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The Treatment of the Waste Water of Urea Fertilizer Plant with a Combined *Process of Advanced Oxidation* and Microalga *Chlorella pyrenoidosa*, *Nannochloropsis* sp. and *Pseudomonas fluorescens* Bacteria

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Abstract -- The activity of urea fertilizer industry with potential impacts or environmental pollution is the activity of wastewater disposal into the waters. The main by product of the fertilizer urea plant is wastewater containing mostly liquid ammonia. According to the Decree of the ministry of Environmental Affairs No. 122 of the year 2004 and the Decree of the Governor of South Sumatra Province No. 18 of the year 2005, the maximum pollution load for urea fertilizer industry is liquid ammonia level of 0.75 kg/ton (50 mg/L) and pH of 6.0 – 9.0. The treatment of the wastewater of urea fertilizer plant is done by means of combining chemical and biological methods. The technology of wastewater treatment by advanced oxidation principle using strong oxidizing agents. The process of oxidation can be combined with or followed by biological processes involving microorganisms such as microglae *Chlorella pyrenoidosa*, *Nannochloropsis* sp. And the *Pseudomonas fluorescens* bacteria. The findings of the study on the treatment of the wastewater of the urea fertilizer plants using Fenton reagent and advanced oxidation processes are as follows: the capacity to degrade NH_3 is 95% and urea is 65% with a ratio of 1:10 and the use *Pseudomonas fluorescens* bacteria can absorb nitrate and nitrite as much as 92.63% microalgae *Chlorella pyrenoidosa* as much as 99% and *Nannochloropsis* sp. as much as 84%.

Keywords--Advanced Oxidation Process (AOP), *Chlorella pyrenoidosa*, *Nannochloropsis* sp, *Pseudomonas fluorescens*

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I. INTRODUCTION

THERE are six urea fertilizer plants in Indonesia whose wastewater is characterized with high levels of urea and ammonia-nitrogen. The treatment of wastewater with high levels of urea and ammonia-nitrogen is one of the problems faced by urea fertilizer plants in Indonesia. Although the waste water of urea fertilizer plants is not considered as hazardous materials compound, it may cause serious damage to the ecosystem of water bodies. Industrial activities of urea fertilizer plants with the potential impact of causing environmental pollution is the disposal activities of wastewater into the waters. The efforts to improve wastewater treatment by separating ammonia have been done by using variety of methods such as: *Ion Exchange* [20], *breakpoint chlorination* [12], *aerobic-nitrification* and *anaerobic-denitrification electron* [1], *water stripping* [29], *fluidized-bed reactor* [15], *anammox (Anaerobic Ammonium Oxidation)* [21]-[23], *combined nitrification-denitrification and microalgae* [25], *membrane reactors (MBR)* [28]. These ammonia separation methods have limitations, such as not being able to reduce the amount of ammonia up to safe concentration level, needing huge cost and the application of some of these methods in practice still encounters obstacles. This constraint is mainly due to the specific capacity of $\text{NH}_3\text{-N}$ removal is still so low that the output process is still higher than the quality standards that have been set.

Whereas, according to ^[10] biological waste treatment processes (microbes) will not run optimally or will be impaired when toxic chemical compounds are found in the wastewater that will affect the performance of a waste treatment facility. In this study, the treatment of the wastewater of the urea fertilizer plant is carried out by combining chemical and biological methods. The chemical processing technology applied in this study is *Advanced Oxidation Proces* (AOP) or advanced oxidation processes of wastewater treatment which is a wastewater processing techlogy with advanced oxidation principle using strong oxidator. This process of oxidation can be combined or followed by biological processes involving microorganisms such as microalgae *Chlorella pyrenoidosa*, *Nannochloropsis* sp. and the bacterium *Pseudomonas fluorescens*.

II. MATERIALS AND METHODS

A. Material and Tools

The tools used in this study are volumetric flask, pH meter, measuring pipette, Spectrophotometer, scales, aerator, transparent plastic tubing measures $\frac{3}{4}$ 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. pyrenoidosa* 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 *Nannochloropsis* 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, $MgSO_4 \cdot 7H_2O$ 0, 75 g, glycerol 7, 5 ml, drilled water 500 ml). Variable measurement including pH, density, NH_3-N level and nitrogen, H_2O_2 , $FeSO_4 \cdot 7H_2O$, Measurement variables include pH, NH_3-N , urea, nitrate, nitrite, TKN, COD, TSS.

