Toxicity Of Nitrogen Industry Wastewater On Microalgae *Chlorella pyrenoidosa*, *Nannochloropsis sp.* And Bacteria *Pseudomonas fluorescens*

Marhaini[1)], M. Faizal[2)], M. H. Dahlan[2)], Arinafril[2)], Marsi[2)]

[1)] Student of Environmental Science Study Program, Graduate School of Sriwijaya University, Palembang, Indonesia,

[2)] Lecturers of Graduate School of Sriwijaya University, Palembang, Indonesia

ABSTRACT

has six Nitrogen industries containing high level ammonia-nitrogen in wastewater as their . This characteristic is one of problem faced by Nitrogen industries in Indonesia. The Nitrogen Industry that could potentially cause environmental pollution is wastewater disposal waters. Commonly, by product of nitrogen industry wastewater is Ammonia. Although, wastewater does not include as B3 compound, but it can disturb the equilibrium of water can lead eutrophication, inhibits the animals to live and metabolized, even it can lead that caused lung damage and death. Based on the knowledge of characteristics and toxicity wastewater, microalgae Chlorella pyrenoidosa, Nannochloropsis sp and bacteria fluorescens is needed in order to prevent and control pollution caused by activities of wastewater. This research is aimed at finding the value of IC_{50} within 96 hours after industry wastewater as toxicant into microalgae Chlorella pyrenoidosa, Nannochloropsis Pseudomonas fluorescens. The value of IC_{50} of toxicant nitrogen industry wastewater on Chlorella pyrenoidosa is 626,646 ppm, Nannochloropsis sp is 559.854 ppm and bacteria fluorescens is 723.219 ppm.

C.pyrenoidosa, *Nannochloropsis* sp., *P. fluorescens*, toxicity

PENDAHULUAN

is well known as basic commodity that is important in Indonesia industry. But, on the other ammonia included as a dangerous pollutant. At the certain concentration Ammonia containing in able to disturb the equilibrium of ecosystems because it may cause eutrophication in water, aquatic animals to live and metabolized, even it can lead poisoning that caused lung damage and principle, nitrogen components contained in the waste that caused pollution is the ion of (NH3-), nitrite ion (NO2-), and nitrate ion (NO3-). The activities of Nitrogen Industry that could cause environmental pollution are wastewater disposal to waters.

byproduct of Nitrogen industry in wastewater is ammonia liquid. Based on the Minister of Decree No. 122 of 2004 and Governor of South Sumatra, No.18 of 2005, the maximum for nitrogen industry is 0.75 kg / ton (50 mg / L) and pH 6, 0 to 9.0 for the level of ammonia

ciples of nitrogen industry wastewater treatment using microalgae species *C. pyrenoidosa*, sp and *Pseudomonas fluorescens* is potential to be developed because at a certain nitrogen industry wastewater containing organic and inorganic materials can be used by C. pyrenoidosa, Nannochloropsis sp and Pseudomonas fluorescens bacteria as a source of growth.

of nature, characteristics and potency of nitrogen industry wastewater toward organisme for the prevention and control of pollution caused by activities of nitrogen industry. By doing we are able to know the highest concentration of nitrogen industry wastewater that have no effect (*Upper Bound*), the lowest concentration of waste water that has real effect (*Lower* waste concentration and the permitted discharge waste disposal into the waters, so that the can be used as a guide in the setting of environmental quality standards.

Organism used for testing are microalgae *C. pyrenoidosa, Nannochloropsis sp and Pseudomonas fluorescens* by using Asean Canada Cooperative Programme on Marine Science (ACCPMS) (1995) procedure in 96-hour test. The selection of organisms test is based on its importance in the food chain as producers in the aquatic environment and sensitive to environmental changes

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, drilled water 500 ml). Variable measurement including pH, density, NH_3-N level and nitrogen

3. RESULTS AND DISCUSSION

1. Microalgae Growth patterns and morphology of bacteria

a. Microalgae growth pattern

Based on observation of microalgae culture density on media technical nitrogen for 7 days, we obtained growth curve as shown in Figure 1.

