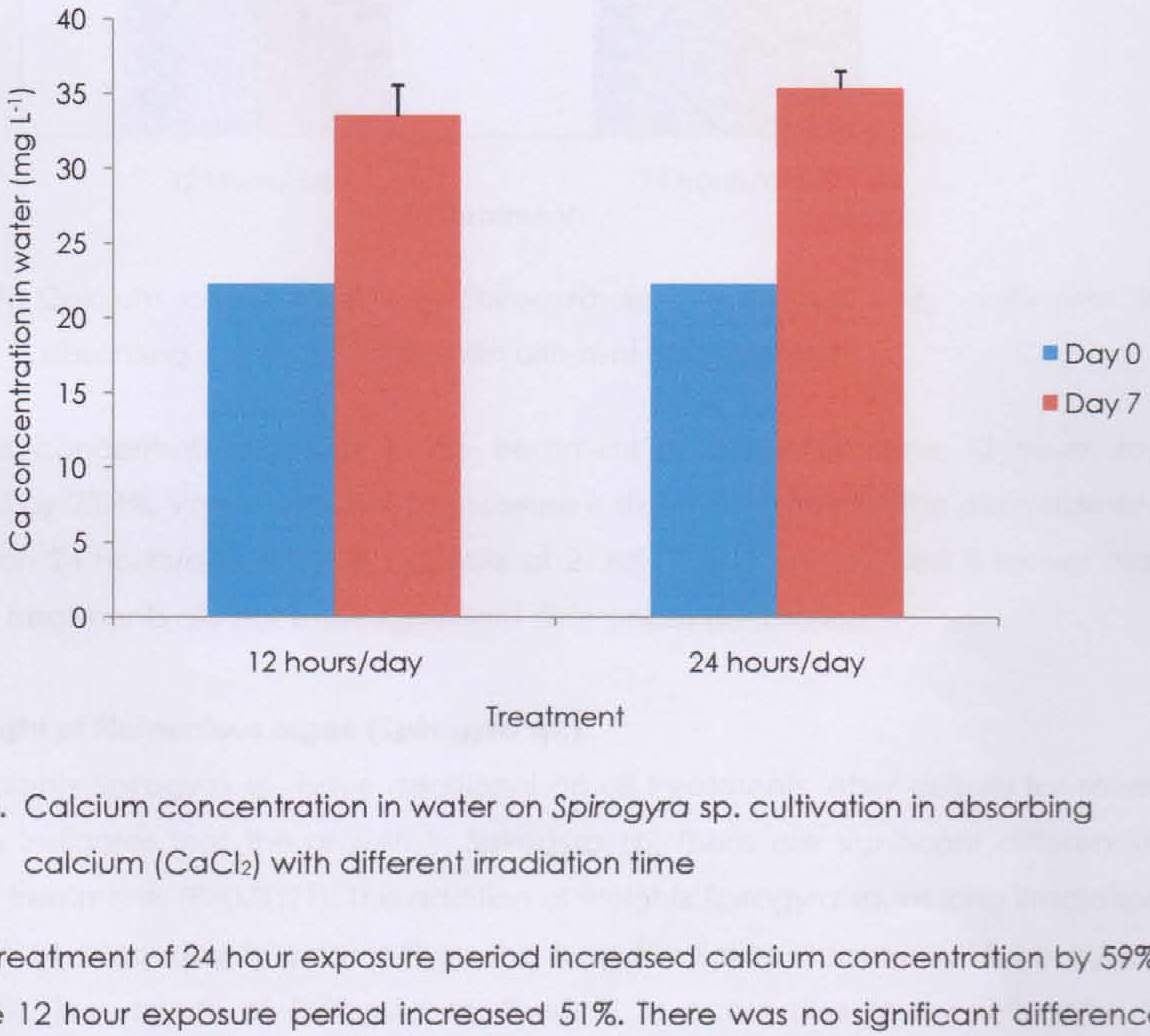


Figure 18. Calcium concentration in water on *Spirogyra* sp. cultivation in absorbing calcium ($CaCl_2$) with different irradiation time

Treatment of 24 hour exposure period increased calcium concentration by 59%, while the 12 hour exposure period increased 51%. There was no significant difference between treatments (P=0.4804). The presence of calcium in water media trial alleged besides comes from the addition of calcium ($CaCl_2$), can also be derived from the breakdown of water Ca contained in the culture medium.

