

# THE EFFECT OF MICROSTART ENZYMES ADDITION AND AERATION TIME ON COD REDUCTION

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**ABSTRACT**---In a wastewater treatment plant in one of the companies in Karawang, some enzyme was added in the aeration tank which aims to reduce the COD content in wastewater. In this study, an observation will be conducted on how much influence the addition of enzyme doses on COD decrease of wastewater. The research method uses the Design of Experiments (DOE) method. Variables include COD content as an dependent variable and enzyme doses as an independent variable with variations in concentration: 0 ppm, 50 ppm, 100 ppm and 150 ppm and variations in aeration time: 0, 1, 2 and 3 hour, data collected directly based on the results of sampling which will be analyzed for COD content. Based on the results of research on enzyme doses significantly influence the decrease in COD content base on the value of  $f$ -count  $>$   $f$ -table Anova factorial  $RAL = 43\ 177.30 > 2.82$ . In addition, the aeration time also has a significant effect on reducing the COD content. This can be seen from the value of  $f$ -count  $>$   $f$ -table Anova factorial  $RAL = 838.63 > 2.82$ . Enzyme dosage and contact time has a significant effect on decreasing COD content. Multiple Linear Regression is  $Y = 101.303 - 0.055X_1 - 19.444 X_2 + e$ . The correlation coefficient number is 95.7% and the coefficient of determination value is 91.6%. From the research data it can be seen that the dose of the microstart enzyme and the optimum contact time is 100 ppm with a contact time of 2 hours. This is indicated by the declining value of COD content reaching 56.3%.

**Keywords**---COD, enzyme and aeration time

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## I. INTRODUCTION

According to PP No.82 of 2001 [10], "Waste is the liquid residue from a result and or activity business". Where the community lives, businesses or activities are carried out, there are various types of waste that will be generated. The wastewater produced must be treated to a level of waste below the quality standard so as not to endanger and pollute the environment. According to PP No.82 of 2001 [10], "Water pollution is the entry or inclusion of living things, substances, energy and or other components into water by human activities, so that the quality of water drops to a certain level that causes water to not function in accordance with designation ". The level of danger produced by a waste is determined by the type and characteristics contained in the waste. If wastewater is channeled directly into the river without treatment, the river will be polluted and cause the water ecosystem to be disrupted.

Industrial wastewater is one of the causes water pollution. Therefore, each industry producing liquid waste must meet the quality standards set by the government. According to PP No.82 of 2001 [10], "Quality standard of wastewater is a

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measure of the limit or content of pollutant elements and / or the amount of pollutant elements which are tolerated in wastewater to be discharged or released into water sources from a business and or activity".

In the context of controlling environmental pollution by industry, Head of the Integrated Investment and Services Agency issues: "Decree Head of the Integrated Investment and Services Agency of Karawang Regency No. 503 / Kep.486 / 2 / IPALASA / BPMPT / 2012 concerning the Granting of Wastewater Disposal Permits to Water or Water Resources (IPALASA) to one of the companies in the Karawang region. The allowable discharge of wastewater to the Cikalapa River is 10000 m<sup>3</sup>/h. This permit requires the company as one of the managers of a large industrial estate in Karawang to conduct wastewater treatment until it meets the quality standards for the disposal of liquid waste into the environment.

The waste processing company is very concerned in meeting the established quality standards and supports the Citarum Harum government program, this can be seen from the results of the wastewater analysis discharges that are below the Quality Standard of liquid waste issued by the government, namely "Ministry of Environment Regulation PERMEN LH No. 3 of 2010 concerning Wastewater Quality Standards for Industrial Estates [7] ". This is indicated by the results of the wastewater analysis discharges below the quality standard threshold.

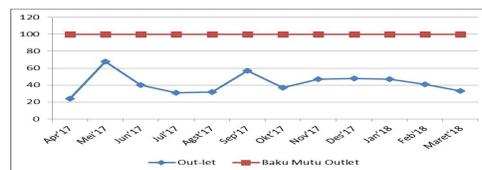


Fig 1. COD Content of Outlet Waste IPAL period April 2017-Maret 2018.