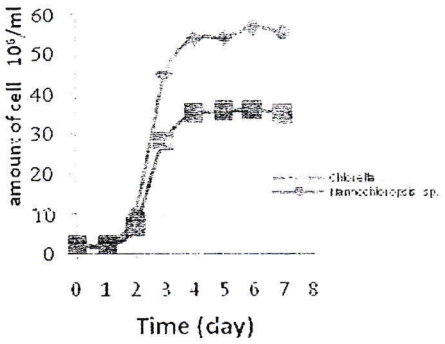


Figure 1. Growth curve of microalgae culture *C. pyrenoidosa, Nannochloropsis sp* within 7 days

Time and phase of *C. pyrenoidosa* and *Nannochloropsis*.sp phase gain from daily density (Figure 1). Based on the curve above, adaptation (lag phase) occurred on day 0- 1st day, where is in this phase the addition of microalgae cells is very low, even it can be said there has no increasing the number of cells. This was due to microalgae cells are still in the process of physiological adaptation to the growing medium, so the metabolism to grow became lower.

On the 1st day to 2nd day occurred logarithmic (log) phase or exponential, i.e. the acceleration of growth and increment of population sharply or increase intensively. When the cultivation condition is optimum, the growth of this phase is able to reach a maximum value. This is the best phase for harvesting microalgae for fish feed or industrial purposes.

At the beginning of 3rdday to 4th day occurred the decreasing rate of growth. (Declination). This phase was characterized by occurring cell division, but not as intensive as in the previous phase, so that growth becomes lower than the previous phase.

On 4th day and 6th day occurred stationary phase, this phase is characterized by the rate of reproduction and mortality rates are relatively similar, so the increment of cell number is same as before. Cell density curves resulting from this phase are a flat line.

At the beginning of day 6th to 7th day- occurred deaths phase (mortality). This phase is characterized by a greater mortality rate than the rate of growth, so that there was decrease in the number density of cells in the cultivation place. This phase is characterized by the changing of media conditions such as color. Through the growth curve of microalgae cells toward time it can be detected the appropriate time for inoculation when the growth of microalgae at the peak i.e 3rd day to 4th and also able to see the growth pattern that has reached a density of 10^6 cells / ml so that it meets the criteria as test organisms.

Culture in this study is closed media culture, so the nutrients come derived from technical fertilizers. Growth media has macro and micro elements that are needed by microalgae to grow. The dominant macro elements contained in the technical fertilizers is N that functions in lipid production and play a role in the production of chlorophyll.

According to Anderson (2005), microalgae do not only fixation CO_2 but also make use nutrient in the body water on the photosynthesis process. Nutrient in this process derived from additional material and also from wastewater material. The use of wastewater as input nutrient will reduce operational cost, improve absorption CO_2 emission instrument and also improve the quality of wastewater in such industrial area. (Green *et al.*, 1995).

The growth of P. *fluorescens* Bacteria

Before conducting Minimum inhibitory Concentration (MIC) test and P. *fluorescents* testing as bio indicator, conducted grow bacterial by using media Kings B. It can be seen in Figure 2.

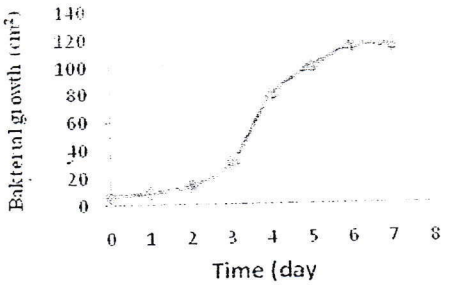


Figure 2. The curve of *P. fluorescents* bacteria cultures growth within 7 days.