Calcium concentration in filamentous algae (*Spirogyra* sp.)

Ca concentration in *Spirogyra* sp. also increased at the end of the observation in all treatments (Figure 19). *Spirogyra* sp. while irradiation in the treatment of 12 hours/day is thought to have relatively better ability to absorb Ca than long irradiation treatment 24 hours/day.

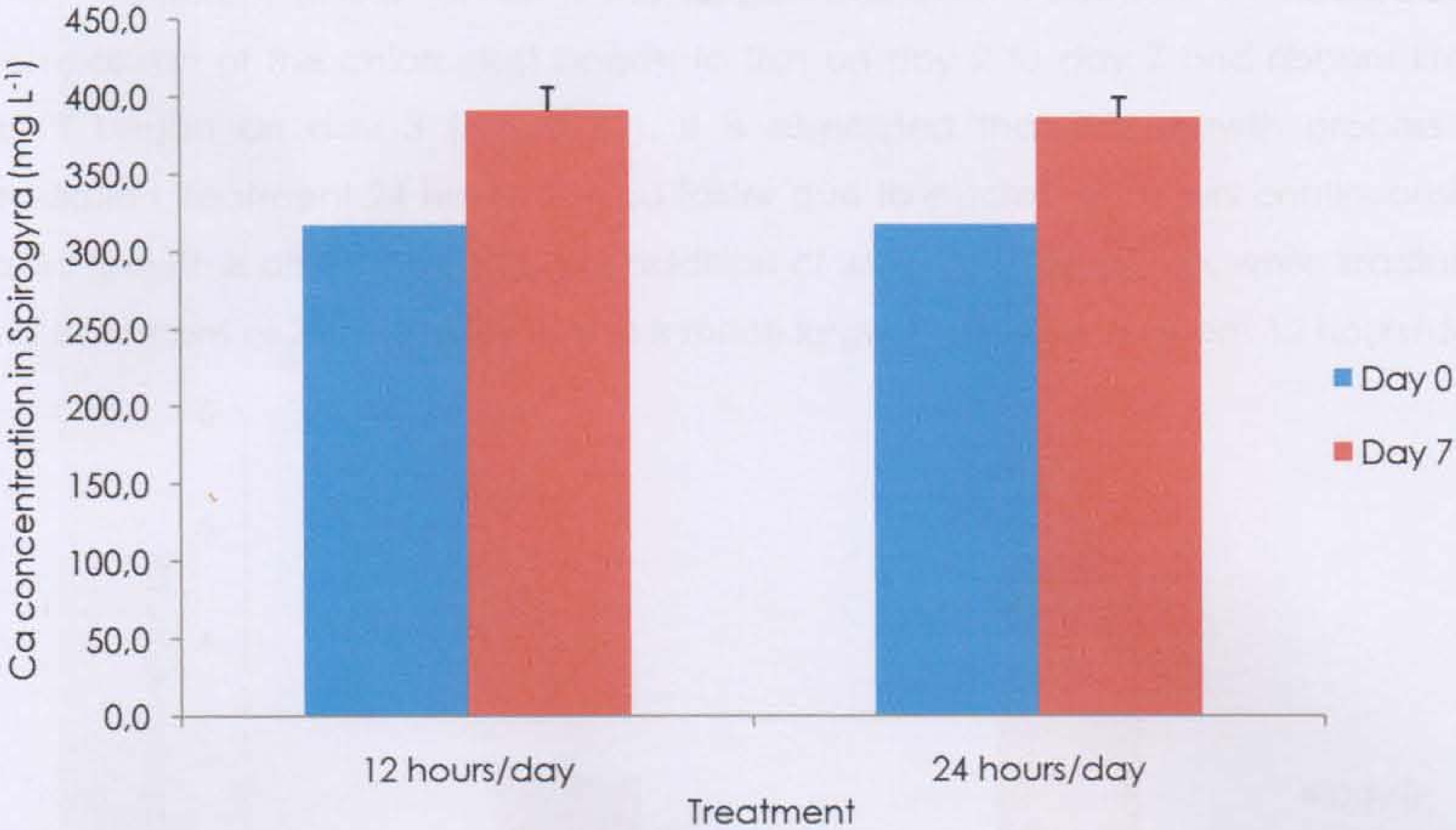


Figure 19. Calcium concentration in *Spirogyra* sp. on *Spirogyra* sp. cultivation in absorbing calcium ($CaCl_2$) with different irradiation time

The concentration of Ca in the treatment of irradiation time 12 hours/day increased by 23.4%. Value percentage increase is slightly higher than the old treatment of radiation 24 hours/day, with an increase of 21.8%. Based on the t test is known that between treatments did not show significant differences (P=0.8086).

