

The Control of *Microcystis* spp. Bloom by Combining Indigenous Denitrifying Bacteria From Sutami Reservoir with *Fimbristylis globulosa* and *Vetiveria zizanoides*

Bayu Agung Prahardika, Catur Retnaningdyah*, Suharjono

Biology Department, Faculty of Mathematics and Natural Sciences, Brawijaya University, Malang, Indonesia

ABSTRACT

The purpose of this research is to know the ability of polyculture macrophyte (*Fimbristylis globulosa* and *Vetiveria zizanoides*) and the combination of both with consortium of indigenous denitrifying bacteria from Sutami reservoir that was added by *Microcystis* spp. or not to reduce the concentration of nitrate, dissolved phosphate and the carrying capacity of *Microcystis* spp. The experiment was done in a medium filled up with Sutami reservoir water enriched with 16 ppm of nitrate and 0.4 ppm of phosphate. The denitrifying bacteria used in this research were DR-14, DU-27-1, DU-30-1, DU-30-2, TA-8 and DU-27-4 isolated from Sutami reservoir. The treatments were incubated within 15 days. *Microcystis* spp. abundance was calculated every day, but the measurement of the concentration of nitrate and dissolved phosphate was done every six days. The results showed that both treatment and the combination of both macrophytes with a consortium of denitrifying indigenous bacteria were added or not either *Microcystis* able to reduce nitrate at 99% and 93-99% orthophosphoric. The combination of macrophytes with denitrifying indigenous bacterial consortium from Sutami reservoir was able to inhibit the carrying capacity of *Microcystis* spp. highest up to 47.87%. They could also significantly reduce the abundance of *Microcystis* from 10^7 cells/mL in earlier days of the treatment into 0.35×10^4 cells/mL after fifteen days of incubation.

Keywords: control of *Microcystis*, denitrifying bacteria, *Fimbristylis globulosa*, Sutami reservoir, *Vetiveria zizanoides*

INTRODUCTION

Sutami reservoir located in the Karangates Village, Sumber Pucung District, Malang, is the largest reservoir in the province of East Java. Sutami reservoir is one of the artificial aquatic ecosystems that are multifunctions. Some of them include electricity generation, flood control, irrigation for agriculture, fishery as well as one of tourist attractions in Malang [1]. In 2002, the Sutami Reservoir once bloomed *Microcystis* spp. The result of the monitoring carried out in 2004-2006, *Microcystis* spp., together with *Ceratium* and *Synedra* always had the highest number of population in the reservoir [2].

This phenomenon was probably caused by an increase in nutrients resulting in nutrient

concentration and imbalance ratios that existed in these waters (eutrophication). Microalgal explosion resulted in the reduced productivity of fisheries. *Microcystis* spp. is one type of cyanobacteria that can produce toxins which are harmful to other living things [3]. Accordingly, it is used in this research effort control, using bioremediation techniques to incorporate the denitrifying indigenous bacterial consortium in Sutami reservoirs and macrophyte. The macrophyte used is Mendong (*Fimbristylis globulosa*) and Akar Wangi (*Vetiveria zizanoides*).

MATERIALS AND METHODS

The experimental study was conducted purely in a laboratory scale using a completely randomized design with treatments as follows:

- a. Sutami reservoir water + Wetland soil, and + *Microcystis* spp.
- b. Sutami reservoir water + Wetland soil, and without *Microcystis* spp.
- a. (*F. globulosa* + *V. zizanoides*) + Sutami reservoir water + Wetland soil, and +

*Corresponding author: Catur Retnaningdyah
Biology Department, Faculty of Mathematics and Natural Sciences, Brawijaya University, Jl. Veteran, Malang, Indonesia 65145
Email: catur@ub.ac.id

- Microcystis* spp.
- b. (*F. globulosa* + *V. zizanooides*) + Sutami reservoir water + Wetland soil, and without *Microcystis* spp.
3. a. StokKulturBakteri + Sutami reservoir water + Wetland soil, and + *Microcystis* spp.
 - b. StokKulturBakteri + Sutami reservoir water + Wetland soil, without *Microcystis* spp.
4. a. StokKulturBakteri + (*F. globulosa* + *V. zizanooides*) + Sutami reservoir water + Wetland soil, and + *Microcystis* spp.
 - b. StokKulturBakteri + (*F. globulosa* + *V. zizanooides*) + Sutami reservoir water + Wetland soil, and without *Microcystis* spp.