B. Procedure Research

- Waste water from a pool emergency tube inserted into the control. Sebelum masuk ke umpan wastewater is analyzed (NH_3-N , urea, nitrate, nitrite, TKN, pH, COD, TSS)
- From the tube inserted control samples (waste water) into the reactor of 5,000 (reagent tubes). The tube serves as a reagent tube to react with the fertilizer plant wastewater. Fenton reagent and various comparisons are $FeSO_4 : H_2O_2$ 1: 2, 1: 4, 1: 6, 1: 8, 1: 10.
- By using a magnetic stirrer at 100 rpm stirring speed setting selama 120 minutes, and then the samples were taken after 20 min settling. Then the waste water from the feed tube in the analysis of reagent (NH_3-N , urea, nitrate, nitrite, TKN, pH, COD, TSS).

- Waste water from in vitro reagent, flowed into aguarium / botol aerasi (aguarium microalgae, bacteria, bacteria + microalgae), the aerobic process. Later on leave for 7-9 days, because the growth of microorganisms reach stationary phase at 4-6 days. So that microorganisms (bacteria and microalgae) can decompose organic substances contained in waste water
- Water processed, the water that comes from aguarium / aeration bottle, then analyzed (NH_3-N , urea, nitrate, nitrite, TKN, pH, COD, TSS), which is useful to know the quality of the waste water from some of the previous process .

C. Circuit Research Tool

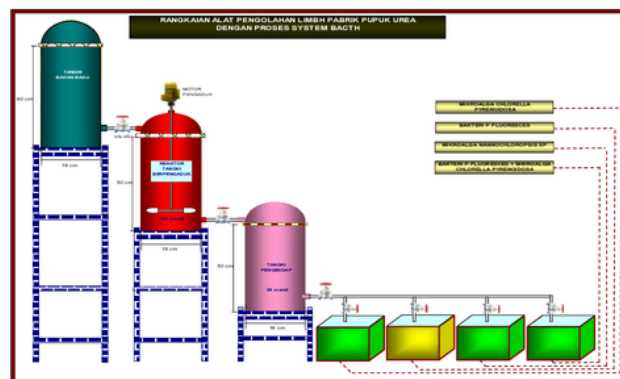


Fig 1. Circuit Research Tools

III. RESULTS AND DISCUSSION

A. Fenton Reagent Result

The treatment of the wastewater of urea fertilizer plant using Fenton reagent of various concentrations and comparisons of $FeSO_4$ and H_2O_2 causes a decline in NH_3-N , Urea, COD, TKN, and TSS, but an increase in levels of pH, nitrate and nitrite as presented in Table 1 . The results of the study show a precipitate $Fe(OH)_3$ which is reddish brown, because the wastewater of the urea fertilizer plant used is that with the acidity (pH) levels of more than 6 ^[9]. The decline in the value of NH_3-N and urea in the results of the study presented in Table 1, is assumed to decompose to form ions and gases, such as nitrate and nitrite molecule, or nitrogen monoxide. This is in accordance with the findings of ^[27] , that the levels of nitrate and nitrite increase. The levels of COD, TSS decrease, indicating that Fenton oxidation can degrade the value of COD and TSS. According to ^[2], Fenton reagent as one of the advanced oxidation processes (*Advanced Oxidation Process / AOPs*) is expected to destroy organic and inorganic pollutants, eliminate color and COD.

TABLE I.
RESULT ANALYSIS TREATMENT WASTEWATER UREA FERTILIZER
PLANTS WITH FENTON REAGENT CONCENTRATION 2500, 2000 AND
1500 PPM

