AMMONIA ABSORPTION IN NITROGEN INDUSTRY WASTEWATER BY MICROALGAE *Chlorella pyrenoidosa, Nannochloropsis sp.* AND BACTERIA *Pseudomonas fluorescens*

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ABSTRACT

The growth of *Chlorella pyrenoidosa* microalgae, *Nannochloropsis sp.* and the bacterium *Pseudomonas fluorescens* on urea nitrogen industry wastewater has been investigated as an effort to study the potential of these microorganisms in the remediation of nitrogen industry wastewater. Microalgae and bacteria to be grown on media in water treatment plants with high levels of urea fertilizer NH3-N and by 50 percent urea for inhibitory Concentration (IC_{50}). The results showed that the microalgae C. *pyrenoidosa* able to grow and tolerant on the levels 626.646 ppm of NH3-N and 1426 ppm urea, *Nannochloropsis sp.* NH_3-N and urea 559.854 1398 ppm, whereas *P. fluorescens* 723.219 ppm and 1623 ppm urea. Microalgae and bacteria growth at that concentration can absorb *C. pyrenoidosa* NH_3-N 53.46% and urea 58%, *Nannochloropsis sp.* NH3-N 57.5% and 53.71% urea, whereas *P. fluorescens* NH_3-N 62.47% and 62.47% urea. Based on the results of these studies, microalgae and bacteria have potency to be developed as a bioremediation agent in absorbing nitrogen industry wastewater, on the bottom of the pool, deposition microalgae can be used as an alternative energy source.

Key words: Absorption, *Chlorella pyrenoidosa, Nannochloropsis* sp. and *Pseudomonas Fluorescens,* nitrogen industry wastewater

1. INTRODUCTION

Wastewater is byproduct of an activity which contains different types of pollutants. One of them is the activity of nitrogen industry wastewater. There are six nitrogen industries in Indonesia which have ammonia- nitrogen in high level in their wastewater industry. The processing of wastewater contains high level urea and ammonia- nitrogen is one of the problems faced by Indonesia fertilizer industry wastewater. Although the $(NH_2)_2CO$ and NH_3-N do not categorize as B3 compound, nitrogen industry wastewater can damage water ecosystem seriously. Ammonia in the water at certain concentrations can harm aquatic life, led to eutrophication, causing corrosion of certain metals, even lead poisoning that will be caused lung damage and death.

The activities of nitrogen industry that potentially caused environmental pollution are wastewater disposal activities to the water. Wastewater is the major byproduct of the nitrogen industry. Based on the Decree of the Minister of Environment Decree 122 of 2004 and Governor of South Sumatra, No.18 of 2005, the maximum pollution load for nitrogen industry wastewater is the ammonia levels of 0.75 kg / ton (50 mg / L) and pH 6.0 - 9.0.

Opportunity to utilize microalgae and bacteria in the contaminated wastewater treatment is possible to be done, because there are many facts that can be observed. Microalgae are microscopic water

plants and potentially be used in the treatment of nitrogen industry wastewater and precipitate them on the bottom and used as an alternative energy source (Aslan and Kapdan 2006, Chisti, 2007).

Utilization of microalgae and bacteria in the nitrogen industry wastewater's treatment will cause a positive impact on the environment, i.e. able to act as a biocatalyst in decreasing the content of ammonia and nitrogen in waste. Processing principles run naturally like natural ecosystems principle and produces secondary waste. Its superiority from other microalgae is in the recycling nutrient process run efficiently and produce biomass that can be used for various purposes (De la noue et al., 1992).

