Study on carbon sinks by classified biofloc phytoplankton from marine shrimp pond water

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Abstract. Study on the carbon sink by biofloc phytoplankton from species *Oocystis* sp. (Chlorophyta) and *Chroococcus* sp. (Cyanophyta) was done from the study area of marine shrimp farm i- sharp Setiu, Terengganu. Three month of database for carbon sinks was collected from the selected phytoplankton in the pond. The biovolume of the phytoplankton was calculated using simple geometrical formula and carbon conversion factor to convert the biovolume of each class of phytoplankton to carbon sink amount of value. Overall, *Chroococcus* sp. was identified as the most abundance species found in the pond where it can sequestrate 14923.39 µ g C L^{-1} amount of carbon dioxide (CO_2) as compared to the amount of carbon can be sequestrated by *Oocystis* sp. which is around 3778.20 µ g C L^{-1}. It also can be claimed that *Chroococcus* sp. was more carbon dense compared to *Oocystis* sp. because of its bigger cell volume. It can be assumed that biofloc phytoplankton was successful in carbon sequestration and contributed as small part of carbon sinker in the world due to its ability to sequester CO_2 and high abundances in number found in the pond make its more efficient in sinking carbon dioxide.
Key words: carbon sink, biofloc, phytoplankton, bio volume, geometrical formula, CO_2 sequestration.

Introduction. In the intensive shrimp farming, ecosystem of the shrimp pond is the basic tool in producing a healthy and highly profitable value of products. For example, overload of nutrient in the farm also will cause eutrophication in the pond and affect the ecosystem and the shrimp itself. Other important factor like the carbon budget in the pond ecosystem also must be in balance to ensure that the pond water is not too acidic to the entire living organisms in the pond (Tucker & D'Abramo 2008). In the shrimp farm that practicing the biofloc technology (BFT), the water quality of the pond can be maintained a long period of time with 0% of water exchange. Biofloc consists of high abundances of microorganisms (bacteria, phytoplankton, zooplankton protozoa and algae) that floc together and become an additional food for shrimp and some of them function as the stabilizer for the water maintenance without needed water exchange (Hargreves 2013; Avnimelech 2012). Nitrifying bacteria such as *Nitrosomonas* sp. and *Nitrobacter* sp. is one of the important decomposers bacteria that degrade the sludge, dead plankton, faeces, wastes and organic matter from the shrimps in the pond. It converts the ammonia to nitrite and nitrate and keeps the pond water in the stable condition (Perez-Rostro et al 2014). Concurrently the carbon budget in the pond also must be in the balance situation. Phytoplankton in the pond that consists of Chlorophyta (green algae), Dynophyta (dinoflagelles), Chrysophyta (golden-brown algae), Cyanophyta (cyanoobacteria) and other type of algae group operates as the carbon sink in the shrimp pond through the photosynthesis process. The phytoplanktons are capable on converting the dissolved carbon dioxide (CO_2) to oxygen (O_2) and energy (glucose) (Lindsey & Scott 2010). Contrary with the decomposers bacteria, they react as the carbon emitter in the pond which taking up O_2 in the pond and create CO_2 in the pond. The shrimp itself also contribute to the release of CO_2 to the pond ecosystem and to the atmosphere. Tonne of shrimp produced each year also will contribute tonne of carbon footprint emission to the atmosphere. Sayre (2010) discovered that microalgae are among the most productive biological systems for generating biomass and as a natural carbon attractor. Microalgae has an ability to transport bicarbonate into cells that makes them efficient for carbon sequestration as the carbon dioxide or bicarbonate are 90% captured by microalgae in the open pond. According to Konoplya & Soares (2011) the method for calculating algal biomass may be direct such as the cell count, biovolume, and estimation of chlorophyll.

Based on these methods, this paper is focusing on the study of carbon sinking by two selected species of biofloc microorganisms which are from Cyanophyta group (*Chroococcus* sp.) and Chlorophyta group (*Oocystis* sp.) using biovolume method of calculation. These two species of phytoplankton were among the most abundance phytoplankton species which can be found in the every sampling station pond and study done on the carbon sequestration by these two species must be valuable in the future.

Material and Method. Water samples consisting of biofloc microorganisms were collected from intensive shrimp farm I-sharp, Setiu, from March 2013 until August 2013. Six sampling points were reviewed, which are main inlet, 4 ponds consisting of cultured shrimp of different ages and, finally, the main outlet. The samplings were done weekly in each pond. YSI 556 multi-probe instrument was used for water parameter analysis and data for each station was taken and recorded. Three litres of water samples were filtered using 20 µm mesh size of plankton net and the filtered samples of microorganisms were preserved in 4% formalin solution. Samples were immediately transported to the laboratory for further analysis. The microalgae and phytoplankton abundance was calculated using haemocytometer and Lackey's methods (APHA 1989). The taxa of the plankton were identified under advance microscope (Nikon 80i) and also a light microscope for direct size measuring purpose. Carbons sink for the microorganisms was calculated using carbon conversion factor for phytoplankton. Cell were counted and sized for height (µm), width (µm) and diameter (µm) at an appropriated magnification using advance microscope (Nikon Eclipse 80i) and the samples volume were calculated using simple geometric formula (Sun & Liu 2003; Vadrucci et al 2007). Volume was converted to the unit of carbon according to Strathmann (1967). Biovolume for phytoplankton was converted to carbon content using conversion factor (Lundgren 1978).

