Ammonia removal from Yogyakarta Domestic Wastewater (WWTP-SEWON) by microalgae reactor with CO₂ addition

A U Farahdiba¹, W Budiantoro² and A Yulianto²

¹Environmental Engineering Department, University Pembangunan Nasional Veteran Jawa Timur, Surabaya 60294, Indonesia
²Environmental Engineering Department, Islamic University of Indonesia, Yogyakarta 55584, Indonesia

Email: auliaulfahfarahdiba@gmail.com

Abstract. Algae technology is an alternative sustainable domestic wastewater treatment. This study analyse the effect of adding CO2 gas to algae reactor systems to reduce ammonia levels in wastewater. The addition of CO2 is expected to improve the performance of algae in photosynthesis thus accelerate the decreasing of ammonia concentration. This research uses algae reactor to treat domestic wastewater from Sewon Wastewater Management Installation Waste, Yogyakarta that conducted in laboratory scale with potential algae in facultative pond in Sewon WWTP. The CO2 supply in rate of 0.2 L / min and 0.5 L / min. Chlorophyll-a is analyzed to determine algae growth. The results show the high correlation between chlorophyll-a and ammonia removal, meanwhile algae reactor without CO₂ is uncorrelated with ammonia removal.

1. Introduction
Nutrient pollution in domestic wastewater is becoming more severe problem, actually in Indonesia. This is not only has substantially negative impacts in environment, but also can affect in the quality of people’s lives. One of these components is nutrients. High content of nutrients that enter the body of water causes the workload of water bodies getting heavier. Increasing nutrients in aquatic ecosystems will cause a vegetation change called eutrophication. The nutrient elements that cause eutrophication include the nitrogen content (N) [1]. Nitrogen in wastewater is found in organic form (40%) and ammonia in the form of NH3 or NH4+ by 60%. Ammonia in wastewater needs to be treated because in high concentrations on the water surface causes fish death in the waters. Moreover, if the pH value in water is high, the amount of low ammonia is highly toxic [2].

The ability of algae in removing ammonia is quite high. Moreover, with the addition of a combined system of algae-bacteria can improve the efficiency of its removal. This happens because the microalgae in the process of ammonia use it as a source of nitrogen to support the growth of microalgae or to form biomass. Meanwhile bacteria utilize oxygen from algae for the process of breaking organic matter in wastewater. This algal-bacterial symbiosis has a mutually beneficial relationship. This relationship by way of bacteria helps solve complex compounds into simpler compounds such as ammonium. Nitrogen, phosphate, and carbon dioxide, so that it can be utilized by algae [3]. While algae can provide nutrients for bacteria, such as vitamin B12 [4]. Bacteria also play a role in increasing algae growth and biomass accumulation during the exponential phase. In addition, algae also facilitate bacterial growth. In the existing study, the use of a combined system of bacterial
algae could remove 78% NH4+ and 92% total phosphorus. If the pH in the reactor is conditioned to 7, the removal of NH4+ and total phosphorus increases to 86% and 93% [5].

As for according [6], in addition CO2 into wastewater can increase the productivity of algae-bacterial biomass so that it can improve the process of nutrient removal in waste water. Additionally with CO2 addition in waste water, CO2 can prevent the process of increasing pH in wastewater. Whereas if the pH rises it will be detrimental in the process of removal of nitrogen and phosphorus elements. So with the regulation of pH 7-9 with the addition of CO2 COD removal process, total N, total P and E.Coli to 84%, 79%, 57% and 93%. And the increase in biomass from the C, N and P contexts was 64.8%, 12.6%, and 2.4% [6].

Analysis of the ability of algae and bacteria in the processing of nutrients in the form of ammonia in domestic wastewater needs to be studied. This is to evaluate the CO2 gas emitters in algae performance capability in reducing nutrients in the form of ammonia. Furthermore, the ability and potential of algae-bacteria with the addition of CO2 in waste water treatment need to be studied to enhance utilization and development of technology using algae.