Microalgae *C. pyrenoidosa* and microalgae *Nannochloropsis sp* are the chlorophyll microalgae that need macro nutrients such as nitrogen and phosphate. Microalgae *C. pyrenoidosa* are able to survive in the environments that contain many nutrients and use them in the process of photosynthesis, breeding and other life activities (Becker, E, W, 1994). Besides that, microalgae *C. pyrenoidosa* and *Nannochloropsis sp* has high concentration of chlorophyll and able to form a new population in every 7-9 hours in the stationary phase occurred in days 5-6 (hansakul, 1993). By the ability of microalgae *C. pyrenoidosa* and *Nannochloropsis sp* to exploit nutrient substance expected that toxic ammonia compounds is reduce, so that it can improve the quality of nitrogen industry waste water.

P. fluorescens is anaerobe bacteria that have ability to fixate molecular nitrogen in photoautotroph manners. Therefore, the bacteria *P. fluorescens* as nitrogen-bonding bacteria have important role in the supplying of nitrogen in some places such as waste pond containing high level nitrogen (Torres, 2010).

2. MATERIALS AND METHODS

The tools used in this study are volumetric flask, pH meter, measuring pipette, Spectrophotometer, scales, aerator, transparent plastic tubing measures ¾ diameters, hemacytometer, microscope, fluorescent lamp and the culture bottles, cork drill, Petri dishes, transparent millimeter paper. While the ingredients needed are water, distilled water, Nessler reagent, liquid ammonia derived from Wetland area of nitrogen industry, microalgae *C. pyre*noidosa and *Nannochloropsis sp,* seeds derived from pure cultures in the uncontaminated condition by zooplankton or other organisms. Water used is brackish (a mixture of sea water and fresh water) as the main growing medium of *Nannocloropsis sp* with 3‰ salinity, while the microalgae C. *pyrenoidosa* using fresh water, pH 8 - 9.5, and temperature of 25-30 ° C. Bacteria P. *fluorescens* seeds derived from pure cultures in the uncontaminated condition. Media Kings B (composition are protease peptone 10 g, K_2HPO_4 0, 75 g, $MgSO_47H_2O$ 0, 75 g, glycerol 7, 5 ml, distilled water 500 ml). Variable measurement including pH, density, NH_3-N level and nitrogen

3. RESULTS AND DISCUSSION

1. Result Analysis of the wastewater quality based on IC_{50} values

Water quality test solutions are important in the study, in which it determined that microalgae *C. pyrenoidosa*, *Nannochloropsis sp.* and *the bacterium P. fluorescens* influence to remediate NH_3-N and urea contained in nitrogen industry wastewater. In which water conditions quality in the test solution based on the concentration IC_{50} values. Water quality measurements on day 0 to day 7th The quality parameters measured include NH_3-N, urea, and pH. Measurement of pH can be measured using a pH meter. NH_3-N and urea are measured by using spectrophotometry. Prior to measurement, the tools used are calibrated in advance. The order of the range of concentrations in the definitive test refers to the logarithmic series.

2. Results analysis of wastewater quality based on Microalgae Concentrations IC_{50}

Based on data from the quality of nitrogen industry wastewater by using microalgae *C. pyrenoidosa, Nannochloropsis sp.* and the bacterium P. fluorescent at concentrations IC_{50} can be seen in Figure 1, 2 and 3

1. The degree of acidity (pH)

The degree of acidity (pH) of water quality tests on each treatment based on the concentration IC_{50}, pH for each treatment do not change because of the toxicant on the provision of wastewater does not directly impact the change of pH in aqueous media test and microalgae *C. pyrenoidosa, Nannochloropsis sp*, can tolerate pH, it can be seen in Figure 1.

This case will facilitate subsequent analysis, in which the factors that truly want to be seen are the effect of the concentration of nitrogen wastewater to pH changes from day 0 to day-7th. According to Cole (1994), pH is not changed due to the natural buffer system CO_2 in the culture medium.