Parameter	Ratio between FeSO ₄ (gram) : H ₂ O ₂ (ml)					
Concentration (2500 ppm)	Initial Analysis	1 : 2	1 : 4	1 : 6	1 : 8	1 : 10
pH	9.3	8.4	8.6	8.9	9.1	9.7
NH ₃ -N	2505	512.23	287.52	298.59	262.27	137.50
Urea	5441	2893	2590	2341	2235	1941
Nitrate	10.8	16.50	19.23	23.12	59.75	113.85
Nitrite	1.04	2.585	3.21	3.725	4.346	7.03
TKN	5007.86	1862.29	1596.18	1391.15	1305.36	1143.30
COD	96	5	5	4	2	2
TSS	66	1.98	1.55	2.20	2.55	2.23
Concentration (2000 ppm)	Initial Analysis	1 : 2	1 : 4	1 : 6	1 : 8	1 : 10
pH	8.2	8.2	8.5	8.7	8.8	9.3
NH ₃ -N	1999	119.47	73.75	11.15	4.625	1.612
Urea	4171	1523	1478	1405	1119	665
Nitrate	6.3	15	18.17	22.89	38.29	86.39
Nitrite	0.767	1.638	2.624	2.579	3.132	4.97
TKN	3917.66	715.5	693.63	657.8	514	307.512
COD	92	5	3	2	2	2
TSS	53	1.66	1.78	2.23	2.12	2.88
Concentration (1500 ppm)	Initial Analysis	1 : 2	1 : 4	1 : 6	1 : 8	1 : 10
pH	8.0	8.0	8.2	8.3	8.5	9.0
NH ₃ -N	1512	17.50	4.25	4.75	0.12	0.023
Urea	3495	1057	905	739	439	32
Nitrate	5.52	15.60	17.12	19.65	29.65	52.34
Nitrite	0.729	1.156	2.611	2.556	3.046	3.48
TKN	3119.7	493.72	417.05	342	201	14.743
COD	80	3	3	2	2	2
TSS	53	1.63	1.33	2.0	2.27	2.87

B. Fenton reagent and Microalgae *Chlorella pyrenoidosa* and *Nannochloropsis* sp.

The study on the treatment of wastewater of the urea fertilizer plants using Fenton reagent and microalgae *Chlorella pyrenoidosa* and *Nannochloropsis* is a further research analyzing the results Fenton reagent which indicates the occurrence of increasing levels of nitrate and nitrite at a concentration of 2500 ppm, 2000 ppm and 1500 ppm. This high level of NH₃-N and urea is expected to be absorbed by the *Chlorella* microalgae *Chlorella pyrenoidosa* and *Nannochloropsis* sp. as nutrients. The results of the study presented in Tables 2 and 3 show no changes in pH value during maintenance by using microalgae *Chlorella pyrenoidosa* and *Nannochloropsis* sp, no increase or decrease of pH value, the pH value is relatively stable during the study. According to [5], the pH did not increase due to the existence the natural buffer system in the form of dissolved CO₂ gas contained in the culture medium. The dissolved CO₂ gas contained in the medium will become carbonic acid which will decompose into ions. The results of the study presented in Tables 2 and 3 show the increase of COD and TSS values indicating that there is an accumulation of organic materials in the wastewater resulting from the growth of microalgae *Chlorella pyrenoidosa* and *Nannochloropsis* sp.

TABLE II.
RESULT ANALYSIS TREATMENT WASTEWATER UREA FERTILIZER PLANTS
FENTON REAGENT AND MICROALGAE *CHLORELLA PYRENOIDOSA*
CONCENTRATION 2500, 2000 AND 1500 PPM

CONCENTRATION 2500, 2000 AND 1500 PPM					
Parameter	Initial Ratio Analysis			FeSO ₄ (gram) : H ₂ O ₂ (ml)	
Concentration (2500 ppm)	1 : 2	1 : 4	1 : 6	1 : 8	1 : 10
pH	8.4	8.6	8.9	9.1	9.7
NH ₃ -N	135	35	105.75	134	129.11
Urea	1914	1803	1685	2075	1816
Nitrate	0.032	1.87	9.37	44.75	99.69
Nitrite	0.093	0.076	0.546	2.546	6.08
TKN	1028.2	763.07	892.08	963.003	983.243
COD	130	105	119	138	110
TSS	31	34	31	37	37
Concentration (2000 ppm)	1 : 2	1 : 4	1 : 6	1 : 8	1 : 10
pH	8.2	8.5	8.7	8.7	9.3
NH ₃ -N	0.25	0.15	0.013	0.013	0.0
Urea	695	713	702	702	529
Nitrate	0.010	0.21	6.35	6.35	76.8
Nitrite	0.056	0.039	0.056	0.056	3.89
TKN	323.65	239.55	327.613	327.613	181.533
COD	140	115	121	121	120
TSS	35	35	35	35	39
Concentration (1500 ppm)	1 : 2	1 : 4	1 : 6	1 : 8	1 : 10
pH	8.0	8.5	8.3	8.5	9.0
NH ₃ -N	0.034	0.15	0.001	0.0	0.0
Urea	157.05	713	37	32.45	0.0
Nitrate	0.034	0.21	3.9	16.93	39.89
Nitrite	0.037	0.039	0.037	0.245	2.7
TKN	73.324	239.55	17.266	15.143	0.0
COD	154	115	150	170	134
TSS	43	35	43	45	40