The *P. fluorescents* bacteria growth during the incubation period will shape the growth curve and the maximum bacterial growth occurred on 4th day after culture. Based on the growth curve of bacterial cultures above, culture adaptation begins on day 0 until 1st day. On 1st day to 4th day occurred increasing growth, while at the beginning 4th day to 6th day occurred stationary phase. On the 7th day decreasing of cell density. The bacteria grow normaly in incubated medium and fulfill the 7th day with characteristic colonies are round, flat edge and fluidal, so easily spread. This is shown in Figure 3.

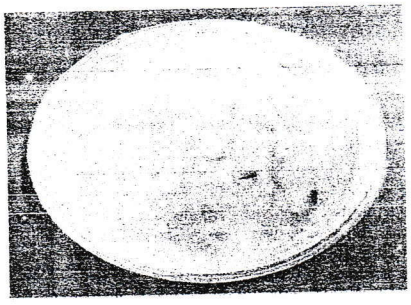


Figure 3. Bacteria colonies P. *fluorescens* almost fulfill Kings B medium

Effect of Nitrogen Industry Wastewater on Cell Growth of Microalgae Bacteria.

Effect of Nitrogen Industry Wastewater on Cell Growth of Microalgae C. *pyrenoidosa*

The result data of microalgae observation growth every 8 hours for 96 hours are presented in Figure 4. To compare the effect of urea industry waste on the growth of microalgae C. pyrenoidosa, the toxicity test

is divided into 5 concentrations: 0 ppm, 1 ppm, 10 ppm, 100 ppm and 1000 ppm. The number of cells at each concentration showed different results. At a concentration 0 ppm, 1 ppm and 10 ppm occurs lag phase (adaptation phase) from hour 0 to 64th hours in which the lag phase is signed by microalgae cells do not increase so that the amount of the population do not growth temporarily (Pelezar and Chan, 1986), but occurred the increasing number of microalgae cells in small amounts because the media of nitrogen industry wastewater having the same compound, in the former cultures that use technical nitrogen. According to Fogg and Thake, 1987 in Prihantini et al.(2005) the adaptation phase will be shorter or even invisible when inoculated cell derived from cultures in exponential phase. At 72nd hour to 96th hour, the increment of the number of cells occurs, this due to the cells of microalgae *C. pyrenoidosa* has entered the exponential phase. On this phase cells of *C. pyrenoidosa* undergo additional population rapidly and constant (FAO, 1990). At a concentration of 100 ppm, from 0 hour until 16th hour, occurred lag phase in which microalgae *C. Pyrenoidosa* descent in the number of cells, this occurs due to the adaptation of microalgae *C. pyrenoidosa* with new media, in high concentrations. At the 24th until the 64th hour, the cells begin to adapt to new media as shown by the increase in the number of cells. In the hour-72, up to 96 hours occurs the increment of number cells rapidly, in which the microalgae C. pyrenoidosa is in an exponential phase. According to Santoso et. al (2011), microalgae population increased drastically and reached peak density in the observation 4th day. Drastic population increment indicates that microalgae have high tolerance to nutrients in the wastewater. The high tolerance of microalgae populations toward wastewater is varying, depends on the given concentration. When microalgae populations increase on the experiment, the absorption of nutrients in wastewater also increased. At a concentration 1000 ppm cell number of *C. pyrenoidosa* microalgae decrease from 0 – 96th hours, this is due to the chemicals surplus in high concentrations on the media. It makes the active ingredient hypertonic to cytoplasm, so that it can affect or inhibit the metabolism in cells and cause plasmolysease (Aslianti, 1986).
According to Connell *in* Haryoto (2004) on the high concentrations accumulation can affect cell growth, because the protection system of organism is not able to offset the effects of toxicity.

