The synergy of microalgae *Chlorella pyrenoidosa*, *Nannochloropsis* sp and the bacteria *Pseudomonas fluorescens* in the bioremediation process of nitrogen industry wastewater

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Abstract

The purpose of this study is the treatment of Urea nitrogen industry wastewater by using microalgae C. pyrenoidosa, Nannochloropsis sp. and bacteria P. fluorescens. By means of this process, it is expected that the potential of the and the bacteria will synergy in degrading the nitrogen wastewater In this study the concentration used is the concentration of NH_3-N level from the analysis of IC_{50}. The response being observed is a decrease in level of NH_3-N, Urea and pH. The result of the study shows a decrease in the concentration of NH_3-N at 723.219 ppm 78,89 %, Urea at a concentration 1623 ppm 62,66 % and a pH of 8.3 to 7.6 on the synergy of microalgae C. pyrenoidosa and the P. fluorescens bacteria. While the synergy of microalgae Nannochloropsis sp and P. fluorescens bacteria at a concentration of 723.219 ppm results in a decrease of NH_3-N of 58,98 %, Urea at a concentration of 1623 ppm results in a decrease of 60 % and a decrease of pH from 9.3 to 7.9. The study concludes that microalgae C. pyrenoidosa, Nannochloropsis sp and P. fluorescens bacteria can potentially be developed as bioremediation agents in the treatment of nitrogen industry wastewater

Keywords : C. pyrenoidosa sp, Nannochloropsis sp, P. fluorescens, Wastewater of Urea Fertilizer Plants

1. Introduction

Ammonia is well known as important raw material of industrial commodities in Indonesia. However, nitrogen industry wastewater is one of harmful pollutants. Ammonia in Urea nitrogen industry wastewater, at certain concentrations can harm aquatic life, led to eutrofication, causing corrosion to certain metals, even lead poisoning that are able to

damage lung and causing death. In principle, the component of nitrogen in the waste that caused pollution is ammonia ion (NH_3-N), nitrite ion (NO_2^-), and nitrate ion (NO_3^-).

Activities of nitrogen industry that could be potential caused environmental pollution is a wastewater disposal activities to the waters.Wastewater which is a major byproduct of Urea nitrogen industry is ammonia. Based on the Decree of the Minister of Environment No. 122 of 2004 years and Governor of South Sumatra, No.18 of 2005, the maximum pollution load for Urea nitrogen industry is the ammonia levels of 0.75 kg / ton (50 mg / L) and pH 6.0 - 9.0 .

Commitment of nitrogen industry to improve environmental management is wastewater treatment by using Wastewater Treatment Plant (IPAL) and Minimize Wastewater Separation (MPAL), as done by Urea nitrogen industry. The Complement efforts of waste management can be developed by using the bioremediation principles

Several studies have shown that biological methods for remediation Urea nitrogen industry can be less expensive and it is environmentally friendly technologies to treat wastewater of nitrogen industry, but the natural bioremediation has limitations in which it require long periods of time, insufficient number of microorganisms, and incomplete decomposition (Alexander, 1999). In order to overcome these limitations, bioremediation can be done by using a combination of bacteria that are able to degrade Urea nitrogen industry wastewater and microalgae, so it would be more effective and more perfect than use of bacteria and microalgae only.

According to Chevalier, P, and J, de la Noue, (1985) microalgae *C. pyrenoidosa* potentially be used to accumulate waste materials, especially for the nitrogen and phosphorus absorption, they are able to eliminate 90% of the ammonium (within four hours) and 100% of the phosphate (within two hours).Microalgae C.*pyrenoidosa, Nannochloropsis* sp potential to remediate of Urea nitrogen industry wastewater. Microalgae C .*pyrenoidosa,Nannochloropsis* sp is expected able to reduce levels of NH_3-N and Urea contained in Urea nitrogen industry wastewater because of its ability to utilize NH_3-N and urea directly as a nutrient, by the help of bacteria *P. fluorescens*, it is converted to be ammonium ion, nitrate and nitrite that are directly absorbed by the microalgae *Chlorella pyrenoidosa,* Nannochloropsis sp. Bandala *et al*, (2006), stated that *P. fluorescens* bacterium is a bacteria that can survive in extreme conditions, that is a condition in which there are certain environmental pollutants there.