High COD level indicates an organic waste pollution. The inorganic materials found are in the form of clay and sand, and the organic matters are in the form of the remains of plants and other biological solids such as algae cells, bacteria and so forth [18]. Based on the results of the study, it can be concluded that the rise of TSS levels is due to the growth of microalgae *Chlorella pyrenoidosa* and *Nannochloropsis* sp.

TABLE III.
RESULT ANALYSIS TREATMENT WASTEWATER UREA FERTILIZER
PLANTS FENTON REAGENT AND MICROALGAE *NANNCHLOROPSIS*
SP. 2500, 2000 AND 1500 PPM

Parameter	Initial Ratio Analysis FeSO ₄ (gram) : H ₂ O ₂ (ml)				
Concentration (2500 ppm)	1 : 2	1 : 4	1 : 6	1 : 8	1 : 10
pH	8.4	8.6	8.9	9.1	9.7
NH ₃ -N	162.25	98	119.2	174	131
Urea	2195	1807	1884	2132	1895
Nitrate	2.63	3.50	13.45	47.75	109.69
Nitrite	0.921	0.99	1.987	3.345	6.84
TKN	1021.95	924.2	775	912.60	1081.36
COD	178	170	166	234	159
TSS	50	40	47	50	53
Concentration (2000 ppm)	1 : 2	1 : 4	1 : 6	1 : 8	1 : 10
pH	8.4	8.5	8.7	8.8	9.3
NH ₃ -N	0.425	0.075	0.073	0.078	0.0
Urea	925	634	930	923	543.34
Nitrate	2.28	1.65	9.5	27.31	71.8
Nitrite	0.567	0.870	1.30	1.30	2.03
TKN	425.92	383.715	335	370.144	31.21
COD	180	180	181	188	162
TSS	52	52	52	52	52
Concentration (1500 ppm)	1 : 2	1 : 4	1 : 6	1 : 8	1 : 10
pH	8.0	8.2	8.3	8.5	9.0
NH ₃ -N	0.062	0.0	0.002	0.0	0.0
Urea	326	296	138	64.04	1.56
Nitrate	1.84	1.34	6.36	19.93	31.14
Nitrite	0.470	0.774	0.567	1.10	1.67
TKN	196.02	103.96	64.402	29.885	0.728
COD	205	208	167	197	177
TSS	52	52	52	52	60

C. Fenton Reagent and *Pseudomonas fluorescens* Bacteria

The treatment of waste water using bacteria *Pseudomonas fluorescens* is a further research, in which waste water of urea fertilizer plants is treated using Fenton reagent of various concentrations ranging from 2500 ppm, 2000 ppm and 1500 ppm. The results indicate that it still contains high levels of $\text{NH}_3\text{-N}$, urea and an increase in the levels of nitrate and nitrite. The results of the study presented on Table 4 show changes in the degree of acidity (pH), namely a decline. The reason is the process of respiration carried out by the bacteria that produce CO_2 . The presence of CO_2 in the water will shift the carbonate equilibrium to the right so that it will lower the pH value. The following is an equilibrium reaction of the carbonate:



With the presence of CO_2 , the reaction equilibrium will shift to the right to form the H^+ ions that will cause a decrease of the pH of the waters. This is in accordance with the opinion of [16], which states that an increase in CO_2 will lower the pH value of the culture. With the increase in maintenance time, the amount of CO_2 will also increase which will further decrease the pH value of the media. The CO_2 in the media is also allegedly derived from decomposition of organic matter and respiration by the bacteria. According to [26], 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 carbonic 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.