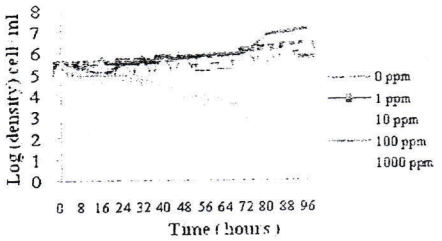


Figure 4. The effect of Microalgae Chlorella *pyrenoidosa* Growth on nitrogen industry Wastewater.

b. The effect of nitrogen industry wastewater to cell growth of Microalgae *Nannochloropsis sp*

The results observations of the influences of microalgae *Nannochloropsis sp* on nitrogen wastewater industry is shown in Figure 5. Based on the data in figure 5, the cell growth of microalgae *Nannochloropsis* sp, at occur lag phase on 0 hour to 32nd. According to Pelezar and Chan, (1986) phase lag is signed by the number of microalgae cell do not change, population do not grow, while on microalgae *Nannochloropsis* sp, occur the increment the number of cell, but the cell increment do not well as *C. Pyrenoidosa.*

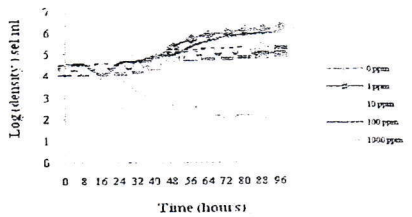


Figure 5. The Effect of Microalgae *Nannochloropsis sp* growth on the nitrogen industry Wastewater

At the 48th hour's microalgae *Nannochloropsis sp,* at concentrations of 0 ppm, 1 ppm and 10 ppm entering the exponential phase at 96th hours. But,at concentrations 1 ppm and 10 ppm the increase of cells number is not big as concentration 0 ppm. This indicates that the wastewater at low concentrations insufficient nutrient on the growth of microalgae *Nannochloropsis sp.*
At concentration 100 ppm, from hour 8th until 24th hours occurs lag phase. In which, the cell number decreased, this proves that the microalgae *Nannochloropsis sp.* Adaptated to new media, at high concentrations. But at the 24th until the 48th hour, microalgae began to enter the exponential phase and

the 48th till 96th hour, there was significant increment in the number of cells, this means the microalgae *Nannochloropsis sp* are in a state of exponential phase. While at the concentration 1000 ppm, from 0 to 96th hours occur the decresement of cell numbers, because of three things i.e the reduced micro-nutrients as a limiting factor because it has been widely used during the phase exponential, the presence of toxic microalgae produced by themselves as a result of the metabolism that poison itself, and the decrease of photosynthesis process as the result of the increment of cells number.So that the part of culture are getting light is the surface only (Riley and Chester, 1971 *in* Nugraheny, 2001). According to Fogg (1965) in *Panggabean*, (2000) and Suantika (2009), due to the formation of toxic compounds in high concentrations and the presence of microalgae esktraseluler products that poisoned themselves, so that the mortality of these microalga increased.

The effect of nitrogen industry wastewater to the growth of *Pseudomonas fluorescens* cell.

Based on the data observation, the growth of *P. fluorescens* bacteria every 1 day during the 7 days are presented in Figure 6. As we know in previous studies, the bacteria *P. fluorescens* can remediate pollutants, including heavy metals and pesticides (Wu *et al.*, 2006, Wasi *et al.,* 2011) In this research note that the bacterium *P. fluorescens* is also thought to be able to remediate nitrogen industry wastewater. It is known from the increment of bacteria growth every day (Figure 6).
This study indicated that Bandala's research (2006) is right. He stated that bacterium *P. fluorescens* is bacteria that are able to survive in extreme conditions, i.e a condition in which there are certain pollutants in the environment and can be converted into compounds that are not dangerous for the environment.