Dissolved CO_2 contained in the media will be carbonic acid that will decompose to carbonate ions and bicarbonate ions. But, mikroalgae *C. pyrenoidosa* and *Nannochloropsis* sp. Able to live on the condition of pH between 8,0 – 9,0. The effect of pH to aquatic organism according to Swingle (1969) in Boyd (1982) is the good growth of microalgae is on the condition of pH 7 – 9. Too acidic water (pH <6.5) and too alkaline water (pH> 10) can damage the cell wall and disturb the growth of microalgae *C. pyrenoidosa* and *Nannochloropsis sp.* Although microalgae *C. pyrenoidosa* and *Nannochloropsis sp* can live in the range of pH 7-9, but the pH should be maintained in the range 7.2 - 7.8. This relates to the toxicity of ammonia,

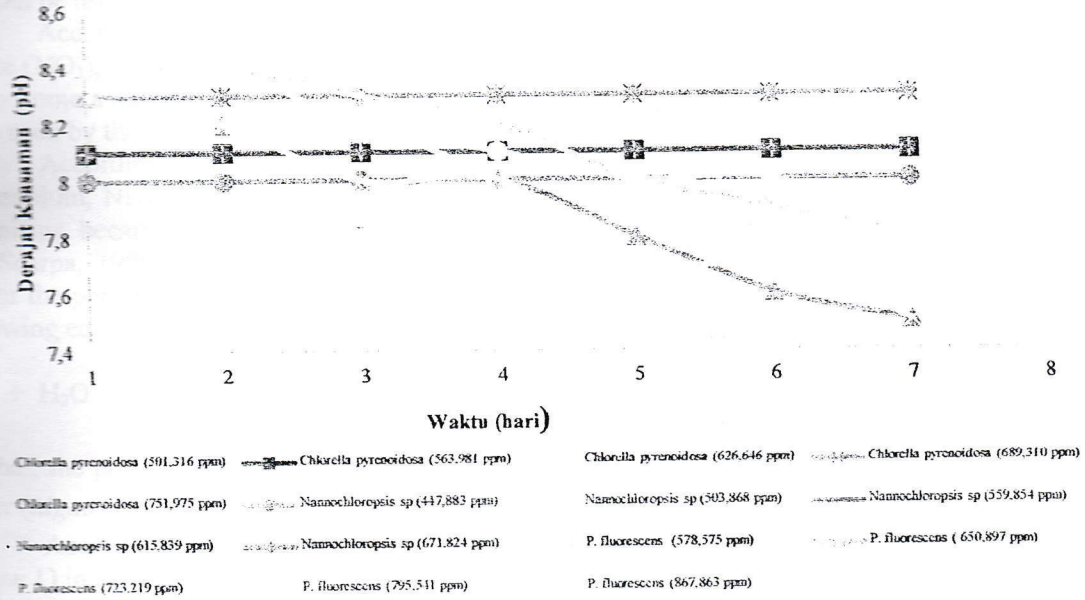

Figure 1. The graph of the quality nitrogen industry wastewater based on concentrations IC_{50} on pH microalgae C. pyrenoidosa, *Nannochloropsis sp.* and *the bacterium P. Fluorescent*

where ammonia toxicity increases with increasing pH. At pH less than 7.8 fraction of ammonia in total ammonia nitrogen decreased about 5% and at pH greater than 9 about 50% of total ammonia nitrogen in the form of ammonia (Van Wyk and Scarpa, 1999). While the pH profile on the quality of nitrogen industry wastewater using bacteria *P. fluorescens* decreased during the maintenance period which is started at day 0 to day7th with varying concentrations, as the increasing maintenance time. During the maintenance occur the decreasing pH values of 8.3 to 7.5.

The values of PH during the maintenance period are influenced by three things, i.e CO_2 in the maintenance media, the addition of sugar and the presence of bacteria *P. fluorescens*. Along with increasing time of maintenance, the amounts of CO_2 become more so that the pH value in the maintenance media tends to decrease. Contribution of CO_2 to the maintenance of media is also suspected originate from the decomposition of organic matter and respiration by bacteria. According to Beristain et

al., (2005) bacterial metabolism involves (a) the oxidation of organic materials that produceCO_2 and energy, and (b) the biosynthesis of cell material bacteria, can be seen in the observations of day-7 th (final pH), because on day 7th, bacteria grow in large numbers, the more bacteria will produce the more amount of CO_2 therefore pH decreasing is greater.