TABLE IV.
RESULT ANALYSIS TREATMENT WASTEWATER UREA FERTILIZER
PLANTS FENTON REAGENT AND *PSEUDOMONAS FLUORESCENS*
BACTERIA CONCENTRATION 2500, 2000 AND 1500 PPM

Parameter	Initial Ratio Analysis FeSO_4 (gram) : H_2O_2 (ml)				
Concentration (2500 ppm)	1 : 2	1 : 4	1 : 6	1 : 8	1 : 10
pH	8.1	8.4	8.7	8.9	9.7
$\text{NH}_3\text{-N}$	76.5	55	76.25	107.25	119.56
Urea	1933	1080	1495	1479	1770.09
Nitrate	1.20	0.98	0.67	32.78	92.45
Nitrite	0.088	0.076	0.043	1.087	6.98
TKN	894.18	727.35	697.91	727.44	928.60
COD	13	11	18	17	15
TSS	10	12	11	13	10
Concentration (2000 ppm)	1 : 2	1 : 4	1 : 6	1 : 8	1 : 10
pH	7.8	8.2	8.3	8.7	9.2
$\text{NH}_3\text{-N}$	0.125	0.08	0.007	0.0	0.0
Urea	593	306	693.67	583.07	300.4
Nitrate	0.94	0.34	0.56	22.08	69.90
Nitrite	0.074	0.034	0.023	1.024	3.053
TKN	273.90	232.84	323.782	272.09	12.290
COD	15	15	15	19	14
TSS	14	15	15	16	14
Concentration (1500 ppm)	1 : 2	1 : 4	1 : 6	1 : 8	1 : 10
pH	7.7	7.8	8.0	8.3	8.6
$\text{NH}_3\text{-N}$	0.075	0.003	0.0	0.0	0.0
Urea	248	15	145.78	1.056	0.0
Nitrate	0.62	0.26	0.91	17.45	69.90
Nitrite	0.064	0.019	0.011	0.019	0.12
TKN	68.95	6.093	0.207	0.482	0.0
COD	20	15	23	19	25
TSS	15	17	15	17	15

The addition of carbonaceous material is proven to be capable of reducing inorganic nitrogen [2]. Bacterial growth is limited by the balance of nutrients in the water. Therefore, the

dynamics of bacteria population are closely related with the availability of nutrients [10]. The data in Table 4, show that the levels of nitrate and nitrite decrease. The decrease is due to the fact that the nitrate and nitrite formed are sufficient for bacterial nutrients that will stimulate the growth of bacteria and the increase of bacterial biomass. Although in low concentrations, nitrite is toxic to fish and other aquatic organisms [13]. Nitrite compounds in fish will be bound in blood that will form methaemoglobin ($\text{Hb} + \text{NO}_2 = \text{Met-Hb}$). The Met-Hb would interfere with the transport of oxygen to the tissues of fish that can cause fish to experience hypoxia. The Met-Hb in the blood will cause the blood to become brown. Therefore, nitrite poisoning is also called brown blood disease [4]-[1]-[24]. The levels of Total Suspended Solid (TSS) and Chemical Oxygen Demand (COD) increase which indicates that there is accumulation of organic materials in the wastewater derived from *Pseudomonas fluorescens* bacteria growing in the waste water of urea fertilizer plants, which is in a state of non-toxic. Whereas the increase of TSS level is due to the occurrence of wastewater suspended solids which form residual components, floating materials, and suspended colloidal components. Suspended solids contain inorganic and organic materials. The inorganic materials are in the form of clay and sand, while the organic matters are in the form of the remains of plants and other biological solids such as algae cells, bacteria, and so forth [17].

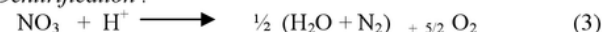
D. Fenton Reagent, Microalgae *Chlorella pyrenoidosa* and Bacteria *Pseudomonas fluorescens*

In further processing of the treatment of the waste water of the urea fertilizer plants which contains high levels of ammonia and urea using Fenton reagent, it is expected that it will be continued with the use of a combination of microalgae *Chlorella pyrenoidosa* and bacteria *Pseudomonas fluorescens*. The microalgae and the bacteria will work together in degrading the waste water. The results of this study presented on Table 5. in general show that pH values tend to decrease with the increasing of maintenance time. This fluctuating value is allegedly due to the addition of the nitrification and denitrification bacteria into the maintenance media. The mechanism of bacterial nitrification and denitrification which can affect pH level can be described by the following equation [17].

