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The Potential of Swampland Microalgae as Nitrogen Provider

Primastya Ayu Lestari[1*], Nuni Gofar[2] and Dedik Budianta[2]

[1]Sriwijaya University, [2]Department of Soil Science, Faculty of Agriculture, Sriwijaya University
Jl. Padang Selasa No. 524 Bukit Besar, Palembang 30139, Indonesia, e-mail: primastya@student.pps.unsri.ac.id

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ABSTRACT

This study aimed to identify and explore the potential of microalgae from swampland of South Sumatra as nitrogen contributor for rice plants grown on swampland. Determination of sampling points was done by looking at the presence and abundance of microalgae in the sampling locations. The method used in the sampling is purposive sampling method. The samples were grouped into 3, *i.e.* culture 1 (B1) derived from rice cultivation area, culture 2 (B2) derived from land that was not cultivated with rice, and culture 3 (B3) derived from swamp water samples. The medium used to culture the microlagae was Johnson's medium. The variables measured were the number of microalgae cells and ammonium concentrations on day 1, 4, 8 and 16 of culturing. Three species of Cyanophyceae class from the swampland were identified, *i.e.* thread algae, *Synedra sp.* and *Melosira sp*. Those microalgae may contribute the maximum amount of available nitrogen of 21.41 µg mL^{-1} in the form of ammonium to the rice plants on days 10-12. Microalgae in culture 1 can contribute nitrogen for rice plants in the form of ammonium about 16.23% - 48.71% with the cell density of 7.48 cells mL^{-1}.

Keywords: Microalgae, nitrogen fixation, swampland

ABSTRAK

Penelitian ini bertujuan untuk mengidentifikasi jenis mikroalga dan mengeksplorasi potensi mikroalga asal rawa lebak Sumatera Selatan dalam menyumbangkan nitrogen bagi tanaman padi yang ditanam pada lahan rawa lebak. Penentuan titik sampling dilakukan dengan cara melihat keberadaan dan kelimpahan mikroalga di lokasi pengambilan sampel. Metode yang digunakan dalam pengambilan sampel ini yaitu metode *purposive sampling*. Sampel dikelompokkan menjadi 3, yaitu biakan 1 (B1) berasal dari area pertanaman padi, biakan 2 (B2) berasal dari tanah yang tidak ditanami padi, dan biakan 3 (B3) berasal dari air rawa yang tidak ditanami padi. Media yang digunakan untuk menumbuhkan biakan adalah medium Johnson. Variabel yang diamati adalah jumlah sel mikrolaga dan konsentrasi amonium yang dilakukan pada hari ke 1, 4, 8 dan 16 setelah aplikasi. Ditemukan 3 spesies mikroalga kelas Cyanophyceae dari lahan rawa lebak, yaitu Alga benang, *Synedra sp*. dan *Melosira sp*. Mikroalga tersebut dapat menyumbangkan nitrogen tersedia dalam bentuk amonium untuk tanaman padi maksimum pada hari ke 10-12 sebesar 21,41 µg mL^{-1}. Mikroalga pada biakan 1 dapat menyumbangkan kebutuhan nitrogen untuk tanaman padi dalam bentuk amonium sebesar 16,23% - 48,71% dengan kepadatan sel sebesar 7,48 sel mL^{-1}.

Kata kunci: Fiksasi nitrogen, mikroalga, rawa lebak

INTRODUCTION

Microalgae are marine organisms which are known as phytoplankton. Cyanophyta Division is the only microalgae that can fix nitrogen so they often being used in agriculture as biofertilizer. According to Fadilah and Ariesyadi (2013) microalgae can cause some nutrients in water to dissolve and the nutrients will be taken up by microalgae. Therefore, microalgae are considered to be potential as a contributor of nitrogen.

Microalgae can grow well in swamp areas because the swamplands provide nutrients which are needed by microalgae to live without disturbing the plants that grow in the same area. Swamplands in Indonesia are quite wide, *i.e.* around 33.4 - 39.4 millions ha, which spread predominantly in Sumatra, Kalimantan, Sulawesi and Papua (Djaenudin 2009).