2. Amonia (NH_3-N)

Based on the analysis of NH 3-N that have been made to the microalgae C. *pyrenoidosa* and *Nannochloropsis sp*, on the nitrogen wastewater industry are able to decrease in each treatment based on the concentrations IC_{50} values observed ranging from 0 to the day 7th, this is presented in Figure 2. In which from several concentrations conducted occur a decline in NH_3-N. This means that the substrate utilization of many nitrogen sources obtained from nitrogen industry wastewater. Thus, during maintenance occurs the utilization of nitrogen content by microalgae *C. pyrenoidosa* and *Nannochloropsis sp* to be used as a substrate so that the levels are decreasing, but the higher decline occurs on the microalgae *C. pyrenoidosa* rather than *Nannochloropsis sp.* According to Chevalier, P, and J. de la Noue (1985) microalgae *C. pyrenoidosa* could potentially be used to accumulate waste materials, especially for the absorption of nitrogen and phosphorus, were able to eliminate 90% of the ammonium (within four hours) and 100% of the phosphate (within two hours). Ammonia is not the source of nitrogen that can be used directly by the *microalgae C. pyrenoidosa.*

According to Effendi (2003), the source of nitrogen that can be used directly by aquatic plants is nitrate (NO_3), ammonium (NH_4) and nitrogen (N_2). Nitrate is the main form of nitrogen in natural waters and become a major nutrient for plant growth and microalgae. Therefore, it is able to known that in order to be used by the microalgae, the ammonia must be oxidized to nitrate first.

According to Masser et al (1999) there are two forms of ammonia in water, which is ionized (ammonium, NH_4 +) and non-ionized (ammonia, NH_3).Non- ionized ammonia is not harmful to aquatic organisms, because it is toxical. NH_3 value depends on the pH value and temperature of water (Van Wyk and Scarpa, 1999; Masser et al., 1999; Boyd, 1990). The higher water temperature and pH water, the higher the percentage of NH_3 (Boyd, 1990). The comparison between NH_3 and NH_4 + can be seen in the following equation

$$NH_3 + H_2O \longleftrightarrow NH_4^+ + OH^-$$

So that, the presence of NH_3-N in excessive number can stimulate the explosion growth of Algae in the water (*algae bloom*).

According to Davis dan Cornwell (1991) there are three reasons that make nitrogen dangerous, as follow 1) in a high concentration of NH_3-N toxic to fish, (2) low concentrations of NH_3, and NO_3-can lead blooming algae, (3) conversion of NH_4^+ to NO_3-requires large amounts of oxygen.

Values NH_3-N by using the bacteria *P. fluorescens* in degration NH_3-N during the maintenance period tends to decline. This observation is seen in day-0 to 7th day. In which the result of observations is shown in Figure 43. NH3-N generally decreased along with the length of maintenance time. Bacteria are the most important groups of microorganisms in wastewater treatment and can oxidize anorganic compounds such as NH_3 for energy.

