Toxicity and Degradation of the Wastewater of the Urea Fertilizer Plants, Oxidation of Fenton and *Pseudomonas fluorescens* Bacteria

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Abstract--- Treating the wastewater with high level of urea and ammonia-nitrogen is one of the problems faced by urea fertilizer plants in Indonesia. The alternative treatment being studied is the one which uses Fenton oxidation process which is continued with the use of *Pseudomonas fluorescens* bacteria. This study is conducted with the concentration of ammonia-nitrogen of 2500 ppm, 2000 ppm, and 1500 ppm. The response being observed is the level of ammonia-nitrogen (NH_3-N) and nitrate-and nitrite in the influent and the level of ammonia-nitrogen (NH_3-N) and nitrate-nitrite in the effluent. This study also aims to estimate the IC_{50} (Inhibition Concentration), NOEC (No Observed Effect Concentration) and LOEC (Lowest Observed Effect Concentration) for 96 hours after being given toxicant in the form of the wastewater of the urea fertilizer plants against the development of the number of cells of *P. fluorescens.* The value of IC_{50} after 96 hours of being given the toxicant of the wastewater of urea fertilizer plants against *P.fluorescens* is 723,219 ppm, while the value of LOEC is 393, 992 ppm and that of NOEC is 2533,658 ppm. The result of the study shows that the biggest average percentage of decline of ammonium is that of the level of ammonium-nitrogen of 2500 ppm in a ratio of 1:10 which is 94.50%. Further study using *P. fluorescens* results in a decrease of nitrate-nitrite in the ratio of 1 : 4 and 1 : 6 which satisfies the quality standards specified in the Environment Minister's Decision No.122 of the year 2004 and the Decree of the Governor of South Sumatra No. 18 of the year 2005. The result of this study provides a fairly high efficiency, hence it is expected that it can be applied in the industrial world.

Keywords---*Ammonia-nitrogen, Fenton oxidation, P.fluorescens, Toxicity*

I. Introduction

IN Indonesia, there are six urea fertilizer plants with wastewater characteristics of high levels of ammonia-nitrogen and urea.

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Up to the time of this study, the process of sewage treatment of those plants is by containing the wastewater in large pools with no special treatment or setting of operating conditions, therefore the output process does not always satisfy the quality standards specified in the Environment Minister's Decision No.122 of the year 2004 and the Decree of the Governor of South Sumatra No. 18 of the year 2005.

Ammonia compound has been widely known as an important raw material for some important commodities in the industrial world. On the other hand, ammonia is also one of harmful pollutants. Ammonia compound in the water at a certain concentration can disrupt ecosystems because it causes eutrophication of aquatic ecosystems, inhibits the metabolism of aquatic animals, and it can even lead to poisoning resulting in organ damage and death. In principle, the nitrogen compounds in the wastewater which can cause pollution are: ion of ammonia (NH_3), nitrite ions (NO_2-) and nitrate ions (NO_3-) [22] Biological waste treatment processes (microbes) will not run optimally or will be impaired when the waste contains toxic chemicals that will affect the performance of a waste treatment facility[15] . This advanced oxidation process can be used as an alternative method of treating industrial wastewater of the urea fertilizer plants which is quite economical. The use of this process can save space and energy, and it is safe and simple, and processing and reaction time is relatively fast and it is easily applied and controlled.