Source: Company Data

In Aeration Tank the Mirostar Enzyme is added which aims to accelerate the process of decomposition of water organic waste dissolving. The addition of enzymes in the aeration surface bath is not yet known for the effect of reducing COD content in wastewater.

This is the base for researchers to conduct research on the effect of adding microstart enzymes in active sludge to decrease COD content in wastewater. And this research is fully supported by company.

The main problem for research object is to determine the effect of adding microstart enzymes on active sludge and aeration time to reduce the COD content in wastewater. So this study aims to determine the effect of the addition of microstart enzymes in active sludge and the aeration time on the reduction of COD content in wastewater.

## II. LITERATURE REVIEW

### Wastewater

According to PP No.82 of 2001 about Water Quality Management and Water Pollution Control [10],

"Liquid waste is the residue from a result of a liquid based activity". Industrial wastewater is one of the reason of water pollution. Therefore, each industry which produced liquid waste must meet the quality standards set by the government. According to PP No.82 of 2001 [10], "Quality standard of wastewater is a measure of the limit or content of pollutant elements and / or the amount of pollutant elements which are tolerated in wastewater to be discharged or released into water sources from a business and or activity".

### **Lumpur Aktiv (Activated Sludge)**

"The basic principle of an active sludge system is that it consists of two main process units, which is bioreactor (surface aeration tank) and a sedimentation tank (settling tank). In active sludge systems, liquid waste and biomass are mixed perfectly in a reactor and aeration. In general, aeration also functions as stirring tools for those suspensions. The biomass suspension in liquid waste is then channeled to the sedimentation tank. Some of the deposited biomass is returned to the bioreactor, and treated water is discharged into the environment "according to (Badjoeri et al., 2002) in the journal (Nuraini Santi, 2017) [8].

The same thing was discussed also in the journal Estiningsih, I. K. (2007). Effect of Active Sludge Volume and Contact Time on Reduction in BOD levels in Hospital Liquid Waste. Research and Development Journal. Semarang State University [4] and Ternes, T. A., Kreckel, P., & Mueller, J. (1999). Behavior and occurrence of estrogens in municipal sewage treatment plants — II. Aerobic batch experiments with activated sludge. Science of the Total Environment, 225 (1-2), 91-99 [14].

### **Microstart Enzymes**

According to PT Mitrarech (2018), "Micro Star is a consortium of waste-decomposing bacteria that is the result of microbiological culture from a collection of pure probiotic bacteria that is beneficial and can decompose organic liquid waste by working specifically and synergistically on industrial wastewater effluents so that the quality of the water environment is clean".

Micro Star is a natural live microbial fermentation product and the best alternative to replace use of synthetic chemical products.

Micro Star is made from environmentally friendly materials so it is safe for humans, animals or nature.

These are benefits of Micro star according to PT. Mitrarech as follows:

- a. Speed up the process of decomposing organic waste dissolved in water.
- b. Remove unpleasant odors in wastewater and improve the color of wastewater.
- c. Reducing BOD, COD and TSS levels in wastewater.
- d. Reduce development of pathogenic micro organisms (*E-coli* sp and *Salmonella* sp) and increase the dominance of beneficial bacteria.

### **Chemical Oxygen Demand (COD)**

According to G. Alaerta, SS Saantika (1984: 1496) [2] "Chemical Oxygen Demand is the amount of oxygen (mg O<sub>2</sub>) needed to oxidize organic substances present in 1 liter of water samples, where oxidizing K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> is used as a source of oxygen ( oxidizing agent). The COD rate is a measure for water pollution by organic substances which naturally can be oxidized through microbiological processes, and reduced dissolved oxygen in the water ".

### **Design Of Experiment (DOE)**

"The design of an experiment is a complete step that needs to be taken long before the experiment is carried out so that the data that should be needed can be obtained so that it will lead to objective analysis and conclusions that apply to the problem discussion." According to Sudjana (1984) [12] .