The swampland comprises of 23.1 millions ha of tidal swampland and 13.3 millions ha of non-tidal swampland (Kasrina *et al*. 2012). The existence of swampland area is widely enough for microalgae habitat.

Agricultural cultivation carried out in swampland is mainly rice cultivation. Efficiency of N taken up from urea fertiilizer by swamp rice is around 30-40% (Tamad 2002). The use of chemical fertilizers continuously causes the role of chemical fertilizers to be ineffective. Organic fertilizers and biological agents are now widely developed, this is because the use of organic fertilizers and biological agents have no negative impacts on soil fertility. The amount of nitrogen that is biologically taken up is mainly derived from the fixation of N_2 from the atmosphere, which is further converted into ammonium ions. Microalgae were reported significantly enhanced rice production including total number and weight of grains per spike and total weight of 100 grains (Chittapun *et al*. 2017). The inoculation of cyanobacteria can help to save 25-40 % of chemical N fertilizers (Nain *et al.* 2010). Therefore, an organism that is able to convert unavailable-N to available-N is needed especially in swamplands. Microalgae are abundant in the swampland, in this research we will identify the species and the ability of swampland's microalgae to provide nutrients for plants.

MATERIALS AND METHODS

Sampling of Microalgae

Sampling of microalgae was conducted in a swampland at Faculty of Agricultue, Sriwijaya University in January to February 2017. Determination of sampling points was done using purposive sampling method by observing the presence and abundance of microalgae in the sampling locations. The sampling was conducted on the area with rice cultivation and without rice cultivation using plankton net with the size of hole nets of 30-50 μm. About 1 L of water sample was taken, then put in a plastic bottle and labeled. The samples were grouped into 3, *i.e.* B1 was derived from rice cultivation area, B2 was derived from land that was not cultivated with rice, and B3 was derived from swamp water samples.

Culturing Microalgae

The microalgae were cultured in Johnson's medium (Kim and Lee 2006). Microalgae samples obtained from the field were then transferred to an erlenmeyer containing Johnson's medium of 500 mL. The growth of microalgae was accelerated by adding walne and vitamin B12 fertilizer about 1 mL L^{-1} on the culture medium. The microalgae culture was then identified.

Identification of Morphology and Species

Identification of the morphology and species of microalgae was done by observing the morphological differences of microalgae below a microscope. According to Sari (2011) identification of morphology and species of microalgae was performed under a light microscope with magnification of 400 or 1000 times.

Propagation of Isolated Microalgae

Identified microalgae belonging to Cyanophyta are propagated in Johnson's media. Propagation of isolates was done by taking samples of the microalgae then put them in erlenmeyer that already contained the Johnson's medium. Isolates that have been propagated then were grown to a density of at least 2×10^7 cells mL^{-1}, the calculation of cell density was done using dilution method.

Culturing Microalgae in Greenhouse

The experiment in the greenhouse was conducted using microalgal isolates with a minimum initial density of 2×10^6 mL^{-1} cells (Susanti *et al.* 2013). The experiment was conducted by applying 3 treatments and 3 replications. Culturing microalgae in the greenhouse was done by placing microalgae isolates in 3500 mL trays that contained Johnson's medium without N addition, then growing them for 16 days.

Calculation of Cell Density

Calculation of the number of cells was performed on day 1, day 4, day 8 and day 16 after application of microalgae in the greenhouse. Calculation of microalgal cell density was done using dilution method under a microscope with 1000 times magnification with haemocytometer and hand counter as supporting tools. The sampling was performed using volumetric method of 1 mL for each experimental unit.