Nitrification:



Denitrification :



Through this equation it can be seen that nitrifying bacteria in the process to convert NH_4^+ (ammonium) to NO_3^- (nitrate) produce H^+ ions that can make the pH of the maintenance medium drop. Since in a large-scale microalgae always associate with bacteria, the interaction between algae and bacteria will be able to purify river water. Metabolic activities of heterotrophic aerobic bacteria produce CO_2 , NH_4 , NO_3 , PO_4^{3-} , and so on. The microalgae absorb those

compounds and produce organic matters, O₂, and H₂O. The oxygen produced by microalgae is used by heterotrophic aerobic bacteria for the reaction of nitrification and is used by anaerobic bacteria for denitrification. Through the process of photosynthesis, microalgae using CO₂ from aerobic bacteria and ammonia form cell protoplasm and release oxygen molecules [6] - [7] - [19] - [22].

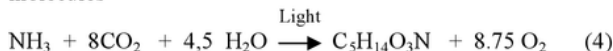


TABLE V.
RESULT ANALYSIS TREATMENT WASTEWATER UREA FERTILIZER PLANTS WITH FENTON REAGENT AND MICROALGAE *CHLORELLA PYRENOIDOSA* AND *PSEUDOMONAS FLUORESCENS* BACTERIA 2500, 2000 AND 1500 PPM.

Parameter (ml)	Initial Ratio Analysis FeSO ₄ (gram) : H ₂ O ₂				
Concentration (2500 ppm)	1 : 2	1 : 4	1 : 6	1 : 8	1 : 10
pH	8,2	8,3	8,8	8,8	9,7
NH ₃ -N	22,34	73,12	96,43	103,89	125,78
Urea	1856	1620	1421	1500,03	1823,23
Nitrate	0,97	0,57	0,56	41,43	103,89
Nitrite	0,076	0,767	0,230	1,767	6,076
TKN	853,88	746,13	663,56	763,90	986,62
COD	101	93	100	115	100
TSS	59	59	59	59	59
Concentration (2000 ppm)	1 : 2	1 : 4	1 : 6	1 : 8	1 : 10
pH	7,8	8,0	8,3	8,7	9,2
NH ₃ -N	0,375	0,078	0,004	0,0	0,0
Urea	597	520	485	660,65	0,0
Nitrate	0,73	0,230	0,61	21,23	32,98
Nitrite	0,062	0,729	0,096	0,729	0,97
TKN	273,995	239,278	226,33	308,30	0,0
COD	136	99	105	119	114
TSS	60	60	60	69	62
Concentration (1500 ppm)	1 : 2	1 : 4	1 : 6	1 : 8	1 : 10
pH	7,7	7,9	8,1	8,3	8,7
NH ₃ -N	0,025	0,003	0,001	0,0	0,0
Urea	205	59	0,75	0,0	0,0
Nitrate	0,26	0,86	0,98	16,98	32,98
Nitrite	0,056	0,597	0,074	1,597	0,97
TKN	94,32	2,309	0,346	0,0	0,0
COD	138	101	111	125	114
TSS	62	50	62	62	62

The main source of nitrogen that can be used by the microalgae are nitrate and ammonia-N. The bacteria utilize the organic materials produced by or derived from dead microalgae as a carbon source for the synthesis of new cells and to establish the energy to form the final product such as CO₂, NH₄⁺ during the process of respiration and synthesis. Microalgae use CO₂ as a carbon source for photosynthesis.

IV. CONCLUSION

1. The best ratio between FeSO₄ : H₂O₂ in treating waste water of urea fertilizer plant using traditional Fenton reagent which meets the quality standards of waste water quality issued by the Minister of Environmental Affairs of the Republic of Indonesia No.122 of the year 2004 and the Decree of South Sumatra Governor No.18 of the year 2005, is 1 : 4, at a concentration of 1500 and 2000 ppm.
2. The treatment of waste water of urea fertilizer plants by means of advanced oxidation using microalgae *Chlorella pyrenoidosa*, *Nannochloropsis* sp. *Pseudomonas fluorescens* and the synergy between microalgae *Chlorella pyrenoidosa* and bacteria *Pseudomonas fluorescens* results in a decrease of pH, NH₃-N, Urea, TKN, Nitrate and Nitrite and an increase of levels of TSS and COD. And the best treatment was using *Pseudomonas fluorescens* bacteria.

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