- a. Experimental Design Principles

According to Sudjana (1984) [12], "The basic principles that are commonly used and known in experimental design are:

- Replication
- Randomization
- Local control

b. Effects and Interactions

According to Sudjana (1984) [12] "For design purposes, free variables will be called factors and values or classifications rather than factors will be called factor levels. Factors are usually expressed in small letters a, b, c, d, and so on, while the level of factors is expressed by the numbers 1, 2, 3, and so on which are written as an index for the related factor".

c. ANOVA Model Factorial Experiment Design.

According to Sudjana (1984) [12] "Factorial experiments are experiments that all (almost all) the level of a particular factor is combined or crossed with all (almost all) the level of each other factors that exist in this experiment".

d. Factorial ANOVA RAL Hypothesis

Hypothesis for the effect of Enzyme Dose, Aeration Time and the Effect of Interaction of Enzyme Dose with Aeration Time is

Ho: No effect on COD content.

H1: Affects the COD content

Decision making based on F values is as follows:

- F-hit value < F-table, then Ho is accepted and H1 is rejected.
- F-hit value ≥ F-table, then Ho is rejected and H1 is accepted

As has also been reported by Nursanti, M. and Fadryani, F. [9] using two factor analysis variant in repeated observations.

e. Multiple linear regression

According to Imam Ghozali (2018; 96) [5], "Multiple linear regression analysis is used to measure the strength of the relationship between two or more variables, also shows the direction of the relationship between the dependent variable and the independent variable".

Common forms of multiple linear regression are:

$$y = \alpha + \beta_1x_1 + \beta_2x_2 + e$$

y = Dependent variable

$\alpha$  = Regression of coefficient

$\beta_1x_1$  = Independent Variable 1

$\beta_2x_2$  = Independent Variable 2

e = Error score

f. Coefficient of determination test

According to Imam Ghozali (2018: 97) [5] "The coefficient of determination (R<sup>2</sup>) Is a tool to measure how far is the ability of the model in explaining the variation of the dependent variable. The coefficient of determination is between zero and one. A small R<sup>2</sup> value means that the ability of independent variables to explain the dependent is very limited and vice versa.

g. Hypothesis Testing

• Partial Hypothesis Test (t-Test)

According to Imam Ghazali (2018: 20) [5] "T-test is used to determine individually whether there is an influence between the independent variables with the dependent variable. Partial testing for each regression coefficient is tested to determine the effect of partially between the independent variable and the dependent variable, by looking at the significance level of the t value at 5% of the formula used:

The hypothesis to be tested is as follows:

- H0:  $\beta_1 = \beta_2 = 0$ , then X1, X2 have no significant effect on Y.
- H1:  $\beta_1 \neq \beta_2 \neq 0$ , then X1, X2 have a significant effect on Y.

• Simultant test (F-Test)

According to Imam Ghazali (2018: 22) [5] "To test together between independent variables with dependent variables by looking at the level of significance (F) at  $\alpha$  5% using formula:

The hypothesis for the effect of enzyme dose is

- Ho: No effect of enzyme dosage on COD content.
- H1: There is an effect of an enzyme dose on the COD content

Testing each regression coefficient together is said to be significant if

a) Based on the value of F

• If the value of F-count  $>$  F-table so independent variables is significantly influence the dependent variable or Ho is rejected and H1 is accepted

b) Based on the significance value

• If the significance value  $>$  0.05, which means that the independent variable (X) simultaneously has no effect on the dependent variable (Y). Then Ho is accepted and H1 is rejected.

• If the significance value  $<$  0.05 which means that the independent variable (X) simultaneously influences the dependent variable (Y). Then Ho is rejected and H1 accepted.

The use of experiments in previous studies include López, C., Moreira, MT, Feijoo, G., & Lema, JM (2011) [6] and Aber, S., Salari, D., & Parsa, MR (2010) [ 1]. In addition, research on the use of linear regression as a research method was reported by Bandpei, AM, Mohseni, SM, Sheikhmohammadi, A., Sardar, M., Sarkhosh, M., Almasian, M., ... & Rezaei, S. (2017 ) [3] and Raghunathan, TE, Lepkowski, JM, Van Hoewyk, J., & Solenberger, P. (2001) [11].