2. Materials and methods

The tools used in this study are volumetric flask, pH meter, measuring pipette, Spectrophotometer, scales, cork drill, Petri dishes, and aerator, transparent millimeter paper, transparent plastic tubing measures ¾ diameter, *hemacytometer*, microscopy, and TL Lamps, culture bottles. While the material required is ammonia wastewater that derived from Wetland area of urea nitrogen industry, water distilled, Nessler reagent, the bacterium *P. fluorescens*, and uncontaminated seeds derived from pure cultures. Kings B

medium (protease peptone 10 g composition, K_2HPO_4 0.75 g, MgSO $7H_2O_4$ 0.75 g, 7.5 ml glycerol, akuadest 500 ml) and *Chlorella pyrenoidosa* microalgae and Nannochloropsis sp. Seed derived from pure cultures that are uncontaminated by zooplankton or other organisms. Water used is brackish (a mixture of sea water and fresh water) as the main growing medium of *Nannocloropsis* with a salinity sp 3%, while the microalgae C. *pyrenoidosa* using fresh water, pH 8 - 9.5, and temperature of 25-30 °C. Measurement variables include pH, density, levels of NH_3-N and urea.

3. Result and discussion

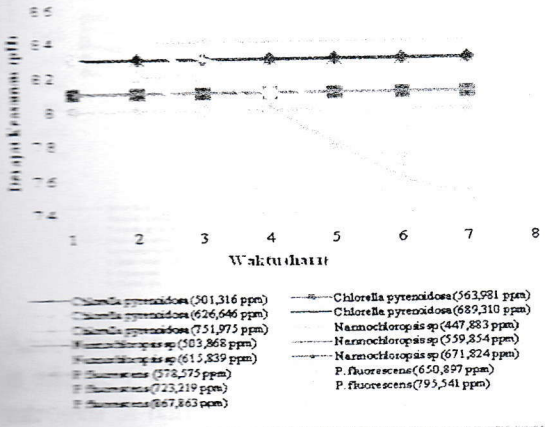
3.1. Results analysis of Wastewater Quality Based on concentration IC_{50} on Microalgae Chlorella pyrenoidosa, Nannochloropsis sp and Pseudomonas fluorescens

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

3.1.1.The Degree of Acidity (pH)

The degree of acidity (pH) of quality water tests on each treatment based on the IC_{50} concentration, pH for each treatment did not change because of giving toxicant on the wastewater did not give effect directly toward the changes of pH in aqueous media test and microalgae C. *pyrenoidosa* and *Nannochloropsis sp,* can tolerate pH, it can be seen in Figure 1. This is a test that will facilitate subsequent analysis, where the truly factors that want to be seen is the effect of concentration nitrogen industry wastewater to pH changes from day 0 to-7. But microalgae *C. pyrenoidosa* and *Nannochloropsis sp* can survive in conditions of pH 8.0 to 9.0. According to Swingle (1969) in Boyd (1982) about the Effect of pH on aquatic organisms is the well growth of microalgae is at the condition pH 7-9. Water that is too acid (pH <6.5) and too alkaline (pH> 10) can damage the wall cell and disturb the growth of microalgae C. *pyrenoidosa* and *Nannochloropsis sp*.