2. Research Methods
2.1. Research Time and Location
Algae and bacteria cultivated from the Wastewater Treatment Plant (WWTP), Sewon Bantul. Algae and Bacteria Culture is cultivate from the first facultative pond in water tubal Sewon Bantul, Yogyakarta. This research conducted in Hazardous Waste room, Sewon Laboratory with open (roofed) condition exposed to direct sunlight. The running process takes place from April 3, 2017 to April 21, 2017. Parameter is analyze at Biotechnology Laboratory, Water Quality Laboratory, and Clinical Pathology Laboratory Islamic University of Indonesia.

2.2. Seeding and Acclimatization
Algae was obtained directly from wastewater in Sewon. Majority algae was analyze with the Biological Binocular CX-22 Olympus Philippines microscope. In the seeding process, NPK fertilizer was added as a nutrient for microalgae with ±35 mg/L each day. Seeding and acclimation process was carried out for two weeks by observing algal growth with algal cell density calculations. Algae was prepared to reach in dense of $6 \times 10^6$ cells/mL [7].

2.3. Experimental Setup
The reactor define five reactors with a reactor consisting of bacterial, algae, algae and bacterial combinations, a combination of algae and bacteria with a CO2 supply of 0.2 L/min, and a combination of algae and bacteria with a supply of CO2 with levels of 0, 2 L/min [7]. CO2 air flow is delivered to the reactor using a dose pump through a diffuser [8] at a rate of 0.2 L/min and 0.5 L/min [9]. CO2 injected to reactor in 10 hours per day. All reactors get the same light intensity from morning to afternoon (natural light). Research conducted in 14 days, with every two days analyze the main parameter (chlorophyll and ammonia). Environment condition was controlled every day.

Optimize algae was grown with wastewater in the reactor, with size 60 cm x 60 cm x 30 cm from translucent glass and a thickness of 5 millimeters. Sampling method and preservation sample are guided by SNI 06-2412-1991 (Water Quality Sampling Method). Ammonia testing refers to SNI 06-6989.30-2005 by spectrophotometer in phenate. Testing of chlorophyll a refers to SNI 06-4157-1996 with spectrophotometer. Population density of cells and identification of algae is calculated using haemocytometer. Statistical analysis conducted with MINITAB 16. The density of the cells can be calculated with the following equation:

$$\text{The number of Cell (cell/mL)} = \frac{\text{The number of cells x dilution Factor}}{\text{Volume}}$$  

The rate of ammonia removal by processing using algae-bacteria in this algae reactor can be calculated on the basis of each reactor. Calculations can be done by looking at the initial concentrations of ammonia and the final concentration of ammonia. The allowance efficiency (%) can be calculated using the formula:
\[
(\%) = \frac{C_{\text{in}} - C_{\text{out}}}{C_{\text{in}}} \times 100\%
\]

Description:
\(C_{\text{in}}\) = Initial Concentration of Ammonia
\(C_{\text{out}}\) = Final Concentration of Ammonia

2.4. Data Analysis
Research was conducted with statistical analysis with MINITAB 16, determine relationship each parameter (correlation). This method could improve connection the additional CO2 in algae reactor.

3. Result and Discussion
3.1. Microalgae Identification
Algae derived from the facultative ponds of the Sewon Sewage Treatment Waste Management, Bantul, Yogyakarta. The potential algae from wastewater pond was analyze with considered to have the ability to remove nutrients contained in wastewater itself. Majority species of algae in facultative ponds was *Chlorella sp.* Morphological identification of algae in Sewon pond found with morphological features have thin cell walls such as spheres. Cell diameters between 2 μm to 10 μm. Solid green at the main cell which shows a single parietal content of chloroplasts that almost fill the entire cell (this shows the classification of green algae) [10].