Some strong oxidizing agents such as peroxide is relatively inexpensive and easy to obtain and can be used as an oxidizer in advanced oxidation processes. Hydrogen peroxide (H_2O_2) has long been known as a strong oxidizing agent and is able to oxidize organic and non-organic compounds and is widely used in various industries. Hydrogen peroxide is an oxidizing agent which is safe enough in terms of its end product in which after the process it will be split into H_2 and O_2 at the temperatures above 80^{o}C. Fenton reagent is a peroxide compound which is reacted with catalyst Fe $^{2+}$ ($FeSO_4$) which will produce hydroxyl radicals (^{O}OH) which are effective compounds to oxidize contaminants or waste water. Fenton reagents have been developed in many places to process organic materials of *Biological Oxygen Demand / Chemical Oxygen Demand* (BOD / COD), Total Suspended Solid (TSS), color, nitrogen, phosphorus and some metals contained in domestic and industrial waste water and drinking water [18] . Fenton reagent is capable of oxidizing organic and inorganic bonding of toxic compounds in waste water. A setting condition of a rapid mixing speed of 100 rpm for 120 minutes is capable of eliminating hydrocarbon in the waste water in the amount of 2000 mg / L and COD of 4200 mg / L [18] It is also known that the *Pseudomonas* bacterium, particularly *P. fluorescens* which is a soil bacterium is used to detect the presence of pollutants in the ecosystems [24] [31]. These bacteria are easily cultured in the form of culture. They can survive in an environment contaminated by pollutants and pesticides, which is an expression of bacteria that can degrade pesticides [22] [27]. [31] It has been known that an environment contaminated with heavy metals such as lead, mercury, and cadmium can be remediated by *P. putida* bacteria [29] [31]

II. Materials And Methods

A. Materials and Equipment

The tools used in this study include: measuring flask, pH meter, measuring pipettes, spectrophotometer, scales, cork drill, petri dish, transparent millimeter paper. A set of wastewater treatment equipment using Fenton oxidation. While the materials required are waste water containing ammonia taken from the wetland areas of the urea fertilizer plants, *Pseudomonas fluorescens* bacteria of the seedlings which are in a state of pure cultures which are not contaminated, Kings B media (the composition of 10 g peptone protease, K_2HPO_4 of 0.75 g, $MgSO_4.7H_2O$ of 0.75 g, 7.5 ml of glycerol, aquadest of 500 ml), $FeSO_4.7H_2O$ and hydrogen peroxide (H_2O_2).

B. Toxicity Test of Pseudomonas fluorescns

In this test, the wastewater of the urea fertilizer plants, which is diluted at the appropriate concentrations, is mixed with Kings B media which is sterile. The mixture is simultaneously put into a petri dish with a medium dose of 1 ml each. It is shaken until it mixes well and is allowed to freeze. The existing colonies of *P. fluorescens* are moved using cork drill and placed in the middle of a petri dish with a diameter of 14 cm, and then the development of bacteria in each petri dish is observed and the diameter of the development of bacteria in a petri dish is measured. They are calculated by using a transparent millimeter paper.

C. Oxidation of Fenton and Pseudomonas fluorescens

- The wastewater originating from an emergency pool is put into the control tube. Before it is put into the feed the wastewater is analyzed (NH_3-N, nitrate, nitrite,). Out of the control tube some sample of the wastewater of 5,000 ml is put into the reactor (reagent tube). The reagent tube serves as a tube for reacting the wastewater of urea fertilizer plants with Fenton reagent with various ratios of FeSO4 : H_2O_2, namely 1 : 2, 1 : 4, 1 : 6, 1 : 8, 1 : 10. The mixture is stirred by using a magnetic stirrer at 100 rpm stirring speed for 120 minutes, and then the sample is taken after it is settled for 20 minutes. Then the wastewater from the feed tube is analyzed (NH_3-N, nitrate, nitrite).
- The wastewater originating from the reagent tube is flowed into a aquarium / bottle aeration (*P. fluorescens*), in the aerobic process. Then the sample is allowed to stand for 7-9 days, because the growth of microorganisms reach stationary phase at 4 - 6 days, so that the microorganisms (bacteria) can decompose organic substances contained in the wastewater.
- The water processed, namely the water from the aquarium / aeration bottle, is then analyzed (NH_3-N, nitrate, nitrite,) to know the quality of the wastewater after previous processes.