### III. RESEARCH METHODOLOGY

#### Research Method

The stages of this research show the flow of research starting from determining the research objectives to determining the effect of the obtained enzymes to be proposed to the company, these are following stages of research conducted by researchers.

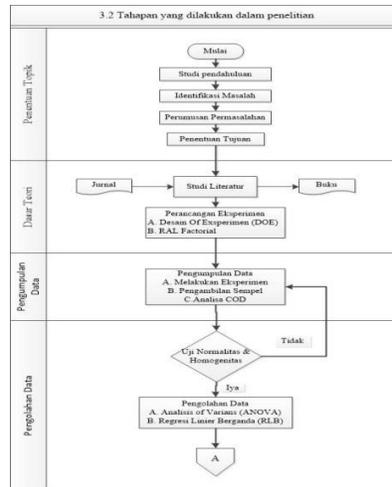


Fig 2. Research Method

**Designing with the Design Of Experiment (DOE) Method**

- a. Determination of Response Variables
  - The independent variable used in this study is the enzyme dose and aeration time.
  - Dependent variable (dependent variable) used in this research is COD content.

b. Determination of Factors and Levels of Factors

The parameters to be studied are

- 1) Enzyme dose (ppm)
- 2) Aeration time of active sludge (hours)

After determining the factors, we determine the level of factors to be examined. The factor levels of each factor are shown in 3.1 Table.

Table 1. Factor and Factor Level

Faktor	Taraf Faktor
Dosis Enzim	0 ppm
	50 ppm
	100 ppm
	150 ppm
Waktu Aerasi	0 jam
	1 jam
	2 jam
	3 jam

c. Research Design

The research design used was a completely randomized design (CRD) factorial. Using the CRD factorial method with the first factor (A) is level 4 enzyme dose and the second factor (B) is the aeration time of active sludge using 4 levels, each level is repeated 3 times, which is:

- First factor (A): Enzyme dose  
 D1: 0 ppm, D2: 50 ppm,

D3: 100 ppm, D4: 150 ppm

- Second factor (B): Aeration time of active sludge

W1: 0 hours, W2: 1 hour,

W3: 2 hours, W4: 3 hours

Table 2. Combination of Treatment

	W1	W2	W3	W4
D1	D1W1	D1W2	D1W3	D1W4
D2	D2W1	D2W2	D2W3	D2W4
D3	D3W1	D3W2	D3W3	D3W4
D4	D4W1	D4W2	D4W3	D4W4

#### IV. Data Collection Methods

##### a. Population, Sample, and Sample decision

- Population

Population in this study is active sludge contained in the research tool.

- Sample

"The sample is part of the number and characteristics that represent the population" (Sugiyono, 2011: 120) [13].

- Determination of the sample

Determination of samples in mini WWTP replication using a 1: 5 comparison scale. Every 1 part of Grit water is compared to 5 parts of active sludge water.

##### b. Data source

In this study the source of the data was came from the research data obtained from direct research, which is the obtained COD content data.

##### c. Data collection

Data collection uses direct observation in the conducted study, which is sampling every decided time, then analyze the COD content in sample.

COD analysis was performed using the Spectrophotometer DR5000 at a wavelength of 620 nm.

#### Data Analysis

Data analysis methods are Factorial RAL ANOVA and Multiple Linear Regression.

##### a. Descriptive Statistic

Descriptive statistics are descriptive data from the variables tested, from N, Minimum, Maximum, Mean Standard Deviation, Amount and others.

##### b. Classic Assumption Test

Before conducting data analysis, we must test the classical assumptions of the data obtained, which is :

- Normality test

1) Graphic analysis

2) Statistic analysis

- Linearity Test
- Multicolinearity test
- Heteroscedasticity Test

## V. RESULT AND DISCUSSION

The results are factorial experimental data of two factors with a completely randomized design is the CRD. The two factors, namely the enzyme dose factor (D) has four factor levels which is "0 ppm, 50 ppm, 100 ppm and 150 ppm" and the aeration time factor (W) has four factor levels "0 hours, 1 hour, 2 hours and 3 hours".