Thus, the treatments in this study consisted of eight repetitive actions of each three times at the same time. Observations made during the fifteen days, including the calculation of the number of *Microcystis* spp., was conducted every day and the measurement of levels of nitrate and orthophosphoric was conducted every six times a day.

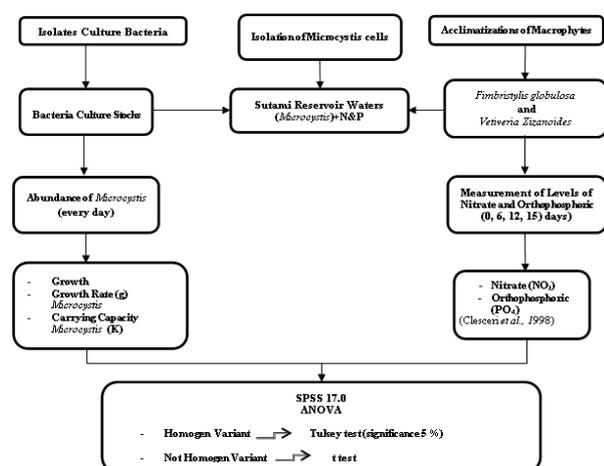


Figure 1. Operational framework of research

Potential test was conducted in a bucket with a diameter of 30 cm. The medium comprised a bucket of water 6 litres Sutami reservoir and 12 kg wetland soil treated with the addition of nitrate administration of 16 mg/L and orthophosphoric as much as 0.4 mg/L. The kinds of macrophyte used in this study were different from one to another depending on the weight of each macrophyte calculated from the area. The weight was valued by closing the Macrophyte that was equal to 25% of the surface area of the bucket. The assay was performed as a preliminary study. All treatments performed an addition of nitrate of 16 mg/L and orthophosphoric as much as 0.4 mg/L. Each

treatment was repeated three times at the same time, and each was carried out in a bucket with a diameter of 30 cm. The observations were made within 15 days and conducted in a greenhouse.

RESULTS AND DISCUSSION

The nitrate concentrations at the beginning of the treatment varied from 18.51 to 23.32 mg/L (Figure 2). This ranging value was previously anticipated because of the variation in nitrate content in the water from Sutami reservoirs, the medium used for the treatment. Based on the t test each time the concentration of nitrate observations is compared with the day-to-zero (Figure 2), it can be concluded that the remediation process resulted in lower nitrate concentrations significantly in each treatment medium from the sixth day of observation, that is to be 3-8 mg/L the percentage of 57-83% reduction. Decrease in the higher on the day of the twelfth and fifteenth, respectively, to 0.02 to 0.65 mg/L and 0.01 to 0.09 mg/L. The percentage decrease in nitrate in the medium each day for twelve observations is 96-99%, whereas on the fifteenth day of observation is more than 99%. Decrease in nitrate concentrations that occur are expected to affect the density of *Microcystis* cells in the medium treatment, which lowers the density of *Microcystis* cells, given the minimum nitrate concentration for the growth of algae in aquatic ecosystems is approximately 0.259 mg/L [4].

The nitrate concentrations in the medium control without the addition of nitrate decreased the number of *Microcystis* in the fifteenth day up to 99.68%, whereas in the control medium with the addition of nitrate, its number decreased approximately 99.74%. The cells of *Microcystis* spp. were able to utilize nitrate as a nitrogen source to form amino acids, chlorophyll and other organic compounds [5]. The decrease of nitrate in the medium macrophyte comprising the combination of *F. globulosa* and *V. zizanooides* without the addition of *Microcystis* in the fifteenth day reached about 99.94%, whereas the macrophyte combination in the medium with the addition of *Microcystis* was approximately 99.84%. The lower decrease in the nitrate macrophyte combination medium with the addition of *Microcystis* was allegedly caused by competition in the utilization of nitrate.