III. The Results And Description

A. The Effect of the Growth of Pseudomonas fluorescens

The data of daily growth observation of the *P. fluorescens* for 7 days are presented in Figure 1. It has been known from the previous studies that *P. fluorescens* can remediate pollutants, such as heavy metals and pesticides [28] [31] This study indicates that possibly *P. fluorescens* is also capable of remediating the waste of the urea fertilizer plants. It is indicated by the daily growth of the bacteria (Figure 1).

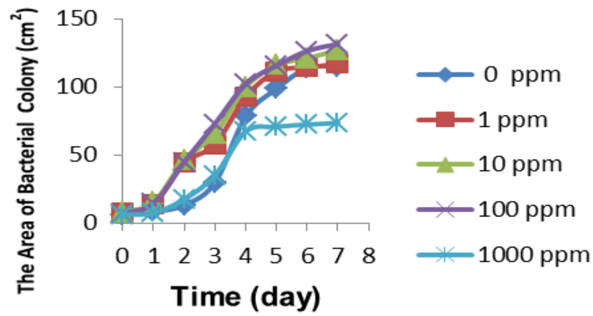

Fig. 1 The Graph Showing the Effect of Cell Growth of Bacteria against the Wastewater of the Urea Fertilizer Plants

The result of this study supports the result of the study by [4] which states that the *P. fluorescens* bacteria are the bacteria that can survive in extreme condition, namely the condition where there are pollutants and through further processing the pollutants can be converted into compounds which are no longer dangerous for the environment.

The data on Figure 1 show that at a concentration of 0 ppm, 1 ppm, 10 ppm, and 100 ppm, the phase lag occurs on day 0 to day 1. On day-1 to-5, the eksponential growth phase occurs, indicated by a sharp expansion of bacterial colonies. On day 5 to 7, the stationary phase occurs. The expansion of bacterial colonies growth starts to be static. The growth rate of the bacterial cells is the same as that of the death. On day 7, there is a decrease in the expansion of bacterial colonies. Whereas at a concentration of 1000 ppm, exponential phase occurs on day 2 to day 4. The stationary phase occurs on days 4 to 7. As shown on Figure 1. according [26] water is the biggest part of a cell, so dissolved nutrients can be easily absorbed by the cells. Beside that, according to Hong (2003), organic and inorganic substances dissolved in the water can also stimulate the activities of the bacteria degrading the wastewater containing organic and inorganic compounds. It is alleged that in the aquatic environment the nutrients required by the bacteria are in a state of dissolved so they can be easily exploited by bacteria to grow. In relation to something similar, [3] states that the nutrients are not only beneficial for the growth of the bacteria, but also for their survival. The nutrients are the materials for the process of metabolism and for producing enzymes for degrading the wastewater.

B. The Results of Analysis of Probit IT50 and MIC50 of P. fluorescens

The test results on the activities of some treatments of the medium of the wastewater of the urea fertilizer plants, show no inhibition of bacterial growth occuring at various concentrations such as 0 ppm (control) 1 ppm, 10 ppm, 100 ppm, and 1000 ppm. This can be seen in Figure. 2.

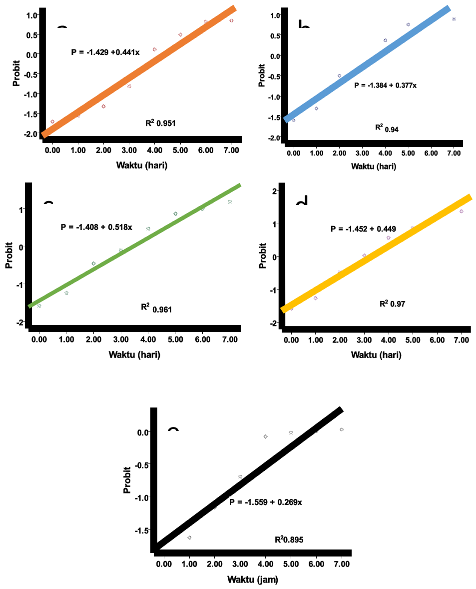

Fig. 2 The Graph of Probit IT50 P. fluorescens Bacteria against the Wastewater of the Urea Fertilizer Plants at Various concentrations (a) 0 ppm (b) 1 ppm (c) 10 ppm (d) 100 ppm (e) 1000 ppm.