3.2. Optimization Microalgae Growth in Seeding and Acclimatization with wastewater
The seeding process is done for two weeks to get a concentrated culture algae. Microorganism seeding process *Chlorella sp.* is done by taking the microalgae seeds and cultured on the pond. After obtaining a culture of dense green, algae cells need to be refreshed by transferring 1 liter of algae from the nursery to a new culture of 2.5 liters. The process is optimize to reach 6 x 10⁶ cells/mL algae [10]. In this seeding obtained 7.97 x 10⁶ cells/mL algae on the fourth day. Acclimatization process distribute by moving the 0.27 liter microalgae into 1 liter. This culture has been added NPK 35 mg/L fertilizer as nutrients performed every 2 days [7].

The cell density in the acclimation process on first day is 2.7 x 10⁶ cells/mL, it increasing until the second day to 7.1 x 10⁶ cells/mL. This shows that algae in the previous culture pond is the logarithmic (exponential) phase of algae growth. The growth phases of *Chlorella sp.* algae in cultures in succession is a 3-day adaptation phase (days 0 to day 2), 3-day exponential phase (day 5 to day 8) [13]. The phase of death 8 days (day 9 to day 15), and then increase again on day 17. This is according to the report (11), stating the adaptation phase of growth of *Chlorella sp.* on a new medium that is 3 days.

The microalga used comes from a four-day acclimatization basin with a cell number of 3.4x10⁶ cells/mL. The use of algae in the expansion phase is done to produce a good culture and algae adaptation process is faster with new medium [12]. Chlorella sp. growth analysis showed differences in the density (number) of Chlorella sp. cells attached to different mediums. In Figure 4 it is seen generally for reactors supplied with CO2 gas that there is an increase in the number of microalgae cells. Unlike reactors without the supply of CO2 which generally on the graph is seen decreasing the number of cells.

3.3. Microalgae Growth
The growth of *Chlorella sp.* under different CO2 condition within 13 days as shown in Figure 1. Microalgae *Chlorella sp.* is phytoplankton that contain chlorophyll-a. Chlorophyll-a is a pigment that plays a role in the process of photosynthesis of microalgae. Thus chlorophyll-a is always associated with the number of phytoplankton or biomass indicators of microalgae in waters [13] [5].

The chlorophyll-a concentration showed increase until the thirteenth day on a reactor with a CO2 supply. The most rapid growth conducted in the thirteenth day chlorophyll-a at a 0.5 L / min CO2 gas supply reactor which reached 657.78 mg / m³. As per the [14] report, the maximum increase of
chlorophyll-a levels is present in the medium supplied by CO2 gas in the Staurastrum sp. microorganisms which reached 10.70 mg / L on the fourteenth day.

The CO2 supply of 0.2 L / min or algae-bacteria without supply has steady increased until the thirteenth day. Furthermore, reactor without Co2 decrease until the last day. Treatment with a combination of algae-bacterial symbiosis may increase algal biomass in terms of chlorophyll-a (Fig 1). In accordance with [15] bacteria also play a role in increasing algae growth and biomass accumulation during the exponential phase.

![Figure 1. Number of Chlorella sp. cells at various reactors](image)

3.4. Light, pH and Temperature Conditions

Supply of CO2 in wastewater causes a change in pH of 8.2 (before the supply of CO2) becomes below 6.5 on every day-to-zero day supply reactor, since in wastewater there is a reaction of bicarbonate acid formation (H2CO3) by reaction of CO2 with water to the reactor [16] [17]. The decrease in pH does not have a long range, this is because the quality of alkalinity of waste water is 120 mg / L which causes the pH of wastewater will be stable, because with alkalinity can neutralize the acid into [18].

In contrast to reactors without CO2 supply pH continues to fluctuate up to ≥ 9. Even on the last day of bacterial pH still around 9. Fluctuating pH occurs due to photosynthesis. The process of photosynthesis results in an increase in pH in water [6]. Until the average mean pH of the director of waste water without CO2 supply is between 7 and 10. While CO2 gas supply water is between 6 to 7, it proves that there is no significant change due to the addition of CO2, because when pH rises during the process of photosynthesis, the pH value will be maintained by the reaction of bicarbonate acid formation (H2CO3) by the reaction of CO2 with water at the reactor. [19] reports, that with the CO2 supply, the pH in the waste water can be controlled so that the pH does not rise.