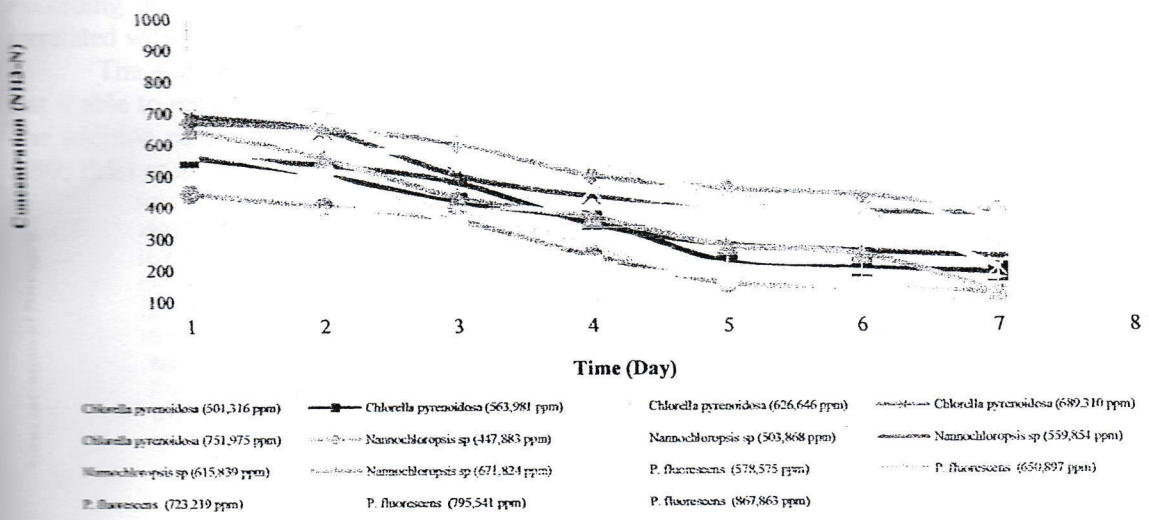

Figure 2. The wastewater quality of nitrogen industry based on concentration IC_{50} of NH_3-N with microalgae *C. pyrenoidosa, Nannochloropsis sp* and bacterial *P. fluorescens*.

According to Willett and Morrison (2006), bacteria will use organic carbon as an energy source, correlated with the nitrogen that will be used for protein synthesis to produce a new cell material.

The addition of carbonaceous material, the bacteria will use the nitrogen contained in culture, so that it able to reduce the concentration of inorganic nitrogen (ammonia) which are toxic to the organism. The addition of carbonaceous materials is proven able to reduce inorganic nitrogen (Avnimelech, 1999; Erler et al., 2005). Bacterial growth is limited by the balance of nutrients in the water. Therefore, the dynamics of bacteria populations is strongly associated with the availability of nutrients (Liu and Han, 2004).

3. Nitrogen

Observation of nitrogen levels on nitrogen industry wastewater by using microalgae *C. pyrenoidosa* and *Nannochloropsis sp* can be seen in Figure 2. It is obvisiously seen that deterioration in the element of urea at the end of study. Therefore, during maintenance occurs the utilization of nitrogen by microalgae *C. pyrenoidosa* and *Nannochloropsis sp,* to be used as substrate,so that the nitrogen levels are decreased, but the higher decreased found in microalgae *C. pyrenoidosa*

On the observations of water quality used bacteria *P. fluorescent* showed that bacterium *P. fluorescent* elements can degrade the elements of nitrogen industry wastewater. In which, there is a decrease on each treatment; this can be seen in Figure 3.

Nitrogen industry wastewater containing high urea, would endanger the waters, is due to the waste contain high levels nitrogen. Nitrogen is an essential element for the growth of microorganisms, plants, and animals are referred as biostimulan. Chemical compounds of Nitrogen are very complex, because nitrogen has several oxidation stages that are able to change the compound of nitrogen. Oxidation process is influenced by living organisms. Nitrogen contained in the water may be in the form of nitrogen gas (N_2), ammonia (NH_3), ammonium (NH_4), nitrite ion (NO_2), nitrate ion (NO_3). This made the content of nutrients in the water increased, also ammonia which is harmful to aquatic organisms. Nitrogen will be used as a source of nitrogen by phytoplankton, algae, plants, and bacteria. But, excessive number of nutrients will lead the algae growth rapidly (blooming) that ultimately result in mass mortality of algae