The data on Figure 2. Show that *P. fluorescens* bacteria do not experience growth inhibition, however when they are in the wastewater of the urea fertilizer plants containing ammonia solution at a concentration of 1000 ppm the growth is small. The results of analysis of probit MIC_{50} with SPSS program, MIC_{50} value of bacteria *P. fluorescens* on the wastewater of the urea fertilizer plants is 723.219 ppm, thus the concentration of the wastewater of the urea fertilizer plants used in the process of bioremediation is 578.575 ppm, 650.897 ppm, 723.219 ppm, 795.540 ppm and 867.862 ppm.

C. The Results of Analysis of Fenton Oxidation Processing and P. fluorescens

a. Analysis of NH3-N Level

Fenton reagent is a solution of hydrogen peroxide and iron catalysts used to oxidize contaminants or wastewater. In the treatment of the wastewater of urea fertilizer plants using Fenton reagent, the concentrations of NH3-N declines as shown by the data of Table 1. The rate of degradation of the organic and inorganic pollutants through the Fenton reaction can increase through higher involvement of iron in degrading pollutants of the wastewater [21] . The decline in the value of NH_3-N in the results of the study shown in Table 1. is possibly due to its decomposition to form ions and gases, such as nitrate, nitrite, or nitrogen monoxide molecule. This is similar to [29] in their study on the method of Fenton Oxidation degradation of nitrogen in organic compounds. The decrease in NH_3-N levels that meet the quality standard of the Minister of the Environment Decree No. 122 of the Year 2004 and the Decree of the Governor of South Sumatra No. 18 of the Year 2005 is the result of the treatment of the wastewater of urea fertilizer plant using Fenton reagent at a concentration of 2000 ppm and 1500 ppm with a range of ratio from 1 : 4 to 1 : 10.

TABEL I.
ANALYSIS OF NH3-N LEVELS AT THE CONCENTRATION OF 2500 PPM, 2000 PPM AND 1500 PPM

Parameter	Indicator and Concentration (ppm)	The Ratio of $FeSO_4$ (gram) : H_2O_2 (ml)				
		1 : 2	1 : 4	1 : 6	1 : 8	1 : 10
NH_3-N	Reagent Fenton					
	2500	512.23	387.52	298.59	262.27	137.50
	2000	119.47	73.73	11.15	4.623	1.612
	1500	17.50	4.25	3.75	0.12	0.023
	P. fluorescens					
	a	76.5	45	76.25	107.25	119.56
	b	0.12	0.08	0.007	0.0	0.0
	c	0.07	0.003	0.0	0.0	0.0

In the treatment of the wastewater of the urea fertilizer plants using *P. Fluorescens* bacteria in absorbing NH_3-N during the maintenance period NH_3-N tends to decrease (Table 1). This is because bacteria are the most important group of microorganisms in wastewater treatment and they can oxidize inorganic compounds such as NH_3 into energy. According to [30] the bacteria will use organic carbon as an energy source, in correlation with the nitrogen to be used for protein synthesis in order to produce new cell materials. With the addition of carbonaceous materials, the bacteria will use the nitrogen contained in the culture so as to reduce the concentration of inorganic nitrogen (ammonia) which is toxic to the organism. The addition of carbonaceous material has been proven to reduce inorganic nitrogen [4] [8]. Bacterial growth is limited by the balance of nutrients in the water. Therefore, the population dynamics of bacteria is closely related to the availability of nutrients [16]

b. Analysis of Nitrate Levels

An increase in nitrate level in the treatment of the wastewater of urea fertilizer plants using Fenton reagent is shown by the data in Table 2. The data on the table shows that the greater the ratio the greater the value of nitrate formation. This is due to the greater amount of NH_3-N compounds which decompose to form ions and gases namely nitrates and nitrites [27] Similarly [33] reported that the removal of ammonia nitrogen at high concentration of H_2O_2 will produce N_2 gas and nitrate. So the higher the concentration of H_2O_2 or the greater the ratio given, the greater the amount of gas N_2, nitrate and nitrite or in other words, high concentration of H_2O_2 can reduce the value of the ammonia and it will continuously occurs in the oxidized wastewater.