Figure 1. Graphs of the quality Urea nitrogen wastewater industry based on IC_{50} concentrations on pH with C. pyrenoidosa, Nannochloropsis sp microalgae and bacterial P. Fluorescent



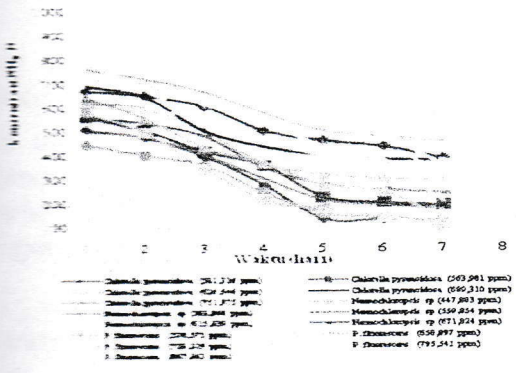
Although microalgae *C. pyrenoidosa* and *Nannochloropsis sp* can survive in the pH range 7-9, but the pH should be maintained in the range 7.2 - 7.8. This relates to the toxicity of ammonia, where ammonia toxicity increases as the increasing of pH. At pH less than 7.8 fraction of ammonia in total ammonia nitrogen decrease about 5% and at pH more 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 waste water using bacteria *P. fluorescents* decreased during the maintenance period at 0 -to-7 day with varying concentrations, as increasing of maintenance time During the maintenance pH values decrease from 8.3 to 7.5 values.

3.1.2. Ammonia (NH_3-N)

In the analysis of NH 3-N that have been done to microalgae *C. pyrenoidosa* and *Nannochloropsis sp* on the waste water of urea fertilizer plant, decrease in each treatment based on the value concentrations of IC_{50} from 0 to7th day, is will be shown in Figure 2, Where from several concentrations conducted, the decreasing of NH_3-N occurs. It means that the substrate utilization of many nitrogen sources obtained from nitrogen industry wastewater. Thus, during maintenance, occurs the utilization of nitrogen by microalgae *C. pyrenoidosa* and *Nannochloropsis sp* to be used as a substrate until its levels decreased, but the higher decreasing occurs to microalgae *C.pyrenoidosa* rather than *Nannochloropsis* sp. According to Chevalier, P, and J, de la Noue (1985), microalgae *Chlorella pyrenoidosa* 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 phosphate (in two hours). Ammonia is not nitrogen that can be utilized directly by the microalgae C. *pyrenoidosa.*

Figure 2. The graphs of quality nitrogen industry wastewater based on IC_{50} algae C. pyrenoidosa, Nannochloropsis sp and bacterial P. Fluorescent.



According to Effendi (2003) Nitrogen sources that can be used directly by aquatic plants is nitrate (NO_3), ammonium (NH_4) and nitrogen gas (N_2). Nitrate is the main form of nitrogen in natural waters and become a major nutrient for plant growth and microalgae.

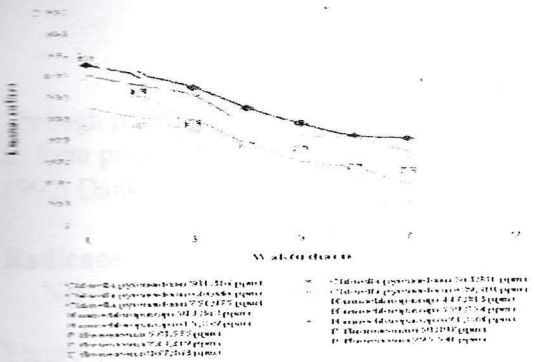
Based on that things Ammonia must be oxidized to be a nitrate in order to be able to use by the microalgae,

NH_3-N levels, using the bacterium *P. fluorescens* in digression NH_3-N during the maintenance period tends to decrease. This observation is seen in day-0 to-7th day. The result of observation will be shown in figure 2. In general NH_3-N decrease as long with maintenance time. Bacteria are the most important microorganisms in wastewater treatment and can oxidize inorganic compounds such as NH_3 for energy.