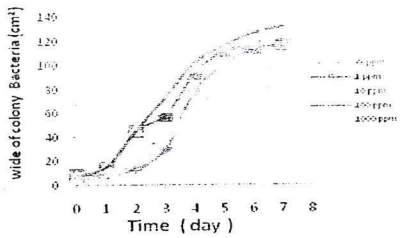


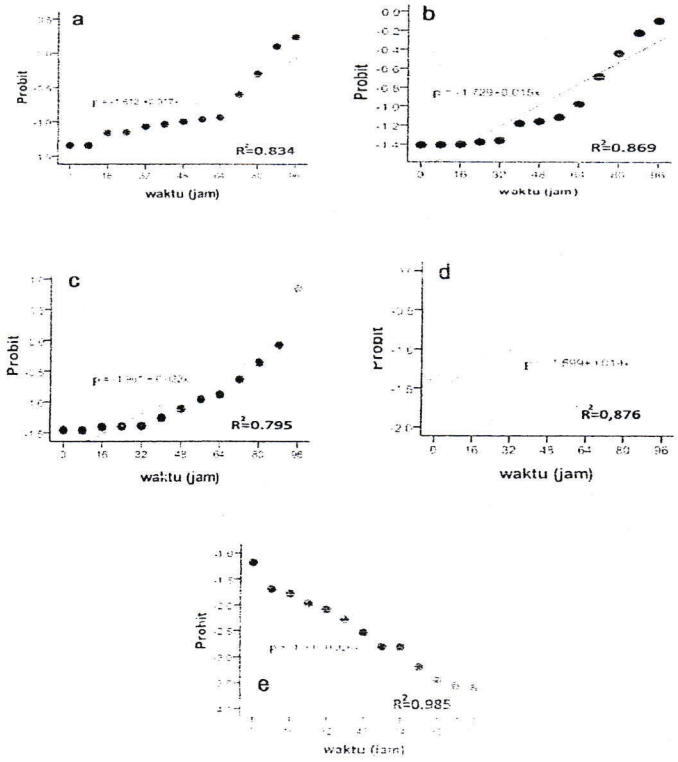
Figure 6. The Effect of Bacteria *P. fluorescens* Growth to Nitrogen Industry Wastewater

As seen in Figure 6. At a concentration 0 ppm, 1 ppm, 10 ppm and 100 ppm, there was a lag phase at day 1 to day 1st. At 1 to day 5th, there was exponential phase, in which there is a incisive expansion of bacterial colonies.On day 5th to day 7th, occur stationary phase, in which the expansion of the growth bacterial colonies begin static, the growth of bacterial cells and death rate are same. On day 7th, occurs the decrease phase in the expansion of bacterial colonies. While at the concentration 1000 ppm, the exponential phase occurred on day 2 to day 4th . Stationary phase occurred on day 4 to day-7th. According Timotus (1987) water is a major part in the cell so that dissolved nutrients are easily absorbed by the cells. While, according to Kadarwati (1985) Organic and inorganic substances dissolved in water are also able to stimulate the activity of bacteria in degrading wastewater containing organic and inorganic compounds. It is alleged in the aquatic environment of nutrients needed by the bacteria in a soluble state that easily utilized by bacteria for growth. Atlas (1984) stated that the nutrients are not only benefit to the growth of microbes, but also for survive. Nutrient is ingredients for metabolic processes and produce enzymes to degrade the nitrogen industry wastewater.

The Result Anlaysis of Probit IT_{50} and IC_{50} Mikroalgae and Bacteria

The result analysis Probit $IT_{50}$and IC_{50} Mikroalgae *C. pyrenoidosa* on Nitrogen Industry Wastewater.

Results analysis of probit IT_{50} by using experimental data from preliminary tests of microalgae *C. pyrenoidosa* growth, which is done at 8 hours to 96 hours in various concentrations (0 ppm, 1 ppm, 10 ppm, 100 ppm and 1000 ppm) on media nitrogen industry wstewater. Based on the analysis using SPSS program is shown in Figure 7. It shown that the value of R^2 = approaching a value of 1, this shows the relationship between two variables that are closely related and positive. This figure 7 also the probit value 5.000 indicates the growth or inhibition of 50% of the population.