The findings of the study shown in Table 2 state that the concentrations which still meet the quality standards specified in the Decree of the Minister of the Environment No. 122 of the Year 2004 and the Decree of the Governor of South Sumatra No. 18 of the Year 2005 are the concentration of 2500 ppm, 2000 ppm and 1500 ppm in a ratio of 1 : 2, 1 : 4, namely below 20 ppm in water quality class 1, and a ratio of 1 : 6 below 30 ppm of water quality in group 2.

During the maintenance, utilization of the nitrate by *P. fluorescens* bacteria occurs to be used as substrates. This causes the decrease of the nitrate level. The resulting nitrate fulfils the nutritional requirements of the bacteria that will stimulate the growth of the bacteria and the increase of bacterial biomass. Nitrate is the end product of ammonia oxidation process.

During the maintenance, utilization of the nitrate by *P. fluorescens* bacteria occurs to be used as substrates. This causes the decrease of the nitrate level. The resulting nitrate fulfils the nutritional requirements of the bacteria that will stimulate the growth of the bacteria and the increase of bacterial biomass. Nitrate is the end product of ammonia oxidation process. Theoretically, the concentration of nitrate is present in large amounts in the waters. Nitrate concentrations in water bodies indicate faecal pollution at the initial level. High nitrate level in drinking water is harmful to children and can cause anemia (hemoglobin meta). According to [14] the bacteria do not require organic materials to perform the activity and growth but they can simply use inorganic compounds (NO_3 and NO_2). Similarly, according to [9] [12] [20] the reduction of nitrate in the wastewater can be done through denitrification. Nitrate is one of the important factors in the process of denitrification because nitrate is used as electron acceptor by the bacteria. The presence of nitrates in the water may cause water quality to decline, lower dissolved oxygen, decline fish population, foul odor, bad taste of the water. Nitrate is a threat to human health, especially to infants. It can cause a condition known as methemoglobinemia, which is also called "blue baby syndrome". Polluted ground water or river water containing nitrate which maybe used to prepare milk for baby causes the nitrates to enter the body of a baby. When the milk gets into baby's gut, the nitrate is converted into nitrite, which then binds to hemoglobin to form methemoglobin and reduce the oxygen carrying capacity of the baby's blood [1] [7]

TABEL II. ANALYSIS OF NITRATE LEVELS AT CONCENTRATION OF 2500 PPM, 2000 PPM AND 1500 PPM

Parameter	Indicator and Concentration (ppm)	The Ratio of $FeSO_4$ (gram) : H_2O_2 (ml)				
		1 : 2	1 : 4	1 : 6	1 : 8	1 : 10
Nitrite	Reagent Fenton					
	1.040	2.58	3.21	3.72	4.34	7.03
	0.767	1.62	2.62	2.57	3.13	4.97
	0.729	1.15	2.61	2.55	3.04	3.48
	P. fluorescens					
	a	0.08	0.07	0.04	1.08	6.98
	b	0.07	0.03	0.02	1.02	3.05
	c	0.06	0.01	0.01	0.01	0.12

C. Analysis of Nitrite Level

The results of observations of nitrite analysis in the treatment of the wastewater of the urea fertilizer plants is presented in Table 3. In the treatment of the wastewater using Fenton reagent, the result shows that the greater the ratio of $FeSO_4$: H_2O_2, the greater the nitrite is formed. According to [26] in their study of Fenton degradation of nitrogen contained in organic compounds, ammonia will be oxidized to form nitrite in small concentrations.