3.1.3. Nitogen

Based on the observations of nitrogen value on the nitrogen industry wastewater treatment synergized with microalgae and bacteria can be seen in Figure 3. Clearly, there is decreasing of urea element at the end of study. On the microalgae *Nannochloropsis* sp *C.pyrenoidosa* occurs the degradation on the nitrogen industry wastewater. Therefore, during maintenance occurs the utilization of Nitrogen level by microalgae C. *pyrenoidosa* and *Nannochloropsis* sp, so the levels are decreased, but the higher decreased occur in microalgae *C. pyrenoidosa*

Figure 3. The graphs of nitrogen industry wastewater quality based on concentration IC_{50} of urea with microalgae *C. pyrenoidosa, Nannochloropsis sp* and bacterial *P.fluorescens*



3.2. The result analysis of Microalgae and Bacteria Synergies Based on the Quality of Wastewater.

In the research of synergy microalgae and bacteria carried out by using various concentration i.e. the concentration of bacteria e.g. 578.575 ppm, 650.897 ppm, 723,219 ppm, 795,541 ppm, and 867,863 ppm on the observation from 0 to 7 th day fond in Figure 4, 5 and 6. The observed observations are NH_3-N, urea and pH.

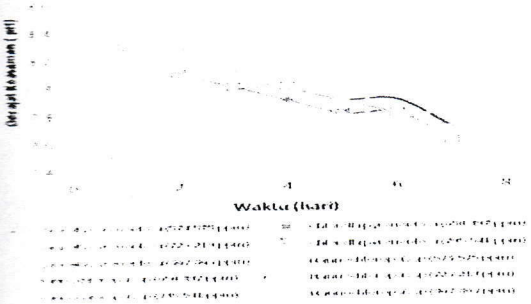
3.2.1. The synergies of Microalgae *Chlorella pyrenoidosa, Nannochloropsis sp* and Bakteria *Pseudomonas fluorescens.*

The use of microalgae *Chlorella pyrenoidosa* and *Nannochloropsis sp* and the bacteria *P. fluorescens* in the process of bioremediation is expected that it synergize potentially in bioremediation of nitrogen industry wastewater.It can be seen in Figure 4, 5 and 6. Based

on the observation on microalgae *C. pyrenoidosa* and *Nannochloropsis sp* with the bacterium *P. fluorescens* in which the decreased of pH, NH_3-N value and urea from each concentration. Based on the result of observation above, it is known that the microalgae *C. pyrenoidosa, Nannochloropsis* sp and bacteria *P. fluorescens* in waste water of urea fertilizer plant occurs in synergy as symbiosis mutualism.

Degradation of waste urea fertilizer plant by bacteria has been elaborate urea fertilizer plant greatly into small compounds that will be used by microalgae for growth i.e. CO_2, NH_4^+, NO_3-, $PO_4^{3-}$and so on. And vice versa microalgae absorb the compounds and produce organic matter, O_2, and H_2O.

Figure 4. pH value on the quality of nitrogen industry wastewater based on synergy microalgae *C. pyrenoidosa* and *Nannochloropsis sp* with the bacteriu *P. fluorescens*

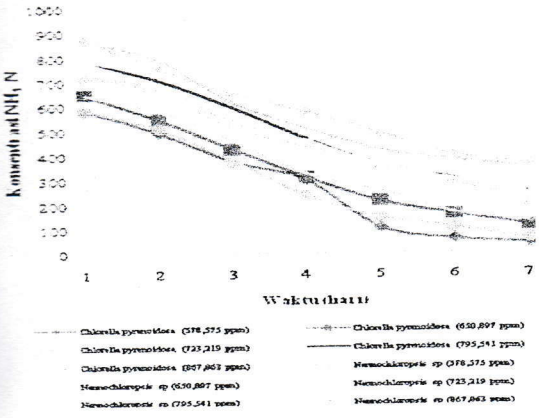


Through photosynthesis, microalgae use CO2 derived from aerobe bacteria and ammonia to form protoplasm cell and produced oxygen molecules (Stein, 1973; Coombs dan Hall, 1992: Danks et al, 1983; Polle et al, 1999):