Measurement of Ammonium (NH_4^+) Concentrations

Ammonium concentrations in the culture were measured using the quantitative Nessler method (Riffiani 2010) using spectrophotometry. About 25 mL of culture samples were taken, added with 1-2 drops of Seignette salt solution and 0.5 mL of Nessler's reagent. After that, the samples were shaken for 10 minutes, then put in a

spectrophotometer with 420 nm wavelength for ammonium measurement. The obtained absorbance was associated with the equation on the ammonium standard curve to determine the ammonium concentrations in the culture samples. Measurement of ammonium concentrations was done on day 1, day 4, day 8 and day 16 after application of microalgae in the greenhouse. This was adjusted to the change of growth phase of microalgae.

The data were obtained from the calculation of cell density of microalgae and analysis of ammonium concentrations in microalgae culture media periodically.

RESULTS AND DISCUSSION

Condition of Swampland

Swamplands located in the Sriwijaya University campus consist of a small part of swampland that has been used as agricultural land, and most of them are just bare swamplands. The swamplands in the Faculty of Agriculture contained total-N of 0.07%-0.22% (very low to moderate). The addition of nitrogen fertilizer in the swamplands was needed if they will be used as agricultural land.

The utilization of swampland for rice and corn cultivations was just done for the first time at the Sriwijaya University campus area. Rice variety that has been used was red rice INPARI 24, and the fertilizer used was compost. The land used for rice cultivation was inundated with 5-10 cm water (Widiawati 2017).

At the time of microalgae sampling, the swampland was being cultivated with rice with the age of 14 days, and fertilization has been done using compost. This condition gives the possibility that the presence of microalgae on the land is abundant, because the microalgae will grow well if the amount of available nutrients are enough in the environment, one of them comes from the application of fertilizer to the soil.

Identification of Morphology and Species of Microalgae

Three types of microalgae with different morphological characteristics have been identified in the water samples of swampland used in the current study. Microalgae with a yarn-like shape were observed in B1 sample, which was the sample obtained from the rice cultivation area. In B2 sample, the microalgae were dominated by round shape, while in B3 sample the morphology of microalgae was rice-like shape. At the beginning of microalgae culturing, these three forms of morphology were predominant in each culture, but on the 32nd day of culturing the predominant morphological form in each culture was round shape. The presence of other morphology of microalgae in each culture because the samples were placed on impure cultures, so there were other types of microalgae derived from the same sample.

The results of the identification on the species of microalgae that has been cultured in artificial media showed that there are 5 species of microalgae, which are from 2 classes of microalgae. In general, microalgae species are commonly found in rice fields. Sari (2011) found 14 species of microalgae from the Cyanophyta genus on paddy fields. Kasrina *et al.* (2012) identified 4 classes and 32 species of microalgae in swamp water samples. Differences of sampling sites affect the type and abundance of microalgae observed, this corresponds to the availability of nutrients in the environment. The results of identification of microalgae species from swampland that has been isolated on the culture media are presented in Table 1.

Chlorophyceae and Cyanophyceae are microalgae that grow on artificial culture media, this is because Chlorophyceae and Cyanophyceae are very adaptable and resistant to extreme conditions. Several species identified from artificial culture media are species that can survive under adverse environmental conditions and partly diatom species that can grow fossilized.

The results showed that *Chlorella sp.* is the predominant species of the three cultures, this is due to *Chlorella sp.* can grow well in a medium that contains enough nutrients such as nitrogen, phosphorus and potassium at optimum temperature of 25^{o}C. *Chlorella sp.* also has high reproductive rate, each *Chlorella sp.* is able to develop into 10,000 cells within 24 hours (Prihantini *et al*. 2005).

Most species of thread algae come from Cyanophyta division of Cyanophyceae class. Cyanophyta widely grows in rice fields with the optimum sunlight, temperature, soil pH and nutrients that support the growth of Cyanophyta (Kim and Lee 2006). Therefore, the thread algae were only found in culture 1, which was isolated from water samples of rice field.

Synedra is the predominant diatom in Indonesia's freshwater. Diatom is microalgae with > 90% of silica cell walls so they can fossilize. Soeprobowati (2010) indicated that Synedra is a tolerant species and is found in many river and lake ecosystems with high organic content.

Abundance of *Melosira sp.* could be used as an indicator of water quality. *Melosira sp.* can grow in a high organic matter environment (Wijaya and

Table 1. Microalgae species from swampland identified in culture media.