Light intensity is measured because it is one of the factors that influence the growth of microalgae. Sunlight (light) is utilized by microalgae as an energy source to oxidize CO2 and H2O, in the process of photosynthesis [6]. Intake of light intensity parameter is done like another sampling that is at 09.00 WIB. Observations can be seen in Figure 4.9:

Temperatures between 20°- 30° C will increase the efficiency of wastewater treatment using microalgae and bacterial mix. At 27°-35 ° C microalgae are still in good photosynthetic temperature range, but there are times when processing is less than optimal because there is a temperature exceeding 30 ° C. Meanwhile the treatment using CO2 from day three, the temperature is in the range
of 30°-33°C. This is due to CO₂ gas absorbs sunlight radiation thus maintaining temperature at the reactor [19].

3.5. Nutrient Removal

The efficiency of ammonia removal in Figure 2 shows that the combination of algae-bacteria premises has an efficiency of > 65%. This is due to the occurrence of algae-bacterial symbiosis, microalgae providing O₂ supply and nutrients for bacteria, such as vitamin B12 and bacteria to help solve complex compounds into simpler compounds such as ammonium. Nitrogen, phosphate, and carbon dioxide, so it can be utilized by algae. The alga-bacterial symbiosis is strengthened by the addition of CO₂ gas at a rate of 0.2 L/min which increases removal efficiency up to 75%. Because the CO₂ rate of 0.2 L/min can make the pH, temperature and chlorophyll-a conditions reach the optimum conditions to remove ammonia in the waste water.

In the algae system occurs fluctuate removal of ammonia until the last reached 30%. This fluctuation is due to the algal system having decreased (death) algae cells resulting in the decomposition of dead algae that form ammonia and inhibits the ammonia removal process due to the limited algae cells [20]. While the fluctuations in the bacterial system on the sixth day due to the death of microalgae sourced from the facultative pond. The efficiency of bacteria that reaches 60% is due to the formation of NH₃ which then evaporates into the atmosphere thereby increasing the efficiency [21]. This efficiency is comparable to that [24], which describes NH₄⁺ removal using alga-bacterial symbiosis between 32.39% to 91.65% for 35 days in artificial waste and aeration rates, and from [19], total nitrogen removal using an industrial CO₂ supply reaches more than 70% for 24 days. While in summer the removal of ammonia (NH₄⁺) reached 85% and in winter of December to January reached 65%, with measurement for 14 days [22].

3.6. Correlation Chlorophyll with Ammonia Removal

High correlation between chlorophyll-ammonia concentration in reactor AB, AB 0.2, and AB 0.5., respectively -0.832, -0.919, and -0.829. There is high relationship between chlorophyll-a and ammonia in reactor with bacteria. High correlation negative means contrary with each parameter. Figure 3 shows increasing chlorophyll will reduce ammonia concentration. This suggests that processing with a combination of algae-bacteria increases the elimination of ammonia. Bacteria helps solve complex compounds into simpler compounds such as ammonium, nitrogen, phosphate, and carbon dioxide, so as to be utilized by algae [3]. While algae can provide nutrients for bacteria, such as vitamin B12 [4]. Figure showed the performance of algae reactor.