Radience

$$NH_3+8CO_2+4{,}5H_2O \longrightarrow C_5H_{14}O_3N + 8{,}75\ O_2$$

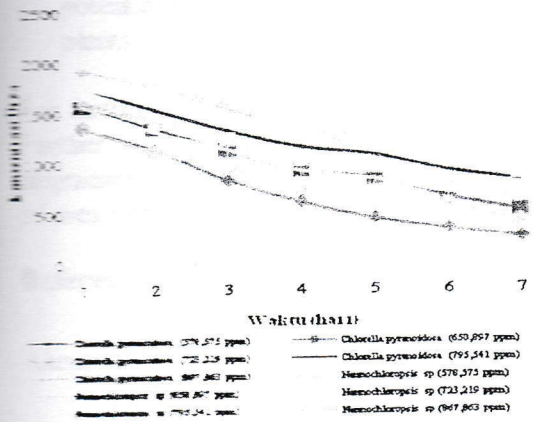
Figure 5. NH_3-N value on the quality of nitrogen industry wastewater by microalgae synergy *C. pyrenoidosa* and *Nannochloropsis sp* with the bacterium *P. fluorescent*



Environmental factors affects the growth (photosynthesis) of microalgae are light intensity, water temperature, pH, macro and micronutrients, and concentration of CO_2 (Suk-Key & Toshiuki, 2002). Although it contains the element carbon, the carbon in Urea cannot be used as a nutrient source (Stein, 1973; Polle et al, 1999), because carbon in the oxidized form and during the hydrolysis released as CO_2 on below reaction:

$$NH_2)_2CO + 2\,H_2O \longrightarrow (NH)_2CO_3 \longrightarrow 2NH_3 + CO_2 + H_2O$$

Figure 6. Nitrogen value on the quality of nitrogen industry wastewater based on synergy *C. pyrenoidosa* microalgae *and Nannochloropsis sp* with. *Fluorescent* bacterium



The bacteria utilize the organic material produced by dead microalgae, as carbon source to synthesize new cells and for the energy needs to produce final product such as CO_2, NH_4^+ in the process of respiration and the synthesis, Microalgae use CO_2 as a source carbon for photosynthesis. Ammonia is used as nitrogen source for phytoplankton, algae, water plants, and bacteria. Presumably the bacteria use ammonium in significant numbers in the waters. Some studies indicated that bacteria used almost 50% of total ammonium in the water. Bacteria do not only use ammonium as a nitrogen source, but the excretion of aquatic organisms (Montoya and Velasco, 2000).

4. Conclusions And Recommendations

Based on the result of research conducted it can be concluded that:

1. Combination of microalgae *C. pyrenoidosa, Nannochloropsis* sp and bacterial *P. fluorescens* is more effective in degrading urea fertilizer plant waste water rather than use microalgae *C. pyrenoidosa* or bacterium *P. fluorescent* only.
2. Microalgae *C. pyrenoidosa* tolerant to live and develop on pH 8 – 9 and able to absorb NH_3-N from concentration 626.646 ppm to 53,46 % and Urea from concentration 1426 ppm able to absorb until 58 %.
3. Microalgae *Nannochloropsis* sp tolerant to live and develop at pH 8 – 9 and able to absorb NH_3-N from concentration 559.854 ppm to 57,5 % and Urea from concentration 1398 ppm able to absorb until 53,71 %

- Bacteria *P. fluorescen* tolerant to live and develop at pH 8 – 9 and able to absorb from concentration 723.219 ppm to 71, 58 % and Urea from 1356 ppm concentration able to absorb until 62, 47 %.
- Synergies between Microalgae *Chlorella pyrenoidosa* and *fluorescent Pseudomonas* bacteria able to decrease NH_3-N on the concentration 723 219 78 ppm 89%, Urea at a concentration 1623 ppm 62.66% and a pH of 8.3 to 7.6.
- Synergies between Microalgae *Nannochloropsis sp* and *P. fluorescen* bacteria on the concentration 723 219 occurs the decreasing of NH_3-N 58,98 %, Urea at a concentration 1623 decreased 62.66% and a pH of 9.3 to 7.9.
- Microalgae C. *pyrenoidosa, Nannochloropsis* sp and bacteria *P. fluorescens* potentially to be developed as bioremediation agents on the urea fertilizer waste water ponds.

For further research are suggested:
Further research needs to be done on the comparison of microalgae and bacteria in degradation nitrogen industry wastewater, so that by knowing that ratio, the degradation of urea nitrogen industry wastewater will be better.

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