Culture Media	Composition		
	Species	Class	Number of Cells per Litre
Culture 1 (B1)	*Chlorella sp.*	Chlorophyceae	18,500,000
	Thread Algae	Cyanophyceae	2,134
	Synedra sp.	Cyanophyceae	32
	Abundance		18,502,166
Culture 2 (B2)	*Chlorella sp.*	Chlorophyceae	16,500,000
	Melosira sp.	Cyanophyceae	3,025
	Asterococcus sp.	Chlorophyceae	2,548
	Abundance		16,505,573
Culture 3 (B3)	*Chlorella sp.*	Chlorophyceae	16,150,000
	Asterococcus sp.	Chlorophyceae	21,974
	Melosira sp.	Cyanophyceae	1,274
	Abundance		16,173,248

Hariyati 2011). *Asterococcus sp.* belongs to the Chlorophyceae class in which this algae is adaptable and tolerant to the extreme conditions. *Asterococcus sp.* can grow under adverse conditions, so that it grows well under sufficient nutritional conditions.

Nitrogen fixation ability could only be found in the Cyanophyta division of Cyanophyceae class. The Cyanophyceae class have heterocyst cells that function to fix free nitrogen from the atmosphere if there is no nitrate in its environment (Sari 2011). In culture 1 the nitrogen fixation is carried out by thread algae and *Synedra sp.* In culture 2 and 3 the fixation of free nitrogen are done by *Melosira sp*.

Microalgae Cell Density

The increase of microalgae cell density is an indicator of the growth of the microalgae. The growth rate of microalgae expressed as cell density is presented in Figure 1. The initial cell density of microalgae at application was 4×10^6 cells mL^{-1} and continued to increase until reaching an optimum density of 30×10^6 cells mL^{-1} on day 8.

In B1, B2 and B3 culture media, the growth of microalgae cells is relatively fast as shown in Figure 1. Thus, the microalgae samples from the swampland showed a good adaptation to the culture environment. The adaptation phase or lag phase will

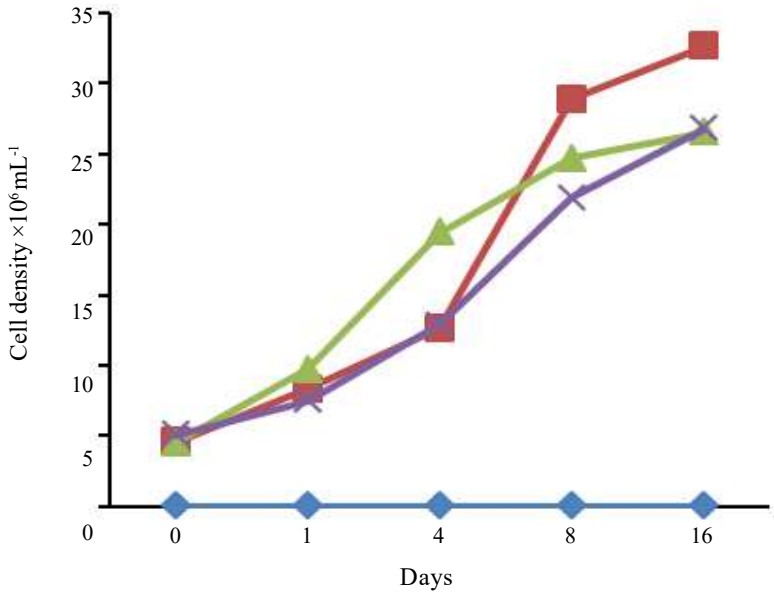

Figure 1. Microalgae cell density on each observation day. K= Control, B1= Culture 1, B2= Culture 2, B3= Culture 3.

be very short when the inoculated microalgae are in the exponential phase (Prihantini *et al.* 2005). Microalgae culture in this experiment experienced an exponential phase on day 4, which was indicated by a rapid growth until day 8. This result is in accordance with the study of Prabowo (2009) that the microalgae growth will reach an exponential phase on day 4 until day 6 of culturing.