Figure 2. Efficiency of ammonia removal
3.7. Correlation pH with Ammonia Concentration

In wastewater, pH affects the distribution of ammonia forms in wastewater. Ammonia in solution may be ammonia (NH₃) or (NH₄⁺) as ammonium ion. If ammonia is a lot in the form of ammonium NH₄⁺ it indicates that ammonium will be absorbed much by the microalgae as a nutrient. As for if ammonia-shaped molecule NH₃ ammonia will easily evaporate. Based on Figure 4 shows the correlation coefficient with high correlation in AB 0.2 and AB 0.5 (-0.869 and -0.736). This is indicates the addition of CO₂ increases the pH relationship to ammonia removal. This statement indicates the addition of CO₂ increases the pH relationship to ammonia removal. Due to Fig. 4 with the addition of CO₂ wastewater pH between the range of 6 to 7, and to the reactor without CO₂ supply of pH range 7-10. Since the pH value is usually less than 8, the majority of the ammonia nitrogen in the form of ammonium ions (NH₄⁺) [23], which is a nutrient used by microalgae.
4. Conclusion
Decreasing of ammonia with the addition of CO2 rate of 0.2 L/min in an algal-bacterial reactor resulted in removal efficiency of 75%. With the addition of CO2 rate of 0.2 L/min makes the ammonia removal process increased with pH, temperature, and chlorophyll-a optimum conditions. Variable water quality parameters such as DO, pH, temperature, light intensity have an effect on the performance of reactor algae in decreasing ammonia. The pH and temperature parameters have a direct influence with the removal of ammonia.

Some suggestions to support the development of future research are as follows: applications with other wastes such as industrial waste by conditioning the same environment. Using different types of microalgae that have the ability to remove higher ammonia. While highly correlated between the addition of CO2 increases the pH relationship to ammonia removal, and chlorophyll with ammonia concentration.

References
[1] Zhu, Liandong., Wang, Zhongming., Shu, Qing., Takala, Josu., Hiltunen, Erkki., Feng, Pingzhong., Yuang, Zhenhong. 2013. Nutrient removal and biodiesel production by integration of freshwater algae cultivation with piggery wastewater treatment. Water Research. 47. 4294-4302
[2] Craggs, Rupert., Sutherland, Donna., Campbell, Helena. Hectare-scale demonstration of high rate algal ponds for enhanced wastewater treatment and biofuel production. 2012. J Appl Phycol. 24:329–337. DOI 10.1007/s10811-012-9810-8
[3] Zhang, Y, Hongyang, Yunna, Chunmin, Zeng Shen, Wenjing, Gang Yan, and Xuefei Zhou. 2012. The effect of bacterial contamination on the heterotrophic cultivation of Chlorella pyrenoidosa in wastewater from the production of soybean products,” Water Res. 46, 5509–5516,
[4] Croft, M.T, Andrew D.L, Evelyne R, Martin J.W, and Alison D.S. 2005.Algae acquire vitamin B12 through a symbiotic relationship with bacteria. Nature. Vol. 438, pages 90–93
[5] Liang Zhijie, Yan Liu, Fei Ge, Yin Xu, Nengguo Tao, Fang Peng, and Minghung Wong., 2013. Efficiency assessment and pH effect in removing nitrogen and phosphorus by algae-bacteria combined system of chlorella vulgaris and Bacillus licheniformis. Chemosphere 2013-05-014
[6] Su, Y., Mennerich, A., Urban, B., 2012. Synergistic cooperation between wastewaterborn algae and activated sludge for wastewater treatment: influence of algae and sludge inoculation ratios. Biore sour. Technol. 105, 67–73.
[7] Mulyanto A, dan Titin H. 2015. “Fiksasi Emisi Karbon Dioksida dengan Kultivasi Mikroalga Menggunakan Nutrisi dari Air Limbah Industri Susu” Jurnal Riset Industri. Vol. 9 No.1 pages 13-21
[8] Kodama dan Fujhisima. 2015. Differences in Infectivity between Endosymbiotic Chlorella Variabilis Cultivated outside Host Paramecium Bursaria for 50 Years and Those Immediately Isolated from Host Cells After One Year of Reendosymbiosis. Journal Biology Open,pages 1-7
[9] Anggriary Dwi. 2012. Perbandingan Kerapatan Sel dan Kandungan Klorofil Synechococcus sp. RDB001 yang Ditumbuhkan pada Suhu 30±5°C dan 50±5°C. Disertation, Departemen Biologi. Universitas Indonesia, 2012
[10] Bellinger E.G, and David C. S., 2010. Freshwater Algae: Identification and Use as Bioindicators. John Wiley and Sons, Ltd, pp. 232-233
[11] Purnawati S. et al.,. 2013. Pertumbuhan Chlorella Vulgaris Beijerinck dala Medium yang Mengandung Logam Berat Cd dan Pb Skala Laboratorium. Seminar Nasional Biologi.
[12] Anggriary Dwi. 2012. Perbandingan Kerapatan Sel dan Kandungan Klorofil Synechococcus sp. RDB001 yang Ditumbuhkan pada Suhu 30±5°C dan 50±5°C. Disertation, Departemen Biologi. Universitas Indonesia
[13] Sihombing R.F, Riris A, dan Hartoni. 2013. Kandungan Klorofil-a Fitoplancton di Sekitar Perairan Desa Sungasang Kabupaten Banyuasin Provinsi Sumatera Selatan. Maspari Journal, 2013, 5 (1), 34-39