The decrease in nitrate concentration in the denitrifying bacteria consortium medium without the addition of *Microcystis* in the fifteenth day reached about 99.59%, while in the denitrifying

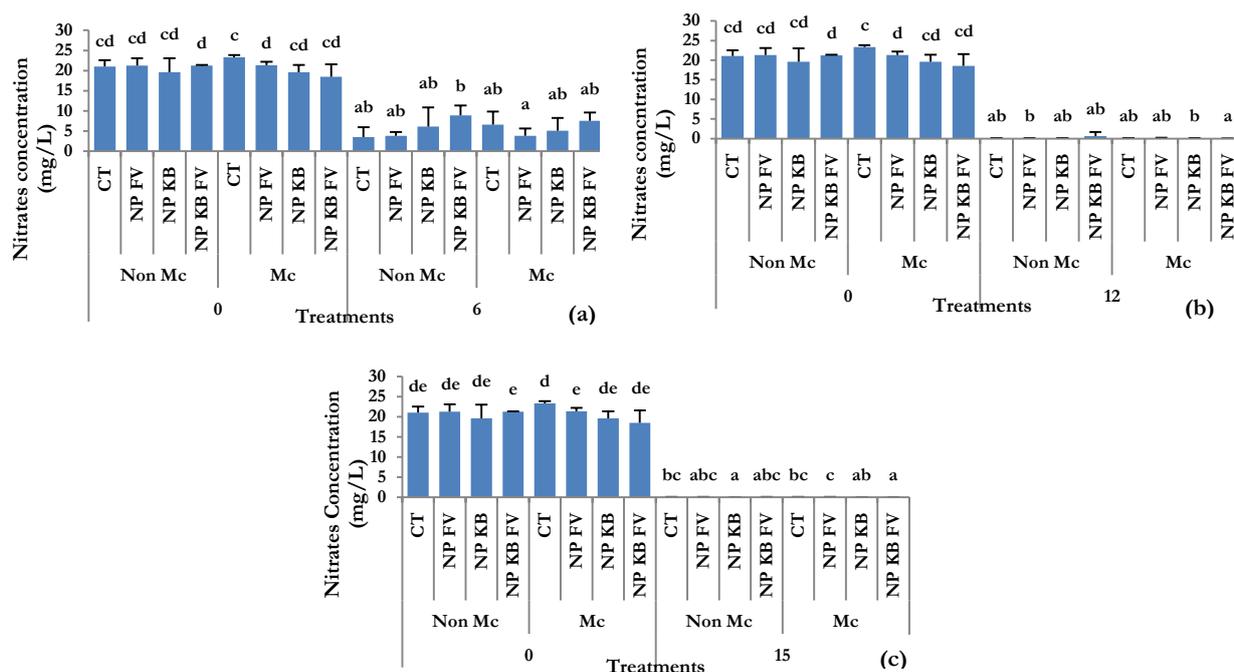


Figure 2. Comparison of the observed 0-6 days (a), 0-12 days (b), and 0-15 days (c). KT=Control; NPFV= Nitrate&Phosphate+(*F. globulosa*), (*V. zizanioides*); NPKB= Nitrate&Phosphate + Denitrifying bacteria indigenous consortium. The same letters on the block showed no significantly different by ANOVA followed by t test α 0.05

bacteria consortium medium with the addition of *Microcystis* the decrease was approximately 99.89%. The medium with the addition of *Microcystis* appeared to enable positive interaction between denitrifying bacteria consortium and *Microcystis*, so both organisms benefited from each other's nitrate contents taken from the environment. The denitrifying bacteria have the ability to secrete enzymes to break down nitrate reductase. The nitrate compounds are used as sources of nitrogen for metabolic processes in the growth period [6]. The decrease in nitrate concentration in the denitrifying bacteria consortium with a macrophyte combination medium, which was able to reduce the nitrate without the addition of *Microcystis* in the fifteenth day reached about 99.81%. Yet, in the same medium treatment with the addition of *Microcystis*, the decrease was approximately 99.93%. The decrease in the nitrate concentration in the sizable amount was allegedly due to the positive interaction between a denitrifying bacteria consortium in the combination of both macrophytes.