The growth of microalgae cells continued to increase until day 16, but the growth was not as fast as on day 4. This is because on day 16 the microalgae has entered the stationary phase, in which the number of increasing cells is balanced by the number of dead cells (Sari 2011). The availability of nutrients in the culture environment would affect the rate of cell division of microalgae. The less nutrient contained in the culture environment the faster the microalgae reach the stationary phase and continued to the declination phase or the phase of death.

The highest growth rate of microalgae was found in culture B1. This could be seen from the ability of microalgae to reach the exponential phase and reach the maximum cell density on day 8. In addition, the adaptability of microalgae in B1 culture could be seen from the difference of cell density and its growth compared to other treatments.

Ammonium Concentrations

Cyanophyceae is microalgae that predominantly grows in rice fields and could help to fertilize soil, due to the microalgae can fix N from the atmosphere freely (non-symbiotic algae). The fixation of N from the atmosphere by microalgae is evident to increase ammonium concentration in the culture media (Figure 2). The media used to culture the microalgae in the greenhouse was Johnson's media without N addition. On day 0, the ammonium concentrations in the culture media were 0 μg mL^{-1}, in the following days the concentrations continued to grow, reaching the highest concentrations on day 8 and experiencing a decline on the next days.

Cyanophyceae fix nitrogen from the atmosphere when the nitrogen levels in the environment are limited or even absent. Cyanophyceae will use nitrogen in any forms as a source of energy and release the nitrogen to the environment in the form that can be taken up by plants, *i.e.* ammonium (Chittapun 2017). If there is enough nitrogen in the environment for the microalgae then the microalgae would utilize the existing nitrogen without having to block nitrogen fixation from the atmosphere.

In this study, the media used to culture the microalgae initially did not contain nitrogen, but after the first day of treatment, the ammonium conncentrations continued to increase until day 8. The increase of ammonium concentrations was caused by the lack of nitrogen content in the media, which triggered Cyanophyceae to inhibit free nitrogen fixation from the air via a non-symbiotic pathway. Nitrogen fixation occured because Cyanophyceae is able to synthesize nitrogenase enzyme.

After day 8 the ammonium content in the culture media decreased, this was due to the uptake of nitrogen by the microalgae itself. As reported by Riffiani (2010) that *Chlorella pyrenoidosa* might decrease the ammonium concentration in fish culture after day 14. Increased ammonium concentrations occured up to day 8 then decreasing after day 9.

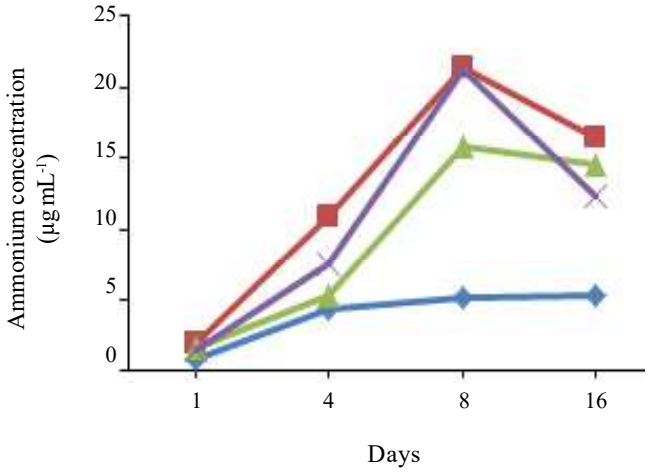

Figure 2. Ammonium concentrations measured in the culture media. K= Control, B1= Culture 1, B2= Culture 2, B3= Culture 3.

Rai (2006) reported that the nitrate and ammonium content at certain concentrations in the culture media could inhibit the synthesis of nitrogenase enzyme in microalgae. In addition, Nilsson *et al.* (2002) reported that the application of nitrogen fertilizer to the soil may inhibit the nitrogen fixation by Cyanophyceae. So, after the ammonium content was available in the media, then there was no nitrogen fixation from the air by the microalgae but the uptake of ammonium in the media by the microalgae itself.