Data collection and pre-processing. Carbons sinking by microorganism (*Chroococcus* sp. and *Oocystis* sp.) were calculated from the bio volume of the organisms by using carbon conversion factor. Conversion factor for phytoplankton, 16% wet weight of Chlorophytes and, 14% wet weight of Dinophytes, 22% of wet weight for Cyanophytes and 11% of wet weight for all others algae (Rocha & Duncan 1985).

Results and Discussion. Through the biovolume calculation, it more focus on the calculation of phytoplankton species of biomass per se rather than estimation of phytoplankton division such as Chlorophyta, Cynophyta, Egulenophyta group through chlorophyll method calculation. From the data analysis of carbon sink by Cyanobacteria from *Chroococcus* sp., it was found that highest carbon sink occurred in May (week 3) which is 14923.39 µ g C L^{-1} (Figure 1). Abundance of *Chroococcus* sp. identified in the water column will help sinking highest CO_2 from the photosynthesis process. This is confirmed by the study of Jansson & Northen (2010), who found out that Cyanobacteria and eukaryotic algae most effectively use bicarbonate and transport bicarbonate as a source CO_2 of for photosynthesis.

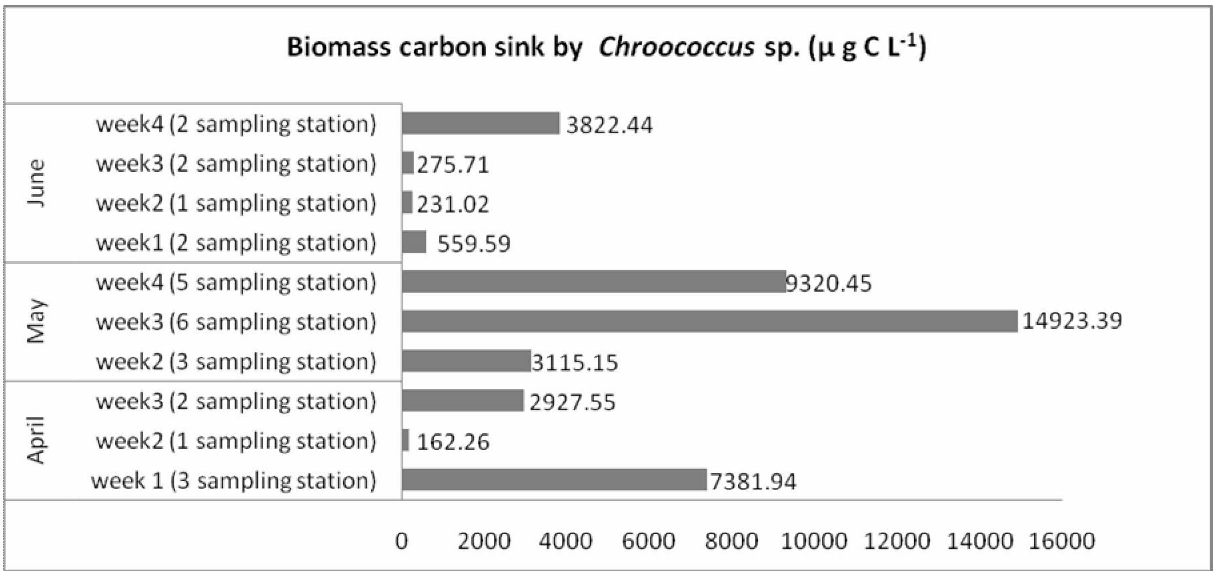

Figure 1. Carbon biomass sinking by *Chroococcus* sp. according to months. Sampling station was from different shrimp pond sampled.

In April 2013, carbon sink was highest in pond 51011 around 7243,86 µ g C L^{-1} in week 1 and also found highest in pond 71312 around 1589.59 µ g C L^{-1} in week 3 (Figure 2).