As was the case in nitrate, the concentration of orthophosphoric at the beginning of each treatment was also different (0.30 to 1.10 mg/L) (Figure 3). This is presumably due to the addition of orthophosphoric content into the Sutami reservoir media as a source of treatment media. The orthophosphoric concentrations in each treatment medium has had a significant

decrease ($p < 0.05$) after six days of incubation ranging from 0.04 to 0.09 mg/L. Nevertheless, on day twelve and fifteen the concentrations increased, each being 0.03 to 0.11 mg/L and 0.01 to 0.15 mg/L (Figure 3). The increased value of the orthophosphoric that occurred in the twelfth and fifteenth day of the observation was allegedly caused by the treatment to the activity of phosphate reducing bacteria that naturally exist in both media, the Sutami reservoirs water and the wetland soil. In addition, the cause of the increased value of orthophosphoric return could be derived from the bacterium that caused the orthophosphoric compounds in the soil medium to become dissolved in the water treatment medium [7].

Orthophosphoric overall decline in the media for each day of the sixth observation was about 74-92%, while the decline within twelve days of observation was about 62-94%. The decrease of the concentration of orthophosphoric on the fifteenth day of observation was 51-99%. The decrease in the concentration of the orthophosphoric occurred in connection with *Microcystis* cell density in the medium treatment, which lowered the density of *Microcystis* cells. The decrease in phosphate concentration in the control medium either without or with addition of *Microcystis* until the fifteenth day of each was about 97.71 and 98.03%. This situation indicates that the Sutami reservoirs water has allegedly already contained microorganisms that can

reduce orthophosphoric. The orthophosphoric's decline in the medium macrophytes addition of *Microcystis* until the fifteenth day of each was about 99.24 and 93.16%. The macrophyte was able to take advantage from the orthophosphoric for metabolic processes [8].

The orthophosphoric decrease in the bacterial consortium medium either without or with addition of *Microcystis* until the fifteenth day was around 51.73 and 54.86%. The use of denitrifying bacteria consortium alone was only able to slightly reduce the concentration of orthophosphoric. This situation was probably due to the many who have contained orthophosphoric content in the wetland soil medium. Meanwhile, the decrease of the concentration of orthophosphoric in the denitrifying bacteria consortium indigenous medium to a combination of both macrophyte without or with addition of *Microcystis* until

combinations *F. globulosa* and *V. zizanioides* both without and with the fifteenth day of each was about 99.65 and 93.69%. The incorporation of denitrifying bacteria consortium with a macrophyte combination was able to reduce the concentration of orthophosphoric in the greatest number. This indicates the positive interaction between the denitrifying bacteria consortium with a macrophyte combination in the use of orthophosphoric. Orthophosphoric is a phosphate compound is dissolved in water, while phosphate is an impetus for the increased use of nitrogen by the organism. When the a sufficient number of phosphate is available, it will trigger the use of nitrate towards the growth of organisms. Yet, when the concentration of phosphate has been exhausted by the growth of organisms, the utilized nitrate will be hampered even if other nutrients are abundant [10].