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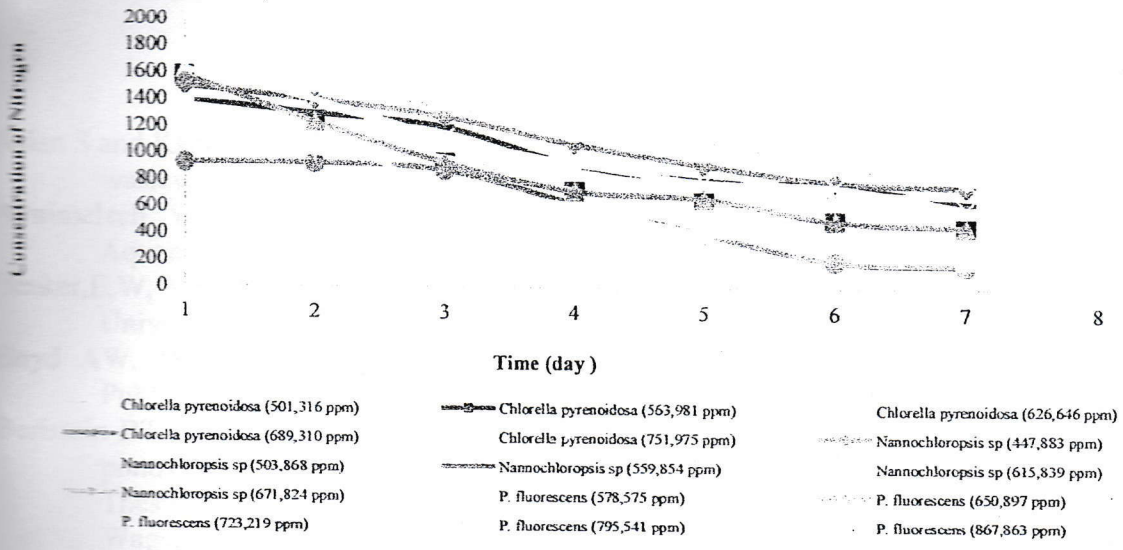

Figure 3. The graphic of the nitrogen industry wastewater quality based on concentration IC_{50} on *C.pyrenoidosa*, *Nannochloropsis sp* and bacterial *P. fluorescent*

Bacterial growth is limited by the balance of nutrients in the water. Therefore, the dynamics of bacterial populations is strongly associated with the availability of nutrients (Liu and Han, 2004).

Ammonium used as a source of nitrogen by phytoplankton, algae, aquatic plants, and groups of bacteria. Presumably the bacteria use ammonium in significant numbers in the waters. Some studies indicated that bacteria use almost 50% of total ammonium in the water. Bacteria are not only using ammonium as a nitrogen source, but also the excretion of aquatic organisms (Montoya and Velasco, 2000).

4. CONCLUSIONS AND RECOMMENDATIONS

Based on the results of conducted research it can be concluded that

1. Microalgae *C. pyrenoidosa* tolerant to live and thrive well at pH 8-9 andable to absorb NH_3-N from concentration 626.646 ppm reached 53.46 % . Nitrogen at the concentration 1426 ppm can absorb up to 58%.
2. Microalgae *Nannochloropsis* sp tolerant to live and thrive well at pH 8 – 9 and able to absorb NH_3-N from consentration 559.854 ppm near 57.5% and nitrogen at the concentration 1398 ppm can absorb 53.71 %
3. Bacteria *P. fluorescent* able to tolerant live to and thrive well at pH 8 – 9 and able to absorb NH_3-N from consentration 723.219 ppm and nitrogen at the concentration 1623 ppm can absorb up 62.47% NH_3-N and 62.47% urea
4. Microalgae *C. pyrenoidosa* and *Nannochloropsis* sp and *P. fluorescen* potentially to be develop as bioremediasi agent in Nitrogen Industry wastewater.

For further research are suggested:

To determine the limit absorption of microalgae *C. pyrenoidosa*, *Nannochloropsis sp* and P. *fluorescent* in the content of NH_3-N and urea on nitrogen wastewater industry at the same concentration continuously added in unlimited time. This is done to know the exact time when the regeneration of microalgae should be implemented.

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