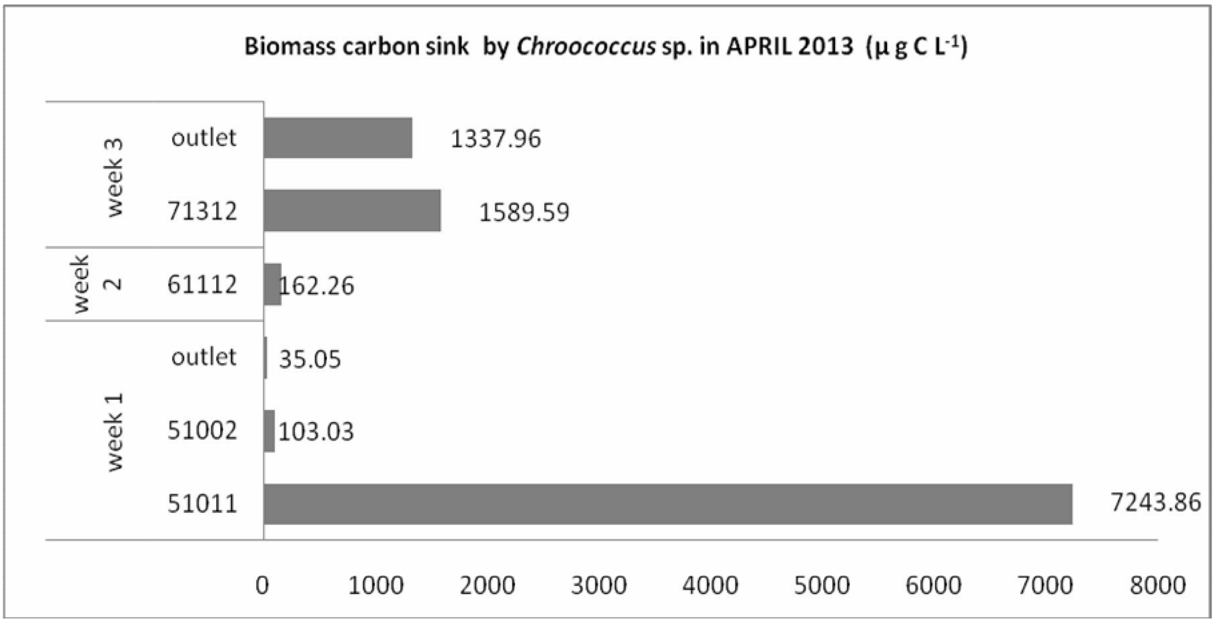

Figure 2. Carbon biomass sinking by *Chroococcus* sp. collected for April 2013.

In May 2013, *Chroococcus* sp. was found mostly in all sampling ponds in week 2, week 3 and week 4. Carbon sink were identified highest in week 3 from main inlet station around 8825.47 µ g C L^{-1} also in outlet around 3044.44 µ g C L^{-1}. In week 4, carbon sink was highest in main outlet followed by sampling pond 20412 the carbon sequestration from the *Chroococcus* sp. is around 2518.44 µ g C L^{-1} (Figure 3).

In June 2013, carbon sink was higher in week 4 from pond 20401 around 3669.9 µ g C L^{-1} (Figure 4). This is because abundance number of *Chroococcus* sp. was identified in the pond same with other species of phytoplankton.