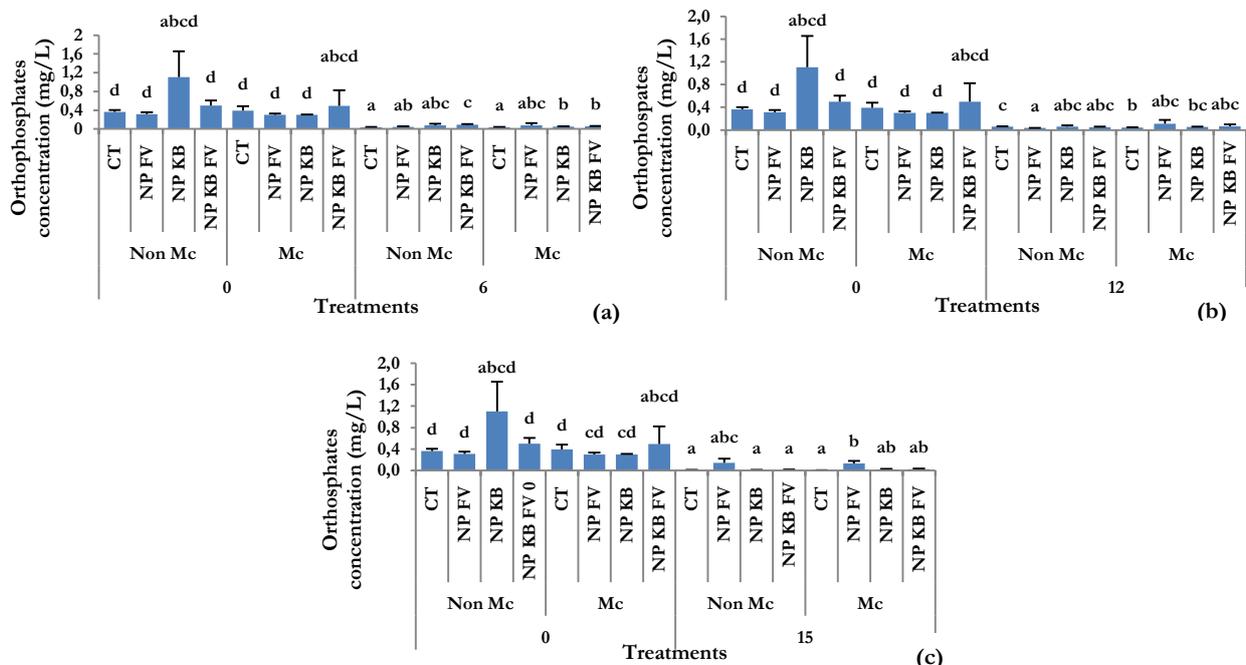


Figure 3. Comparison of the observed 0-6 days (a), 0-12 days (b), and 0-15 days (c). KT=Control; NPFV= Nitrate&Phosphate+(*F. globulosa*), (*V. zizanioides*); NPKB= Nitrate&Phosphate + Denitrifying bacteria indigenous consortium. The same letters on the block showed no significantly different by ANOVA followed by t test α 0.05

The growth curve of *Microcystis* spp. on each treatment medium is shown in Figure 4. The observations indicate that *Microcystis* spp. medium in each treatment did not experience a phase lag. The cells of *Microcystis* spp. did not also undergo the phase of adaptation in the new treatment medium. Not the phase lag in each of the treatment medium is thought to be caused due to the addition of nutrients nitrate and orthophosphoric in accordance with the requirements of living *Microcystis* spp.

Exponential phase of *Microcystis* spp. cells in control medium and the combination of bacterial consortium with macrophyte of *F. globulosa* and *V. zizanioides* medium occurred in the fourth day of observations, each of which was 25×10^4 and 13×10^4 cells/mL. Meanwhile, the exponential phase in combination treatment macrophyte *F. globulosa* and *V. zizanioides* medium occurred on the fifth day of observation with a total of 18×10^4 cells/mL. Then the exponential phase to the treatment of bacterial consortium medium occurred on the fourth day of observation with a

total of 16×10^4 cells/mL. Respective medium control treatment, FV, KB and KBFV in stationary phase but not having directly experienced the death phase. Once passing

through the exponential phase, the cells of *Microcystis* spp cells. directly experienced a significant decline.