Gambar 7. Grafik Probit IT_{50} Mikroalgae *Chlorella pyrenoidosa* on Nitrogen Industry wastewater in various concentration (a) 0 ppm (b) 1 ppm (c) 10 ppm (d) 100 ppm (e) 1000 ppm.

Microalgae *C. pyrenoidosa* in nitrogen industry wastewater. While the value of IT_{50} is a toxicant time that is capable in inhibiting the growth or growth on microalgae *C. pyrenoidosa* by 50% for 96 hours. Upper Bound value is the lowest toxicant time that is being tested and could significantly inhibit the growth of C. pyrenoidosa for 96 hours. Lower Bound value is the value of the highest toxicant were tested and do not influence the growth of *C. pyrenoidosa*.

While the value of IC_{50} is the concentration of toxicant that significantly inhibit the growth or growth occurs microalgae *C. pyrenoidosa* by 50% for 96 hours. Upper Bound value is the lowest concentration of toxicant being tested and could significantly inhibit the growth of *C. pyrenoidosa* for 96 hours. Lower Bound value is the highest toxicant concentration that is tested and did not affect the growth of *C. pyrenoidosa*.

Based on probit analysis on SPSS program, IC_{50} value of nitrogen industry wastewater is 626.648 ppm, it means at that concentration, 50% of the entire population of microalgae *C. pyrenoidosa* has grow or inhibition. Thus, the concentration of nitrogen industry wastewater that is used to final test on bioremediation process is 501.316 ppm, 563.981 ppm, 626.648 ppm, 689.310 ppm and 751.975 ppm.

The result Analysis of Probit IT_{50} and IC_{50} cell Microalgae *Nannochloropsis* Sp.Nitrogent wastewater

The value of IT_{50} microalgae *Nannochloropsis* sp in the nitrogen wastewater will be shown in figure 8. R^2 = approaching 1of value, this shows that the relationship between the two variables are closely correlated and positive.

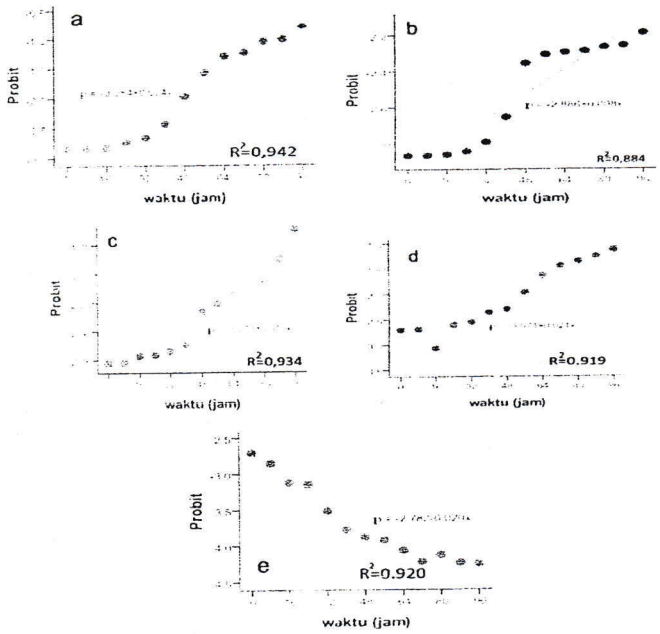
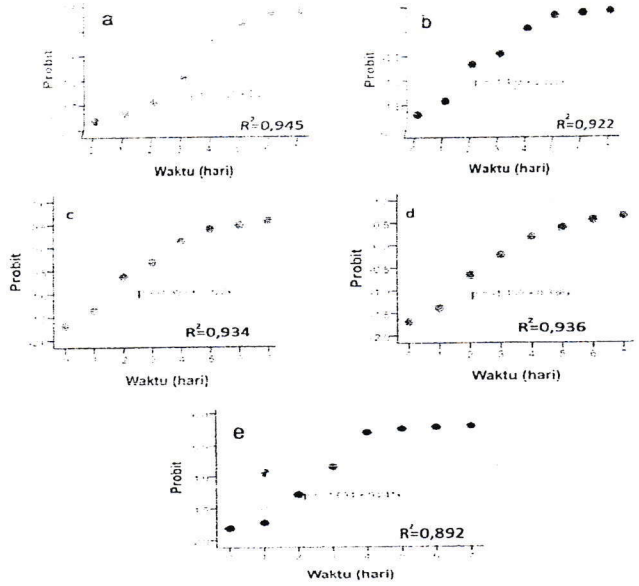