Fresh weight of filamentous algae (*Spirogyra* sp.)

Weights Spirogyra sp. have additional on all treatments, after culture for seven days. This indicates that the growth in *Spirogyra* sp. There are significant differences between treatments (P=0.0021). The addition of weights *Spirogyra* sp. at long irradiation treatment 24 hours/day is greater than the long irradiation treatment 12 hours/day (Figure 20). The growth of *Spirogyra* sp. thought to occur due to the influence of nutrients in the growing medium that provides essential nutrients that algae needs in general.

Based on the microscopic observation of the cells *Spirogyra* sp., during the seven days of culture, there are known changes in the composition of *Spirogyra* sp. Chloroplasts. Chloroplasts at the beginning of treatment (day 0) arranged in neat form spiral ribbons with clearly pirenoid composition. The composition of the chloroplast began to thin on day 4 to day 7 in the long irradiation treatment 12 hours/day, but still spiral ribbons neatly arranged (Figure 21).

Different results found in the longer irradiation treatment 24 hours/day. The composition of the chloroplast began to thin on day 2 to day 7 and ribbons irregular spiral began on day 3 (Figure 22). It is suspected that the growth process while irradiation treatment 24 hours/day go faster due to irradiation occurs continuously. This rapid growth is also marked by the addition of weights *Spirogyra* sp. while irradiation in the treatment of 24 hours/day which is much larger than the treatment 12 hours/day.

Figure 20. Fresh weight of *Spirogyra* sp. on *Spirogyra* sp. cultivation in absorbing calcium ($CaCl_2$) with different irradiation time

Water quality conditions

pH values during the study ranged from 7.66 to 8.92 for radiation period 12 hours on light conditions and 7.57 to 8.74 in dark conditions, whereas the 24-hour exposure period pH values tend to be higher, with a range of values 7.72 -9.01. An increase in the pH value is presumably due to the utilization of inorganic carbon in the form of CO_2 by *Spirogyra* sp. Generally, the pH had a significant increase in the algae culture.

Temperature values during the study period ranged 19,7-22,1°C 12 hours of irradiation on the light conditions and 19,3-20,7°C in dark conditions, whereas the 24-hour exposure period temperature values tend to be higher, ranging from 20,7-22,1°C. This is presumably related to differences in the quantity of heat from the light received by the culture medium.