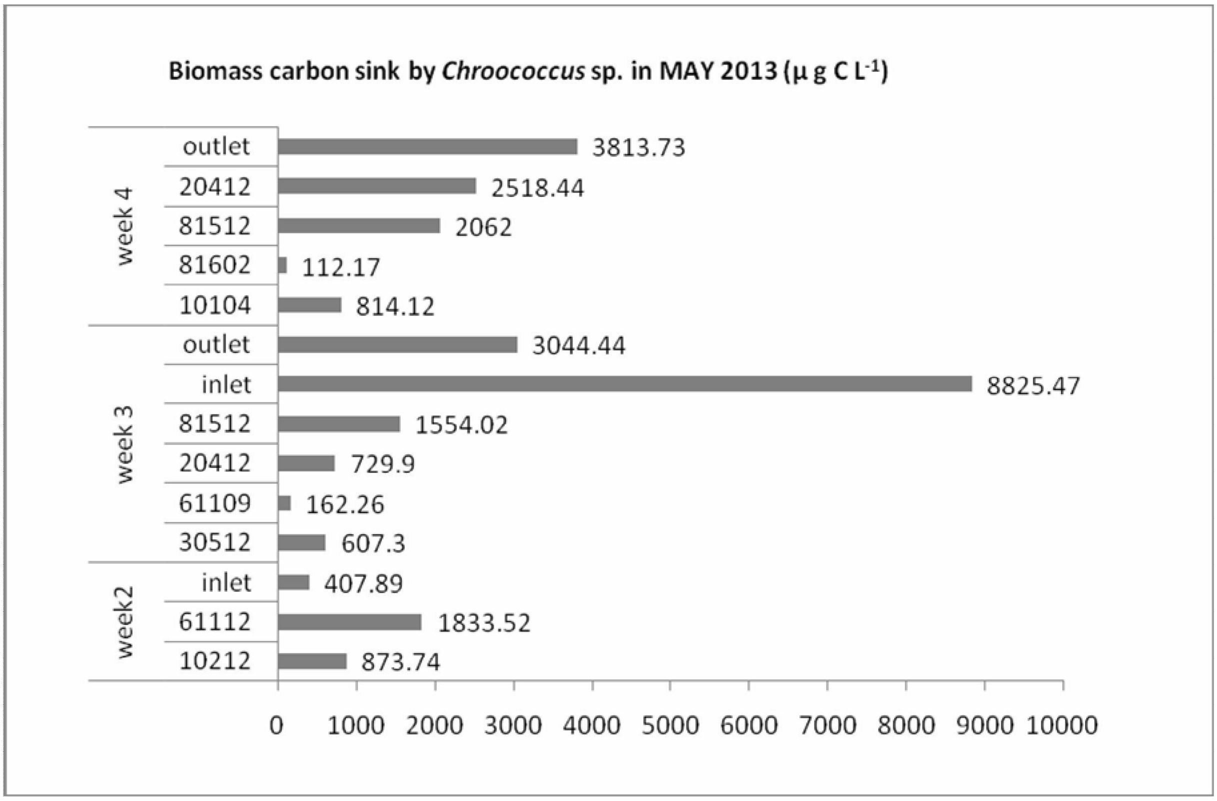

Figure 3. Carbon biomass sinking by *Chroococcus* sp. collected in May 2013.

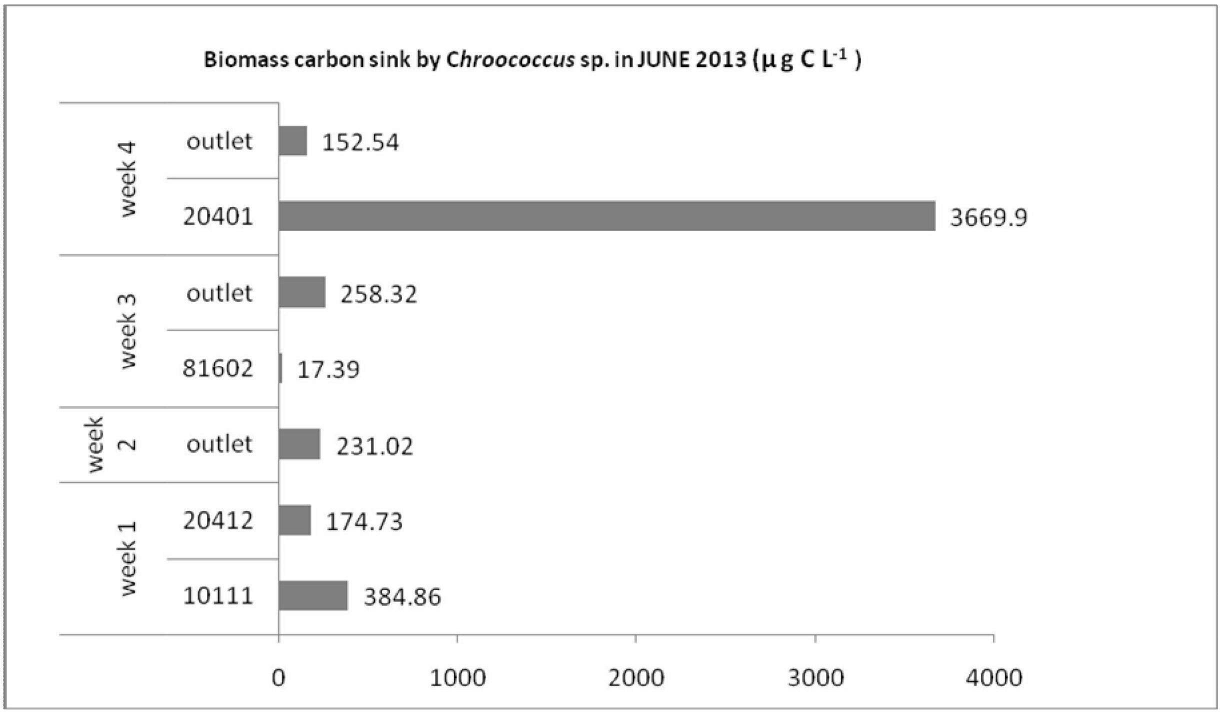

Figure 4. Carbon biomass sinking by cyanobacteria (*chroococcus* sp) collected in June 2013.

For carbon sink by *Oocystis* sp., carbon sink was found higher in March in week 4 around 3778.20 µ g C L^{-1} from main outlet (Figure 5; Figure 6). Higher carbon sink also were found in June, week 2 around 2374.11 µ g C L^{-1} and also found highest in May in week 5, around 2018.14 µ g C L^{-1} (Figure 5).