[14] Agus S, Yeni Y, dan Rizal M., 2011. Pengaruh CO$_2$ Terhadap Pertumbuhan Staurastrum sp. Makara Sains. Vol.9 No. 1

[15] Xiaochen M, Wenguang Z, Zongqiang Fu, Yangling C, Min Min, Yunkai Z, Paul Chen, and Roger Ruan., 2014. Effect of wastewater-borne bacteria on algal growth and nutrients removal in wastewater-based algae cultivation system. Bioresource Technology. 167, 8-13

[16] Hoshida H, Takayuki O, Akira M, Rinji A, and Yoshinori M. 2005. Accumulation of Eicosapentaenoic Acid in Nannochloropsis sp. In Response to Elevated CO2 Concentrations”, Journal of Applied Phycology. Vol.17, pages 29-34

[17] Setiawan Y, Aep S, Prima B, dan Saepulloh. 2014. Pemanfaatan Emisi Gas CO$_2$ untuk Budidaya Spirulina Plantensis dalam Upaya Penurunan Gas Rumah Kaca (GRK). Jurnal Riset Industri. Vol.8 No.2 Hal 83-89

[18] Limbong A., 2008. Alkalinitas: Analisa dan Permasalahannya untuk Air Industri. Karya Ilmiah, Kimia Anlasis, Universitas Sumatra Utara.

[19] Posadas E, Maria M, Cintia G, Gabriel A, and Raul M., 2015. Influence of pH and CO$_2$ source on the performance of microalgae-based secondary domestic wastewater treatment in outdoors pilot raceways. Chemical Engineering Journal 265 (2015) 239-248

[20] Ayu S. et al., 2014. Penambahan Urea sebagai Co-Substrat pada Sistem High Rate Algae Reactor (HRAR) Untuk Pengelohan Air Limbah Tercemar Minyak Solar. Jurnal Teknik POMITS Vol. 3, No. 2

[21] Shi, J., Podola, B., Melkonian, M. 2007. Removal of nitrogen and phosphorus from wastewater using microalgae immobilized on twin layers: an experimental study. Journal of Applied Phycology 19, 417-423.

[22] Godos I, Arbib, Lara E, and Rogalla., 2016. Evaluation of Hight Rate Algae Pond for treatment of anaerobically digested wastewater: Effect of CO$_2$ addition and modification of dilution rate. Bioresource Technology 220. 253-261

[23] Crites, R. W. 2006. Natural Wastewater Treatment Systems. Boca Raton, FL: CRC Press

[24] Tang C, Wei Zuo, YuO Tin, Ni Sun, Zhein W, and Jun Zhang., 2016. Effect of aeration rate on perfomance and stability of algal-bacterial symbiosis system to treat domestic wastewater in sequencing batch reactors. Bioresource Technology 222(2016) 156-164