Besides taken up by microalgae, the decrease of ammonium concentrations in the media may be due to the presence of bacteria in the media that can convert the ammonium into nitrate. Nitrifying bacteria take about 10-14 days to convert ammonium to nitrate (Rai 2006). The existence of bacteria in the culture media was due to the media were not sterilized first, so it triggered the growth of unexpected bacteria.

Figure 2 showed that the ammonium concentrations in the control treatment were significantly different from those in other treatments at day 1, 8 and 16, but they were not significantly different from day 4 of the observation. The increase of ammonium levels in the control treatment was due to the presence of non-nitrogen-fixing bacteria. The working principle of non symbiotic bacteria is by synthesizing nitrogenase enzyme. Nitrogenase enzyme will actively fix N from the atmosphere if the concentration of N in the medium is low (Danapriatna 2010).

The concentration of ammonium in the B1 treatment showed a significant different from that in other treatments only on day 1, while on the next days the concentration was not significantly different from that in the treatments of B2 and B3. This is because the microalgae cell density at the beginning of application was higher in B1 culture than in other cultures, so that the ammonium concentration on the 1st day was already high. On the next days, the ammonium concentrations showed no significant difference among the three cultures because the microalgae in B2 and B3 have undergone an adaptation phase and increased their cell number so that their ability were not significantly different from the microalgae in B1 culture.

On day 8, Cyanophyceae has increased the ammonium concentrations in the media into 15.83 μg mL^{-1} - 21.41 μg mL^{-1}. The results indicated that if the nitrogen requirement of swamp rice plants is about 40-135 kg ha^{-1}, the Cyanophyceae can contribute nitrogen in ammonium form of 11.72% - 15.86%, with the abundance of Cyanophyceae of 106 cells mL^{-1} and the height of water inundation on swampland is about 10 cm.

CONCLUSIONS

Three species of Cyanophyceae class and 2 species of Chlorophyceae class of microalgae were identified in the swampland. The maximum microalgae population from the swampland samples was reached on day 11 of culturing, *i.e.* 30×10^6 cells mL^{-1}. Cyanophyceae might contribute to the maximum amount of available nitrogen in the form of ammonium for rice plants on day 10-12 (21.41 μg mL^{-1}). Based on the results of current study, Cyanophyceae could be applied to rice plant cultivation in swampland in order to substitute N fertilizer. The number of microalgae applied can still be improved to achieve a more optimum of ammonium contribution from the algae.

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REFERENCES

Chittapun S, S Limbipichai, N Amnuaysin, R Boonkerd and M Charoensook. 2017. Effects of using cyanobacteria and fertilizer on growth and yield of rice, Pathum Thani I: a pot experiment. A*ppl Phycol* 30: 79-85. doi: 10.1007/s10811-017-1138-y.

Danapriatna N. 2010. Biokimia penambatan nitrogen oleh bakteri non simbiotik. A*gribisnis dan Pengembangan Wilayah* 1: 1-10. (in Indonesian).

Djaenudin UD. 2009. Prospek penelitian potensi sumber daya lahan di wilayah Indonesia 1). *Pengembangan Inovasi Pertanian* 2: 243-257. (in Indonesian).

Fachrullah MR. 2011. Laju pertumbuhan mikroalga penghasil *biofuel* jenis C*hlorella* sp. dan *Nannochloropsis* sp. yang dikultivasi menggunakan air limbah hasil penambangan timah di pulau bangka. [Skripsi]. Institut Pertaian Bogor. Bogor. (in Indonesian).

Fadilah R and HD Ariesyady. 2013. Analisis kelimpahan dan keragaman mikroalga di kolam stabilisasi instalasi pengolahan air limbah berdasarkan analisis biologi konvensional dan molekuler. [Tesis]. Institut Teknologi Bandung. Bandung. (in Indonesian).