So that the number of treatment combinations is 16, where each treatment combination is repeated 3 times. Then the number of experiments in this study was  $16 \times 3 = 48$  times the experiment. In this study, the initial COD content of Grit water was 416 mg / l and the initial COD content of active sludge was 40 mg / l, then a comparison of samples was 1: 5 (1 grit water and 5 parts active sludge).

### 4.1

Table 3. Data Content of COD Research

**Hasil Penelitian Nilai Kandungan COD**

Analisa awal air limbah : 412 mg/l  
 Analisa awal air sludge : 40 mg/l

Replikasi	Waktu Aerasi	Dosis Enzim (ppm)				Keterangan
		0	50	100	150	
1	0 jam	103	103	102	102	
	1 jam	80	77	70	65	
	2 jam	60	55	45	46	
	3 jam	45	45	45	45	
2	0 jam	102	102	103	102	
	1 jam	81	77	70	65	
	2 jam	61	55	45	45	
	3 jam	45	45	45	45	
3	0 jam	103	103	103	103	
	1 jam	81	76	71	65	
	2 jam	61	55	45	45	
	3 jam	45	45	45	45	

Source: Research results in the IPAL Lab

Based on the table above, it can be displayed by graph 4.4. From the research data it can be seen that the most influential COD content reduction data is at the enzyme dose of 100 ppm and contact time is 2 hours. This is indicated by the COD content value of 45 mg / l which is the lowest point of the research results.

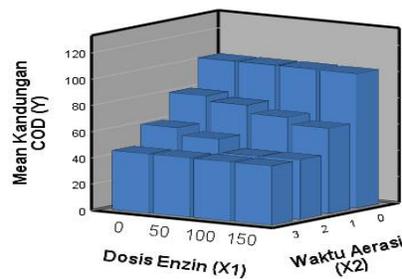


Fig. 3. Research Results Graph

Source: Research results in the IPAL Lab

### Descriptive Statistics

With the help of the application SPSS version 25, we can see the number of samples, minimum numbers, maximum numbers, averages, total numbers, and standard deviations of each variable, recapitulation of the data that has been processed in table 4.2.

Table 4. Descriptive Statistics

Descriptive Statistics					
	N	Minimum	Maximum	Mean	Std. Deviation
Kandungan COD (Y)	48	45	103	68.06	23.180
Dosis Enzin (X1)	48	0	150	75.00	56.493
Waktu Aerasi (X2)	48	0	3	1.50	1.130
Valid N (listwise)	48				

Source: Results of SPSS Data Output Version 25

Based on the test results above, it is known that the N value for all variables is 48, this shows that the research data is 48 data.

- The COD content variable on the results of data processing, obtained an average value of 68.06 with a standard deviation of 23.180. Shows that the standard deviation value <compared with the average value, meaning that in this study the data is normally distributed. While the minimum value is 45 and the maximum value is 103.
- Enzyme Dose Variable on the results of data processing, obtained an average value of 75,00 with a standard deviation value of 56,493. Shows that the standard deviation value <compared with the average value, meaning that in this study the data is normally distributed. While the minimum value is 0 and the maximum value is 150.
- Aeration Time Variable on the results of data processing, obtained an average value of 1.50 with a standard deviation of 1.130. Shows that the standard deviation value <compared with the average value, meaning that in this study the data is normally distributed. While the minimum value is 0 and the maximum value is 3.

### Classical Assumption Test

- Normality test

The normality test results are described as follows:

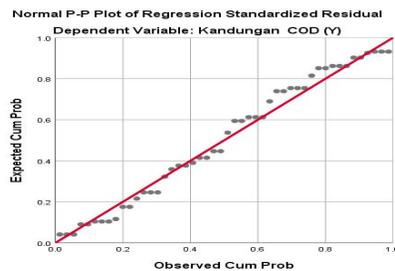


Fig. 4. Normal P-Plot

Source: Results of SPSS Data Output Version 25

Based on the picture above, it can be seen that the data from all data has been normally distributed. This is because all data is spread and follow a diagonal line so that the data meets the normality requirements.