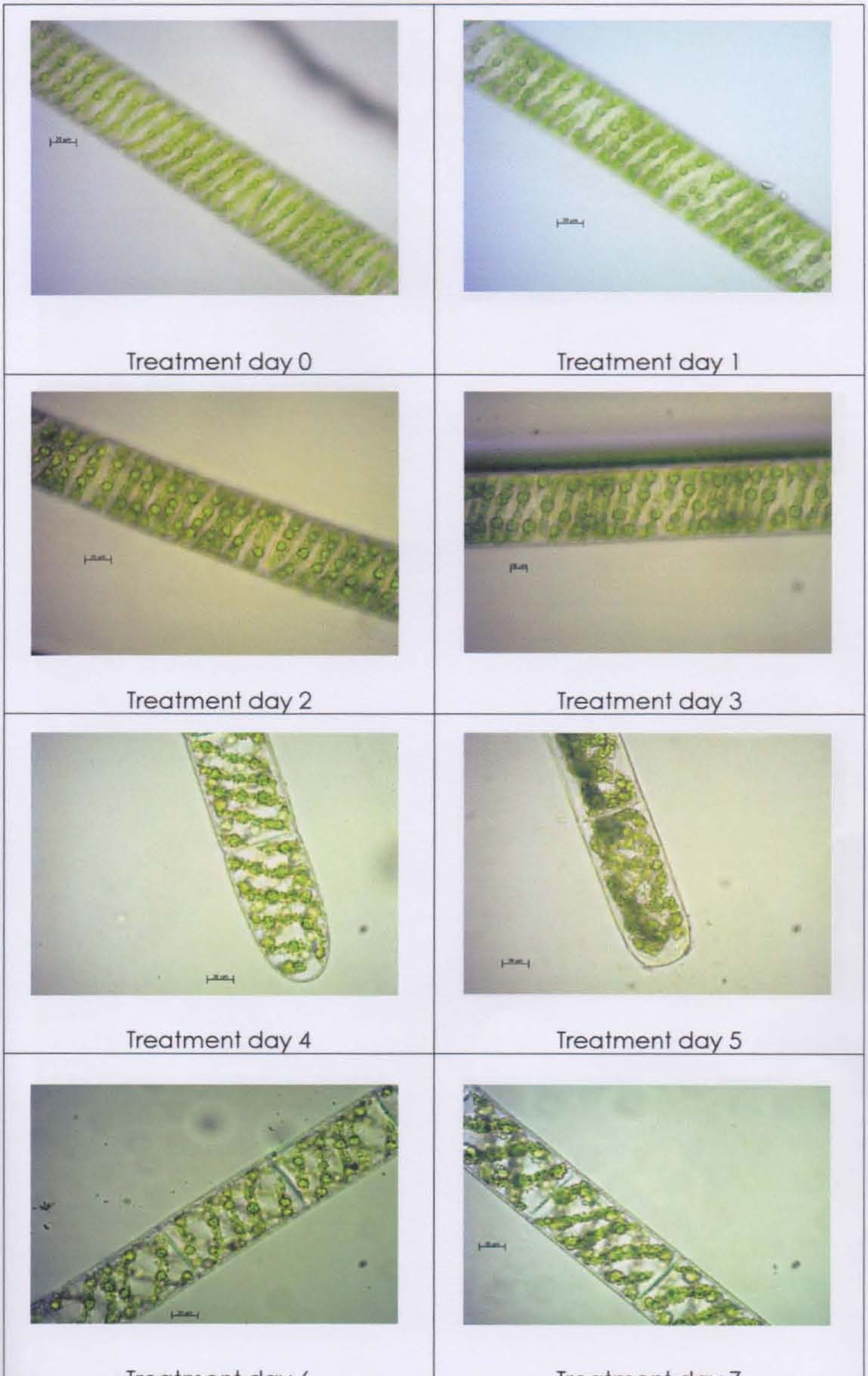


Figure 21. Photo cell *Spirogyra* sp. light treatment 12 hours/day

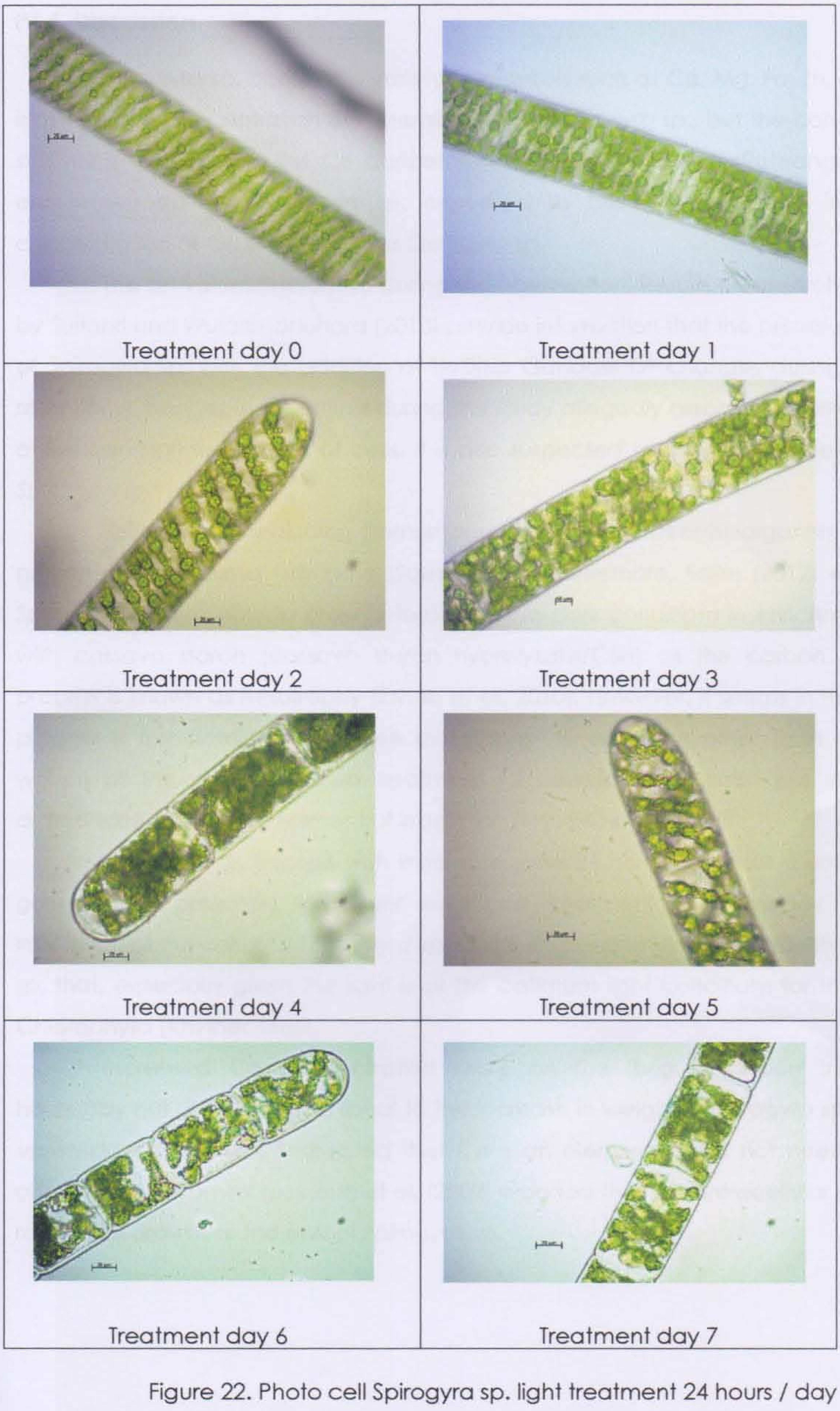


Figure 22. Photo cell Spirogyra sp. light treatment 24 hours / day

IV.4. Discussion

Spirogyra sp. contains a variety of minerals such as Ca, Mg, Fe, Zn, and Cu. Ca is the highest concentration of minerals found in *Spirogyra* sp., but the concentration is still much smaller than the Ca concentration found in *Spirulina* (Rutikanga 2011). Ca enrichment cultures *Spirogyra* sp. expected to be an attempt to increase the concentration of Ca in the biomass *Spirogyra* sp.

The pH values fluctuated during the observation. Results of research conducted by Sulfahri and Wulanmanuhara (2013) provide information that the pH value in cultures of Spirogyra sp. with the addition of fertilizer Gandasil D® changes during the 40-day retention. Changes in pH values during the study allegedly associated with the activity of ion transport in and out of cells. It is also suspected to affect the uptake of Ca by *Spirogyra* sp.

Spirogyra sp. including filamentous algae are autotrophs organisms with faster growth cycle within a few days (Salim 2012). Furthermore, Salim (2012) explains that *Spirogyra* sp. also able to grow heterotrophic in dark conditions in enrichment cultures with cassava starch (cassava starch hydrolyzate/CSH) as the carbon source. This process is known as mixotrophy (Eshaq *et al*. 2010). However, it seems in this study, the process is not dominant because mixotrophy no additions other than carbon, the weight of the long irradiation treatment 12 hours/day was also not increased as dramatically as in the treatment of irradiation time 24 hours/day.