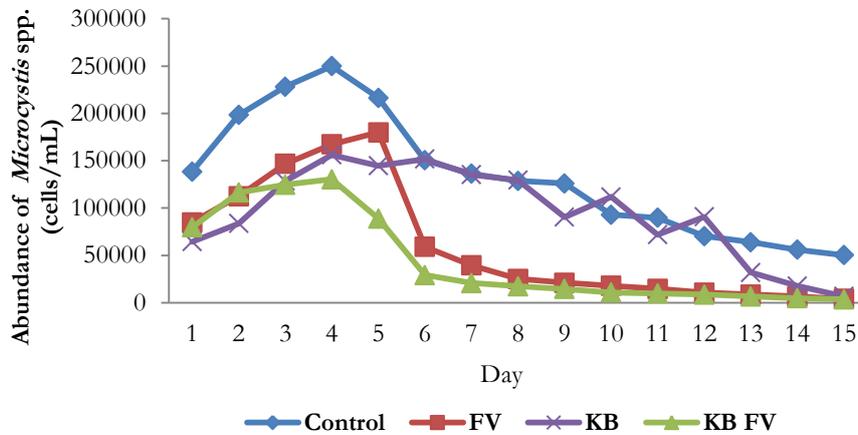


Figure 4. The growth curve of *Microcystis* spp. medium at various treatment. FV: Combination of *F. globulosa* dan *V. zizanioides*; KB: Denitrifying bacteria consortium. *Microcystis* spp. cells in each treatment medium directly into the exponential growth phase characterized by an increase in cell number of the most high.

The observations in the medium combination of denitrifying bacteria consortium indigenous treatment with a macrophyte *F. globulosa* and *V. zizanioides* showed that it was able to inhibit the growth of *Microcystis* cells the most if compared with other treatment media. This was indicated by the maximum abundance of *Microcystis* cells which only occurred until the fourth day of observation with *Microcystis* cell density of 13×10^4 cells/mL. It means that the availability of nutrients that support the growth of *Microcystis*

cells in the medium reduced after the fourth day. Moreover, this medium *Microcystis* cell density on the final day of observation (fifteenth day) has the least density, which is 0.35×10^4 cells/mL. The highest value of *Microcystis* cell growth inhibition in this medium was also supported by the significant decrease in nutrient concentrations of nitrate and orthophosphoric since the sixth day of observation, that are 60% and 86%, respectively.

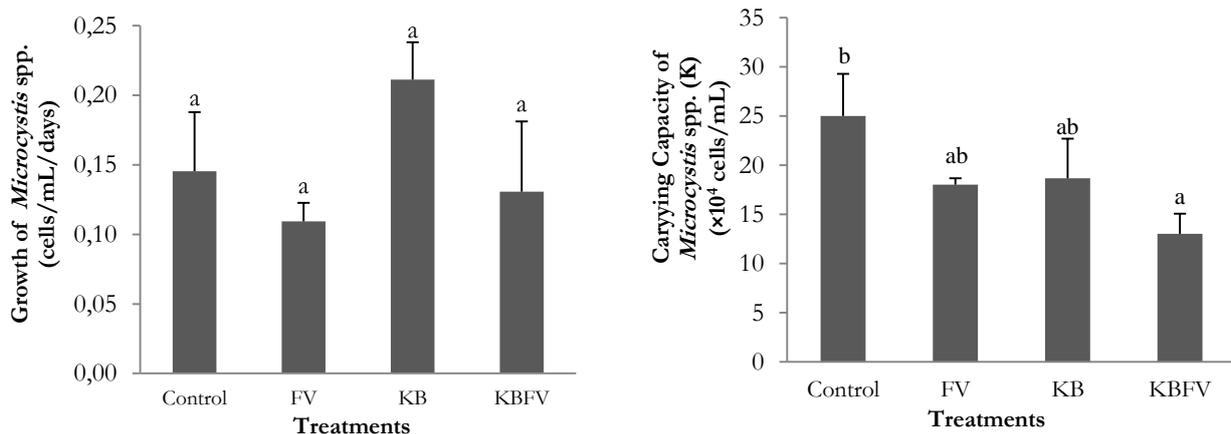


Figure 5. Growth rate (a) and carrying capacity (b) of *Microcystis* spp. in a variety of different treatment medium FV. Combination of *F. globulosa* and *V. zizanioides*; KB. Bacterial consortium. The same letters on the block showed no difference based on ANOVA test followed by Tukey test HSD (BNJ) at α 0.05