One-Sample Kolmogorov-Smirnov Test		Unstandardized Residual
N		48
Normal Parameters <sup>a,b</sup>	Mean	.0000000
	Std. Deviation	6.71233252
Most Extreme Differences	Absolute	.098
	Negative	-.098
Test Statistic		.098
Asymp. Sig. (2-tailed)		.200 <sup>c,d</sup>

a. Test distribution is Normal.  
 b. Calculated from data.  
 c. Lilliefors Significance Correction.  
 d. This is a lower bound of the true significance.

Table 5. Kolmogorov-Smirnov Normality Test Results

Source: Output SPSS Version

Based on the output image of Kolmogorov-Smirnov normality test with SPSS version 25 above, the Asymp.Sig (2-tailed) significance value is known as  $0.200 > 0.05$ , so according to the decision basis in the Kolmogorov-Smirnov normality test, it can be concluded that the data is normally distributed.

b. Linearity Test

The linearity test results in the research data are described as follows:

ANOVA Table						
		Sum of Squares	df	Mean Square	F	Sig.
COD Hasil (Y) * Dosis Enzim (X1)	Between Groups (Combined)	471.729	3	157.243	.279	.840
	Linearity	456.504	1	456.504	.811	.373
	Deviation from Linearity	15.225	2	7.612	.014	.987
	Within Groups	24781.083	44	563.206		
	Total	25252.813	47			

Table 6. Linearity Test Results

Source: Output SPSS Version 25

Based on the image output linearity test with SPSS version 25 above is known:

- Based on the F value of  $0.014 < F\text{-table } 3.21$  ( $F\text{-count } < F\text{-table} = 0.014 < 3.21$ ), then according to the decision basis on the linearity test it can be concluded that there is a linear and significant relationship between the enzyme Dose variables (X) with COD content variable (Y).
- Based on the deviation value of the linear significance (Sig) of  $0.987 > 0.05$ , according to the decision basis on the linearity test it can be concluded that there is a linear and significant relationship between the Enzyme Dose (X) variable with the COD content variable (Y).

c. Multicollinearity Test

Multicollinearity test results on the research data are described as follows:

Coefficients <sup>a</sup>								
Model		Unstandardized Coefficients		Standardized Coefficients			Collinearity Statistics	
		B	Std. Error	Beta	t	Sig.	Tolerance	VIF
1	(Constant)	101.363	2.124		47.731	.000		
	Dosis Enzim (X1)	-.055	.018	-.134	-3.115	.003	1.000	1.000
	Waktu Aerasi (X2)	-19.442	.886	-.948	-21.953	.000	1.000	1.000

a. Dependent Variable: COD Hasil (Y)

Table 7. Multicolinesity Test Results

Source: SPSS Output Version 25

Based on the multicollinearity test output image with SPSS version 25 above, it is known the tolerance value for the Enzyme Dose variable (X1) and the Aeration Time variable (X2) of  $1,000 > 0.10$ . And the VIT value for the Enzyme Dose variable (X1) and the Aeration Time variable (X2) of  $1,000 < 10.00$ , then in accordance with the decision base in the multicollinearity test it can be concluded that there were no symptoms of multicollinearity in the regression model.

Heteroscedasticity Test

Heteroscedasticity test results in the research data are described as follows:

Model	Unstandardized Coefficients		Standardized Coefficients		t	Sig.	Collinearity Statistics	
	B	Std. Error	Beta				Tolerance	VIF
1								
(Constant)	.999	.438			2.281	.027		
Dosis Enzim (X1)	.064	.004	.901		14.826	.000	1.000	1.000
Waktu Aerasi (X2)	.445	.183	.148		2.436	.019	1.000	1.000

a. Dependent Variable: Abs\_RES

Table 8. Heteroscedasticity Test Results

Source: Output SPSS Version 25

Based on the image of the heteroscedasticity test output with SPSS version 25 above, it is known that the significance value for the Enzyme Dose variable (X1) is 0,000. While the significance value for the Aeration Time (X2) variable is 0.019. Because the significance value of the two variables above is smaller than 0.05, according to the basis of the decision in the glacier test it can be concluded that there is a symptom of heteroscedasticity in the regression model.