Similarly, [11] [34] stated that to remove ammonia nitrogen at high concentration of H_2O_2 will produce N_2 gas, nitrate and nitrite. So the higher the concentration of H_2O_2 or the greater the ratio of a given gas the greater the amount of N_2, nitrate and nitrite is formed. The result of the study shown in Table 3 indicates that at concentration of 2500 ppm the ratio is 1 : 2 and at concentration of 2000 and 1500 the ratios are 1 : 2, 1 : 4 and 1 : 6 which still meet the specification of the Decree of the Minister of Environment No. 122 of the Year 2004 and the Decree of the Governor of South Sumatera No. 18 of the Year 2005.

In a study of wastewater treatment using advanced oxidation, it is found that there is an increase in the level of nitrite, then the study using bacteria *P.fluorescens* is carried out. The result of the study shows that there is a decrease in nitrite level as shown in Table 3. The decrease in the level of nitrite is thought to occur due to the use of it by the bacteria as the nutrients. The nitrite compounds is used by the bacteria as a final electron acceptor in the process of metabolism. The mechanism is known as nitrite respiration and the enzyme involved is nitrite reductase [18]

TABEL III. ANALYSIS OF NITRITE LEVEL AT THE CONCENTRATION OF 2500 PPM, 2000 PPM AND 1500 PPM

Parameter	Indicator and Concentration (ppm)	The Ratio of $FeSO_4$ (gram) : H_2O_2 (ml)				
		1 : 2	1 : 4	1 : 6	1 : 8	1 : 10
Nitrate	Reagent Fenton					
	10.80	16.50	19.23	23.12	59.75	113.85
	6.30	16.15	18.17	22.89	38.29	86.39
	5.52	15.60	17.12	19.65	29.65	52.34
	P. fluorescens					
	a	1.20	0.98	0.67	32.78	92.45
	b	0.94	0.34	0.56	22.08	69.90
	c	0.62	0.26	0.91	17.45	32.98

Although in a low concentration, nitrite is toxic to fish and other aquatic organisms [16] Nitrite compounds in fish will be bound to the blood that will form methaemoglobin ($Hb + NO_2 = Met\text{-}Hb$). Met-Hb would interfere with the transport of oxygen to the tissues of fish that can cause fish to experience *hypoxsia*. Met-Hb in the blood causes the blood to look brown. Therefore nitrite poisoning is also called "*brown blood*" disease [6]

[6]states that high content of nitrite in drinking water can cause cancer of the stomach and respiratory tract in adults, because nitrite is toxic nitrogen compound, although it is usually found in a very small quantity. It is also conveyed by [7] that excessive consumption of nitrite in human can lead to disruption of the binding of oxygen by hemoglobin in the blood, which in turn, can form the met-hemoglobin which cannot bind oxygen.

IV. CONCLUSIONS

Based on the results of the study it can be concluded that:

1. The result of MIC_{50} of bacteria *P. Fluorescens* is obtained in the wastewater of the urea fertilizer plants at 723.219 ppm.
2. In the treatment of the wastewater of the urea fertilizer plants using Fenton reagent, the greater the ratio of $FeSO_4$ and H_2O_2, the smaller the decrease of NH_3-N, nitrate and nitrite.
3. The best ratio of $FeSO_4$: H_2O_2 in the treatment of the wastewater of the urea fertilizer plants and the one that meets the quality standards of the wastewater quality specified in the Decree of the Minister of the Environment No. 122 of the Year 2004 and the Decree of the Governor of South Sumatra No. 18 of the Year 2005 is 1 : 4 at the concentration of 1,500 and 2,000 ppm.
4. In the further treatment of the wastewater of the urea fertilizer plants using advanced oxidation by bacteria *P. Fluorescens*, the decrease of NH_3-N, nitrate, and nitrite occurs.

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