The growth rate of *Microcystis* spp. cell in a variety of treatment medium was obtained from the increase in abundance or density of cells every day. The growth rate shows the relative ecological success of a species of organism to be able to adapt well to the environment or the

place of growth medium. The growth rate of *Microcystis* spp cells. in the control medium was 0.14 cells/mL / day, whereas for FV treatment medium, the KB and KBFV were respectively 0.11: 0.21 and 0.13 cells/mL / day (Figure 5). The growth rate of *Microcystis* spp. was relatively

similar to the medium control treatment, FV, KB and KBFV ($p > 0.05$). The carrying capacity of the medium control, FV, KB and KBFV were 25×10^4 , 18×10^4 , 19×10^4 and 13×10^4 cells/mL, respectively. The treatment medium which is the combination of consortium of denitrifying bacteria with macrophyte of *F. globulosa* and *V. zizanioides* was able to inhibit the growth of *Microcystis* the most. This indicated that the carrying capacity of the lowest (13×10^4 cells / mL) was in medium (Figure 5). These results were supported by the highest percentage of the decrease in the abundance of *Microcystis*. This was shown in the denitrifying bacteria indigenous consortium medium combined with a macrophyte *F. globulosa* and *V. zizanioides* which was 47.87%, while in the combination of *F. globulosa* and *V. zizanioides* medium the decrease reached 27.93% and 23.35% in the medium denitrifying indigenous bacterial consortium.

CONCLUSION

The results showed that both treatment and a combination of both macrophytes with a consortium of denitrifying indigenous bacteria were added or not either *Microcystis* able to reduce nitrate at 99% and 93-99% orthophosphoric. The macrophytes combinations with denitrifying indigenous bacterial consortium were able to degrade the carrying capacity of *Microcystis* spp. at the most, reaching up to 47.87%. The *Microcystis*'s number of 10^7 cells/mL in the beginning of the treatment was inhibited as much as 0.35×10^4 cells/mL after incubation for fifteen days.

ACKNOWLEDGMENTS

Thank you for Dr. Catur Retnaningdyah, M.Si. and Dr. Suharjo, M.Si. who have helped to improve this manuscript.

REFERENCES

- Soekistijono (2004) Eutrofikasi di waduk sutami: monitoring, evaluasi dan upaya penanganannya, kolokium hasil penelitian dan pengembangan sumber daya air. Perum Jasa Tirta I. Malang.
- Samino S, Retnaningdyah C (2006) Monitoring dinamika komunitas fitoplankton dan zooplankton di waduk sutami malang periode bulan oktober sampai desember 2004. Research Report Cooperation Jasa Tirta I with the Department of Biology Faculty of Mathematics and Science Certificate No.ID03/0127 UB.
- Codd GA, Lindsay J, Young MF, Morrison LF, Metcalf JS, (2005) From mass mortalities to management measures, In Huisman J, Matthijs HCP, Visser PM, eds, Harmful cyanobacteria-Aquatic Ecology series vol 3, pp 1-23. Springer. Netherlands.
- Fried SBM, Nothwehr E (2003) Nitrate and phosphate levels positively affect the growth of algae species found in perry pond. Biology Department, Grinnell College. Grinnell.
- Ruckert GF, Giani A (2004) Effect of nitrate and ammonium on the growth and protein concentration of *Microcystis viridis* Lemmerman (Cyanobacteria), <http://www.scielo.br>. Accessed on May 24th 2012.
- Vivian CM, Cabello P, Luque MM, Blasco R, Castillo F (1999) Prokaryotic nitrate reduction: molecular properties and functional distinction among bacterial nitrate reductases. J. Bacteriol. 181 (21): 6573-6584.
- Wulandari S, (2001) Efektivitas bakteri pelarut fosfat *Pseudomonas* sp. terhadap pertumbuhan tanaman kedelai (*Glycine max* L.) pada tanah podsolik merah kuning. Jurnal Natur Indonesia 4 (1). 56-63.
- Safaria TN (2012) Ketersediaan dan peranan beberapa bioelement dalam tanah untuk proses pertumbuhan tanaman, <http://file.upi.edu/>. Accessed on June 7th 2012.
- Retnaningdyah C, Suharjo, Soegianto A, Irawan B, (2010) Daya dukung dan laju pertumbuhan *Microcystis* hasil isolasi dari waduk sutami pada berbagai variasi konsentrasi nitrat dan fosfat dalam medium selektif B-12. Biota. 15(3): 354-362.