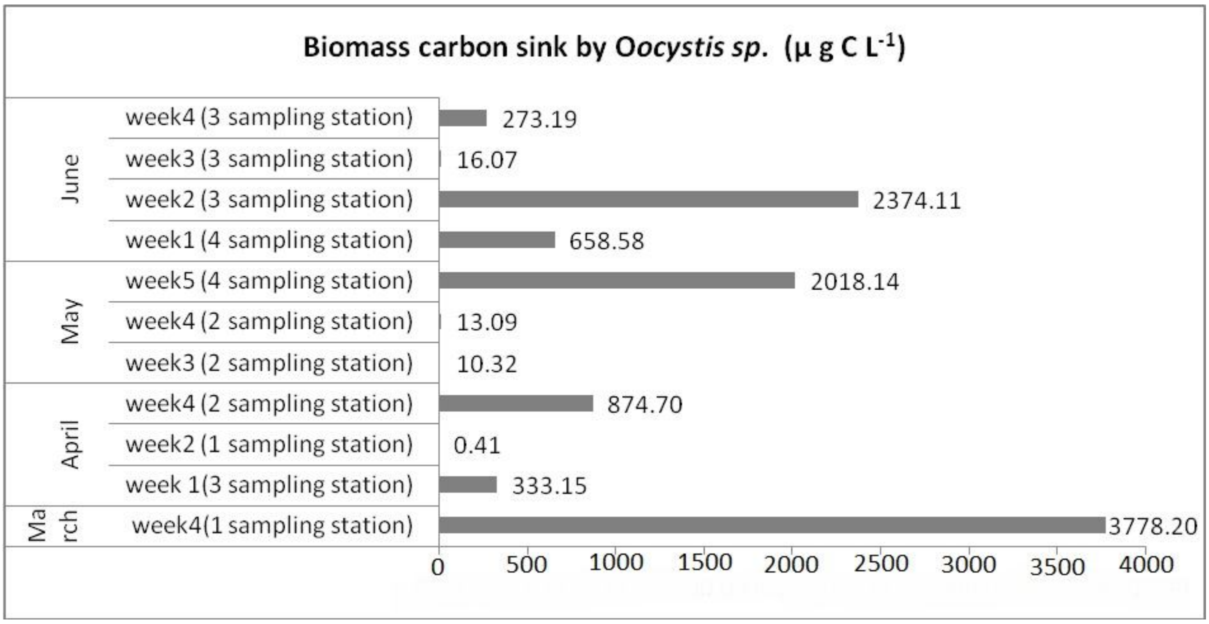

Figure 5. Carbon biomass sinking by *Oocystis* sp. according to months. Sampling station was from different shrimp pond sampled.

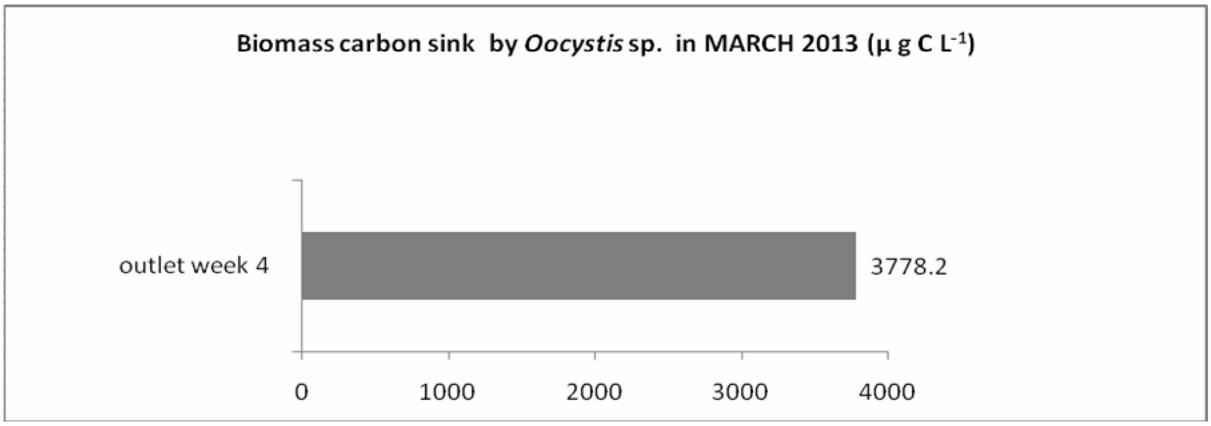

Figure 6. Carbon biomass sinking by *Oocystis* sp. collected in March 2013.

In April 2013, carbon sink was identified highest in pond 71312, around 869.97 μ g C L^{-1} in week 4 and also in week 1 from pond 51011, around 319.19 μ g C L^{-1} (Figure 7).