Figure 8. Probit Graph IT_{50} Microalgae *Nannochloropsis sp* toward nitrogen industry wastewater and various concentrations of (a) 0 ppm (b) 1 ppm (c) 10 ppm (d) 100 ppm (e) 1000 ppm.

analysis of probit IC_{50} by using data on the experiment of the influence of microalgae *Nannochloropsis sp* done at 8 hours to 96 hours to various concentrations of nitrogen industry wastewater, obtained the value of 559.854 ppm, 559.854 ppm means the concentration of 50% of the population of microalgae *Nannochloropsis sp* has grow or inhibition. Thus, the concentration of industry wastewater on the microalgae *Nannochloropsis sp* that used for final test in the remediation process is 447.883 ppm, 503 868 ppm, 559.854 ppm, 615.839 ppm and 671.824.

The results Analysis of Probit IT_{50} and MIC_{50} Bacteria *P. fluorescens* On nitrogen industry wastewater

microalgae *Nannochloropsis sp* in nitrogen industry wastewater will be shown in figure 9. Figure 9 R^2 = approaching a value of 1, this demonstrate that colleration between two variable has storong colleration.



Probit Graph IT_{50} bacteria *Pseudomonas fluorescens* on nitrogen industry wastewater of various concentrations (a) 0 ppm (b) 1 ppm (c) 10 ppm (d) 100 ppm (e) 1000 ppm.

Results analysis of probit MIC_{50} on nitrogen industry wastewater from the data carried on each day to 7t days. Based on the analysis of probit MIC_{50} through SPSS program, MIC_{50} values of bacterium *F fluorescens* on nitrogen industry wastewater is 723.219 ppm, thus the concentration of nitrogen industr wastewater used in the bioremediation process is 578.575 ppm, 650, 897 ppm, 723.219 ppm, 795.54 ppm and 867.862 ppm.

4. CONCLUSIONS AND RECOMMENDATIONS

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

1. Hasil penelitian uji toksisitas air limbah pabrik pupuk urea pada pertumbuhan ata penghambatan pertumbuhan mikroalga *Chlorella pyrenoidosa, Nannochloropsis* sp dan bakte *Pseudomonas fluorescens* menunjukkan bahwa respon yang diberikan oleh mikroorganism tersebut merupakan respon akibat perlakuan yang diberikan bukan dari pengaruh paramete kualitas air.
2. Nilai IC_{50}-96 jam mikroalga *Chlorella pyrenoidosa* pada air limbah pabrik pupuk urea 626,64 ppm, Lower bound 223,593 ppm dan Upper bound 837,692 ppm
3. Nilai IC_{50}-96 jam mikroalga *Nannochloropsis* sp pada air limbah pabrik pupuk urea 559,854 ppr Lower bound -98,331 ppm dan Upper bound 841,433 ppm
4. Nilai MIC_{50}-7 hari bakteri *Pseudomonas fluorescens* pada air limbah pabrik pupuk urea 723,21 ppm, Lower bound 393,992 ppm dan Upper bound 2533,658 ppm
5. Berdasarkan data IC_{50}, Upper bound dan Lower bound bakteri *Pseudomonas fluorescens* leb baik pertumbuhannya pada air limbah pabrik pupuk urea dari pada biota uji mikroalga *Chlorel pyrenoidosa* dan *Nannochloropsis* sp

For further research are suggested:

Konsentrasi yang digunakan saat uji akhir disarankan pada mikroalga Chlorella pyrenoidosa 626,646 ppm, Nannochloropsis sp < 559,854 ppm dan bakteri *Pseudomonas fluorescens* < 723,219 ppm, sehingga akan menghasilkan kualitas air yang baik dalam proses bioremediasi.