Kasrina S, Irawati and WE Jayanti. 2012. Ragam jenis mikroalga di air rawa Kelurahan Bentiring Permai Kota Bengkulu sebagai alternatif sumber belajar biologi SMA. *Exacta* X : 36-44. (in Indonesian).

Kim JD and CG Lee. 2006. Diversity of heterocystous filamentous cyanobacteria (blue-green-algae) from rice paddy field and their differential susceptibility to ten fungicides used in Korea. *Microbiol Biotechnol* 16: 240-246.

Nain L, A Rana, M Joshi, SD Jadhav, D Kumar, YS Shivay, S Paul and R Prasanna. 2010. Evaluation of synergistic effects of bacterial and cyanobacterial strains as biofertilizers for wheat. *Plant Soil* 331: 217-230. doi:10.1007/s11104-009-0247-z.

Nilsson M, J Bhattacharya, AN Rai and B Bergman. 2002. Colonization of root of rice (*Oryza sativa* L.) by simbiotic *Nostoc* strains. *New Phytol* 156: 517-525. doi:10.1046/j.1469-8137.2002.00534.x.

Prabowo DA. 2009. Optimasi pengembangan media untuk pertumbuhan *Chlorella* sp. pada skala laboratorium. [*Skripsi*]. Institut Pertanian Bogor. Bogor. (in Indonesian).

Prihantini NB, B Putri and R Yuniati. 2005. Pertumbuhan *Chlorella* spp. dalam medium ekstrak tauge (met) dengan variasi pH awal. *Makara Sains* 9: 1-6. doi: https://doi/org/10.7454/mss.v9i1.457. (in Indonesian).

Rai MK. 2006. *Handbook of Microbial Biofertilizers*. Haworth press. New York, pp/ 435-436.

Riffiani R. 2010. Penggunaan mikroalga *Chlorella pyrenoidosa* chick amobil untuk meningkatkan kualitas air dalam akuakultur. *Hidrosfir Indonesia* 5: 25-33 (in Indonesian).

Sari WE. 2011. Isolasi dan identifikasi mikroalga *Cyanophyta* dari tanah persawahan Kampung Sampora, Cibinong, Bogor. [Skripsi]. UIN Syarif Hidayatullah. Jakarta (in Indonesian).

Soeprobowati TR. 2010. Stratigrafi diatom danau rawa pening : kajian paleolimnologi sebagai landasan pengelolaan danau. *Makalah Seminar Nasional Limnologi V*. Pusat Penelitian Limnologi – LIPI. Bogor (in Indonesian).

Susanti ET, M Lutfi and WA Nugroho, 2013. Pengaruh penambahan plant-growth promoting bacteria (*Azospirillum* sp.) terhadap laju pertumbuhan mikroalga (*Chlorella* sp.) pada media limbah cair tahu sintetis. K*eteknikan Pertanian Tropis dan Biosistem* 1: 239-248 (in Indonesian).

Tamad. 2002. Pengaruh urea dan *Azolla* sebagai tanaman penutup pada budidaya padi sawah tanah berkapur di sekitar Kupang, Timor, NTT. *Berita Biologi* 6: 515-520 (in Indonesian).

Widianingsih A, Ridho, R Hartati and H Oko. 2008. Kandungan nutrisi *Spirulina platensis* yang dikultur pada media yang berbeda. *Ilmu Kelautan* 13: 167-170 (in Indonesian).

Widiawati. 2017. Potensi pemberian kompos briket dan remah terhadap N tanah dan tanaman serta pertumbuhan padi beras merah dengan sistem penanaman jajar legowo 2.1 di lahan sawah lebak. [Skripsi]. Universitas Sriwijaya. Palembang (in Indonesian).

Wijaya TS and R Hariyati. 2011. Struktur komunitas fitoplankton sebagai bio indikator kualitas perairan danau Rawa Pening Kabupaten Semarang Jawa Tengah. *Anatomi Fisiologi* XIX: 55-61. doi: https:/ doi.org/10.14710/baf.v19i1.2584 (in Indonesian)