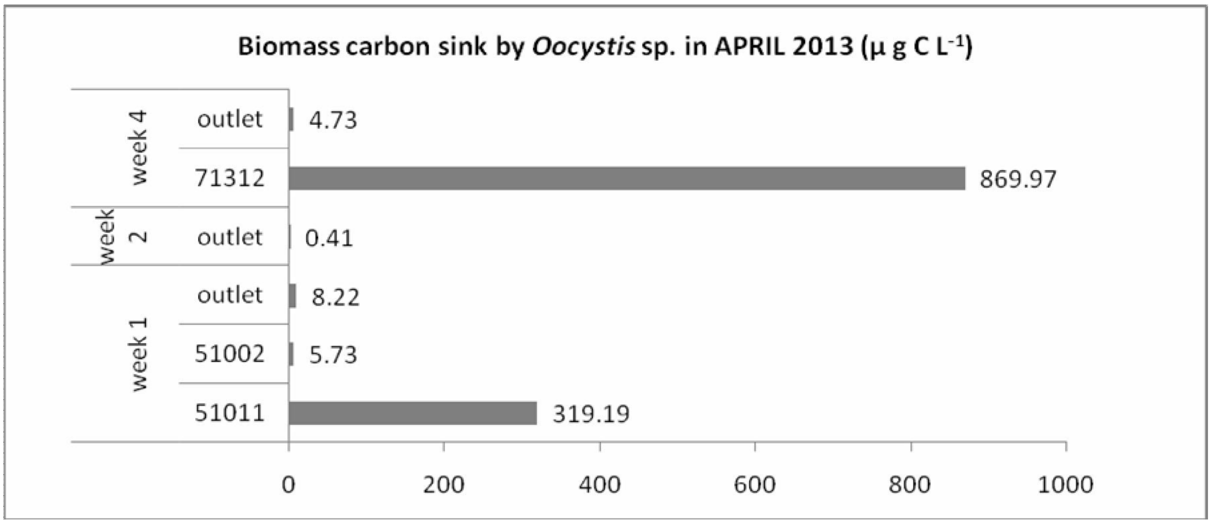

Figure 7. Carbon biomass sinking by *Oocystis* sp. collected in April 2013.

In May 2013 carbon sink was only found higher in week 5 from pond 10104, around 1464.11 μ g C L^{-1}. Carbon sink from other ponds was in small rate in week 3 and week 4 in range 0.24–12.85 μ g C L^{-1} from sampling pond (Figure 8).

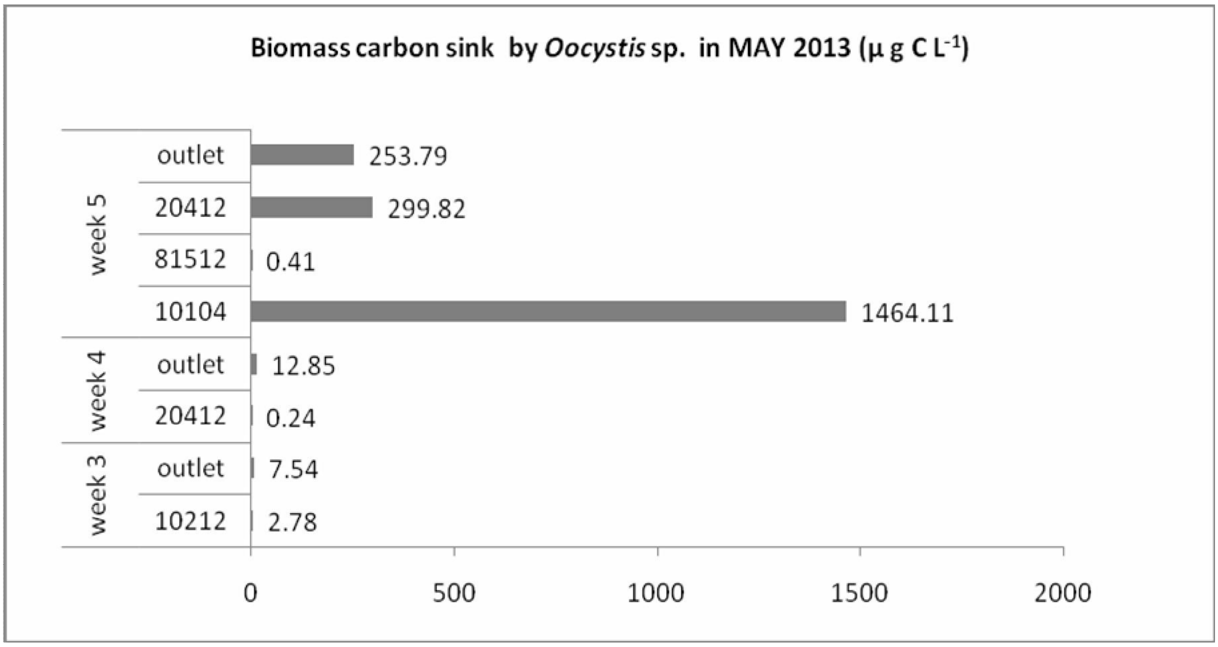

Figure 8. Carbon biomass sinking by *Oocystis* sp. collected in May 2013.