REFERENCES

[1] Anderson, I. C., and J. S. Levine. 2005. Relative rates of nitric oxide and nitrous oxide production b ntrifiers,denitrifiers, and nitrate respirers. Appl. Environ. Microbiol. e.d 51: P.938-945

[2] Asianti, T. S. R. 1986. Pengaruh Zat Perangsang Tumbuh pada Kepadatan *Tetraselmis chuii* Laboratorium.*Jurnal Penelitian Budidaya Pantai Maros*. P.108-112.

[3] Atlas, R.M.,1984. Microbiology, Fundamentals and Application Clasification and Identification Microorganisme: Systematics of Bacteria (ch, 11,p 363-579) Macmilan Publ, Co. New York.

[4] Bandala, E.R., J. Andres-Octaviono, P. Pastrana dan L.G. Torres. 2006. Removal of Aldrin, Dieldr Heptachlor, and Heptachlor Epoxide Using ActivatedCarbon and/or *Pseudomonas fluorescens* Free C Cultures. J. Environ. Sci. Health Part *ed B*41:P. 553– 569.

[5] Food and Agriculture Organization Of The United Nations. Terjemahan Martosudarmo, B, dan Indah Wulai 1990. Petunjuk Pemeliharaan Kultur Murni dan Massal Mikroalga. United Nations Developme Proramme.INS/85/009.

[6] Green, F.B., T.J. Lundquist, and W.J. Oswald. 1995. Energetics of advanced integrated wastewater por system. *Water Sci Technol.*, 31:9-20.

[7] Haryoto dan Wibowo. 2004. Kinetika Bioakumulasi Logam Berat Kadmium oleh Fitoplankton *Chlorella* s Lingkungan Perairan Laut. Fakultas Farmasi, Universitas Muhammadiyyah, Surakarta. Jurnal Penelitian Sair dan Teknologi, Vol. 5(2): 89-103.

[8] Nugraheny, 2001. Ekstraksi bahan anti bakteri dari diatom laut Skeletonema costatum, Skripsi Fakulta Perikanan dan ilmu kelautan, IPB

[0] Pelezar, M dan Chan,1986. Dasar-dasar Mikrobiologi. Terjemahan Ratna Sri H. Teja Imas, Sutarmi T dan S Lestari A. Penerbit Universitas Indonesia (UI-press), Jakarta

[10] Santoso, A.D., R.A. Darmawan, dan J.P. Susanto. 2011. Pengaruh Laju Alir Injeksi gas Emisi pac Fotobioreaktor terhadap penyerapan CO2 oleh *Cholrella sp. Jurnal Teknologi Lingkungan,* 12(1):1-6

[11] Panggabean, L.M.G. dan Sutomo. 2000. Karakteristik Pertumbuhan Beberapa Jenis Diatomae dalam Kultur

[12] Timotus.K.H. 1982, Mikrobiologi Dasar, Universitas Kristen Satya Wacana, Salatiga.

[13] Wasi, S., S. Tabrez dan M. Ahmad. (2011), Suitability of immobilized *Pseudomonas fluorescens*SM1 strain f remediation of phenols, heavy metals, and pesticides from water. Water Air Soil Pollut. DOI 10.1007/s1127(010-0737-x

[14] Wu H.C., T.K. Wood, M. Ashok, dan C. Wilfred. 2006. Engineering plant-microbe symbiosis for rhizoremediatio of heavy metals: Appl. Environ. Microbiol. 72 (2) : 1129.