Spirogyra sp. treated with irradiation time 24 hours/day had a greater weight gain when compared to longer irradiation treatment 12 hours/day. Continuous irradiation of the culture of *Spirogyra* sp. allegedly can stimulate the growth of *Spirogyra* sp. that, especially given the light is at the optimum light conditions for the growth of Chlorophyta (Rhyther 1965).

Increased Ca concentration more on the long irradiation treatment 12 hours/day not directly proportional to the increase in weight of *Spirogyra* sp. during the seven days of culture. Suspected that Ca is an element that is not needed in large amounts by *Spirogyra* sp. Inoue et al. (2002) reported that only intracellular Ca_2^+ plays a role in cell growth at the end of *Spirogyra* sp.

V. CONCLUSION AND RECOMMENDATION

5.1 Conclusion

$CaCl_2$ is the most effective type of calcium that absorbed by *Spirogyra* sp. The best $CaCl_2$ concentration added in *Spirogyra* cultivation was 0.5 mL/L medium at the optimum light intensity (500-750 ft cd). Ca absorption by *Spirogyra* sp. more effective at irradiation time 12 hours/day. Long exposures are directly proportional to the increase in weight of *Spirogyra* sp. Ca is an element that is not needed in large amounts by *Spirogyra* sp. in their growth process.

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