In June 2013, higher carbon sink was identified in week 2 from pond 20412, around 2356.14 μ g C L^{-1}. It is because of higher density of *Oocystis* sp. was available in the pond 020412. In week 1 the carbon sink from 20412 pond was the highest amount compared to other ponds. In week 3, all ponds showed small amount of carbon sink around 0.6-12.72 μ g C L^{-1} for pond 81602, pond 20412 and outlet (Figure 9).

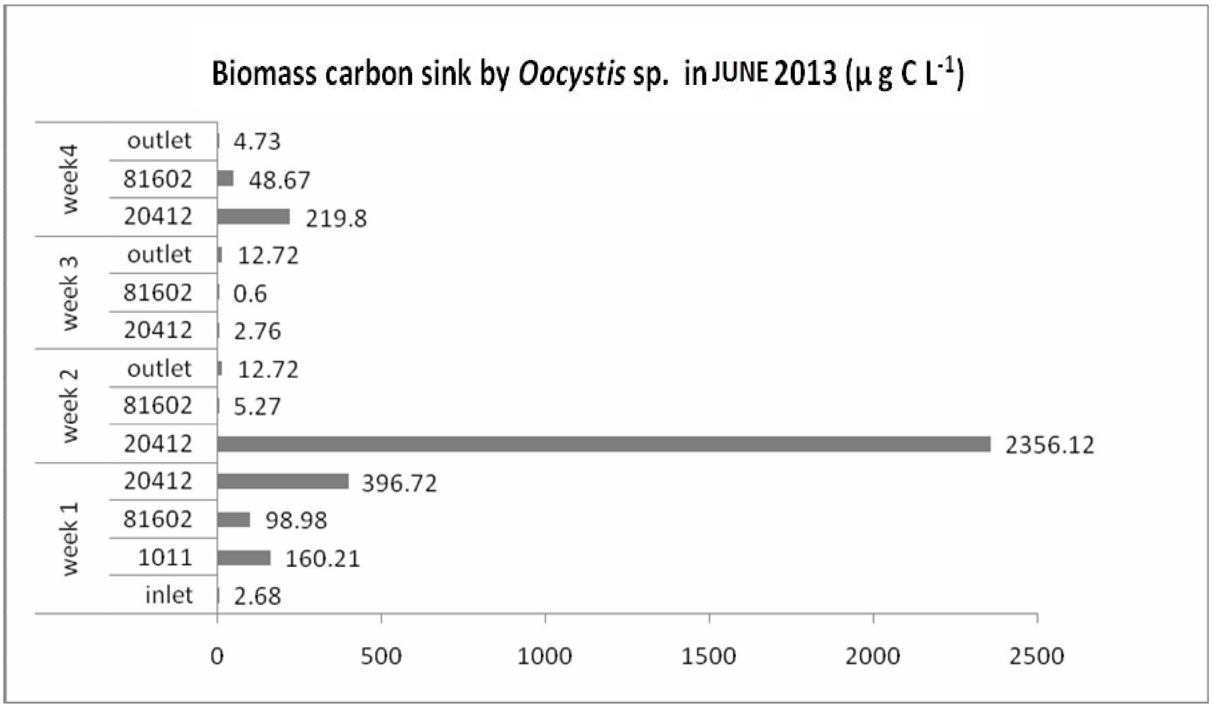

Figure 9. Carbon biomass sinking by *Oocystis* sp. collected in June 2013.

Overall from the data, *Chroococcus* sp. was identified as the most abundant species found in the shrimp farm as it can sequestrate 14923.39 μ g C L^{-1} of carbon dioxide compared to *Oocystis* sp., the green algae group. *Oocystis* sp. highest carbon sink was only 3778.20 µ g C L^{-1} in March (week 4) compared to carbon sink by *Chroococcus* sp. from cyanobacteria group. It also can be claimed that *Chroococcus* sp. (Figure 10) was more carbon dense than *Oocystis* sp. (Figure 11) because of its bigger cell volume. It confirms the literature from the review that the positive biovolume influences the carbon biomass in phytoplankton (Gotsis-Skretas & Ignatiades 2010).

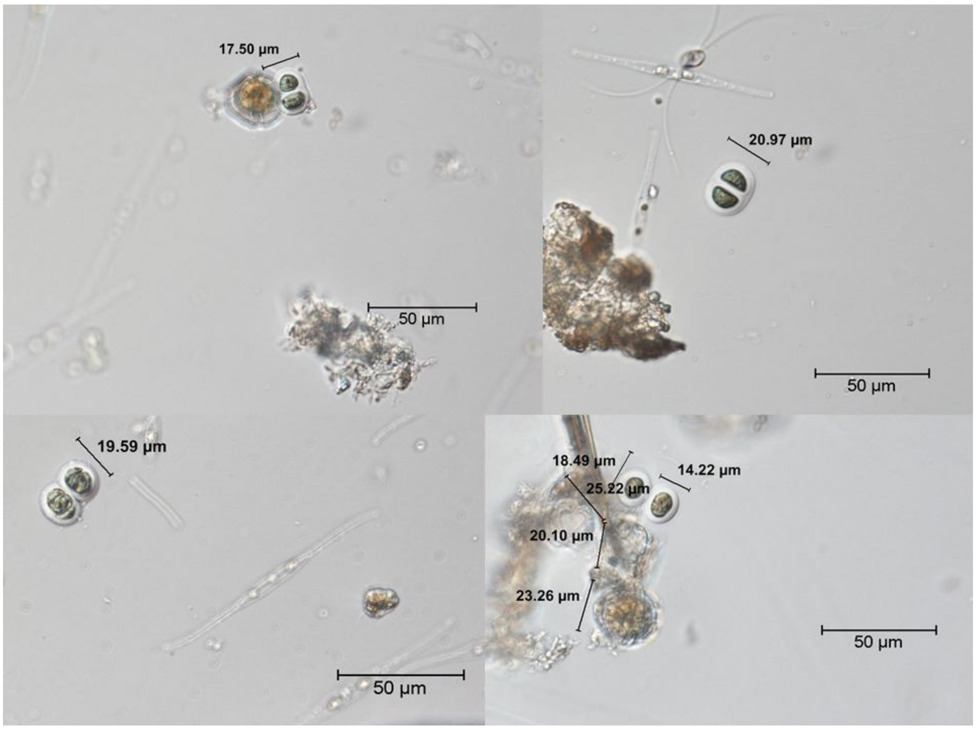

Figure 10. *Chroococcus* sp. bio volume estimation using sphere geometrical shape. Picture was taken and sized using Advance microscope Nikon 80i.

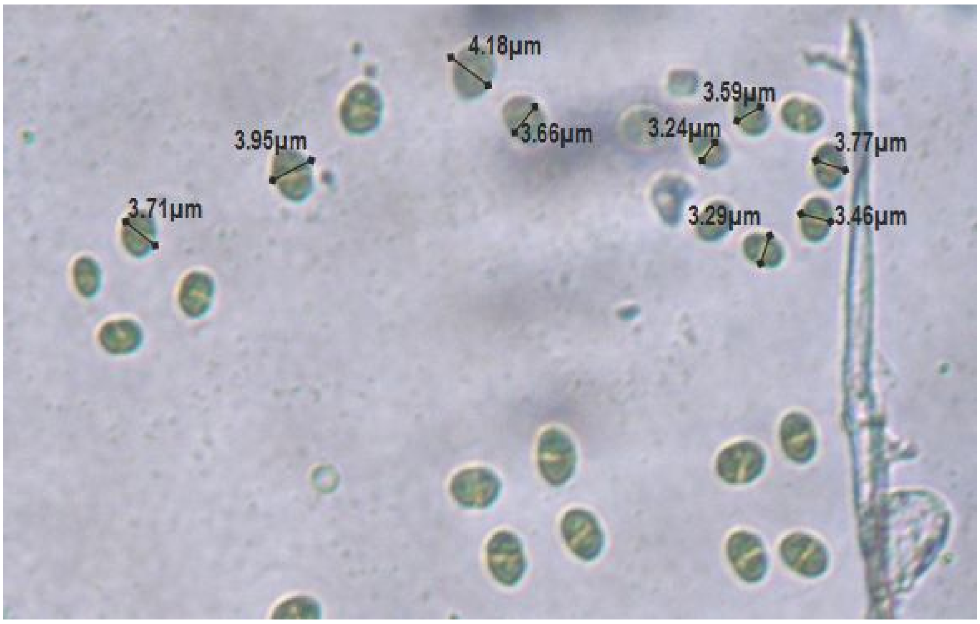

Figure 11. *Oocystis* sp. biovolume estimation using prolate spheroid geometrical shape. Picture was taken and sized using Advance microscope Nikon 80i.

The highest concentration of biomass in phytoplankton from the biovolume estimation confirmed that the biofloc also contributed as the carbon sequestration. Biofloc phytoplankton was also capable to absorb and sequestrate the CO_2 produced from the biological processes and activities done by microbials in biofloc. This is in agreement with

Levinton (2001) and Raven et al (2001) that planktonic algae is an important primary producer and can fix carbon in their biomass by the photosynthetic processes.

Conclusions. It was identified that the phytoplankton aggregates in the biofloc was also helpful in sink carbon and store it in the biomass form. Phytoplankton in biofloc was successful in sequestrated carbon dioxide from the atmosphere as according to the higher biomass identified from the two species of *Oocystis* sp. around 3778.20 µ g C L^{-1} and *Chroococcus* sp. around 14923.39 µ g C L^{-1} measured.

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