### ANOVA RAL factorial

The enzyme dose factor (A) which has four levels, namely "0 ppm, 50 ppm, 100 ppm and 150 ppm", and the Aeration time factor (B) which has four levels namely "0 hours, 1 hour, 2 hours and 3 hours" , so factorial analysis of variance with the basic design of two-factor CRD. The calculations are as follows:

a. Degrees of Freedom (DF)

- Total Degrees of Freedom (DFT)

$$DFT = (axb \times r) - 1 = (4 \times 4 \times 3) - 1 = 47$$

- Degree of free treatment (DF<sub>Trt</sub>)

$$DF_{Trt} = ab - 1 = (4 \times 4) - 1 = 15$$

- Factor A degrees of Freedom (DFA)

$$DFA = a - 1 = 4 - 1 = 3$$

- Factor B degrees of freedom (DFB)

$$DFB = b - 1 = 4 - 1 = 3$$

- Interaction factor AB degrees of freedom (DFAB)

$$DFAB = (a - 1)(b - 1) = (4-1)(4-1) = 9$$

- Error degree of freedom (DFE)

$$DFE = DFT - DF_{Trt} = 47 - 15 = 32$$

b. Correction factors (CF)

$$CF = \frac{\sum Y_{ij}^2}{a * b * r} = \frac{3284^2}{4 * 4 * 3} = 222\ 360,19$$

c. Sum of Square (SS)

- Total Sum of Square (SST)

$$SST = \sum (Y_{ijk})^2 - CF = 25\ 252,81$$

- Sum of Square Treatment (SSM)

$$SSM = \frac{\sum (\sum y_{ij})^2}{r} - CF = 25\ 246,81$$

- Sum of Square Factor A (SSA)

$$SSA = \frac{\sum (\sum y_{i.})^2}{rb} - CF = 24\ 487,23$$

- Sum of Square Factor B (SSB)

$$SSB = \frac{\sum(\sum y_j)^2}{rb} - CF = 471,73$$

- Sum of Square Interaction A\*B (SSA\*B)

$$SSA*B = SSM - SSA - SSB = 478,55$$

- Error Sum of Square (SSE)

$$SSE = SST - SSM = 6,00$$

d. Mean Squares (MS)

- Treatment mean squares ( $MS_{Trit}$ )

$$MS_{Trit} = SSM / DF_{Trit} = 1\ 683,12$$

- Factor A mean squares (MSA)

$$MSA = SSA / DFA = 8\ 095,74$$

- Factor B mean squares (MSB)

$$MSB = SSB / DFB = 157,24$$

- Interaction Factor AB mean squares ( $MSA*B$ )

$$MSA*B = SSA*B / DFAB = 54,21$$

- Error mean squares (MSE)

$$MSE = SSM / DF_{Trit} = 6,00/32 = 0,19$$

e. Frequency value (f-val)

- F-val P =  $MS_{Trit} / MSE = 8\ 976,64$

- F-val A =  $MSA / MSE = 43\ 177,30$

- F-val B =  $MSB / MSE = 838,63$

- F-val A\*B =  $MSA*B / MSE = 289,1$

f. ANOVA Table

Table 8. ANOVA RAL Factorial Table

Sumber keragaman	JK	db	KT	F-hit	F-tabel
P	25 246,81	15	1 683,12	8 976,64	1,99
Dosis Enzim	24 487,23	3	8 095,74	43177,30	2,82
Waktu Aerasi	471,73	3	157,24	838,63	2,82
Dosis Enzim * Waktu Aerasi	478,55	9	54,21	289,1	2,14
G	6	32	0,19		
<b>Total</b>	<b>25 252,81</b>	<b>47</b>			

**ANOVA RAL Factorial Hypothesis**

a. Hypothesis of the Effects of Enzyme Doses

H0: There is no effect of the enzyme dose on COD content.

H1: There is an effect of an enzyme dose on the COD content

Based on the Factorial RAL ANOVA table above, for the Enzyme Dose variable it can be F-value > F-table (43177.30 > 2.82). Then Ho is rejected and H1 is accepted. Means Enzyme Doses affect the COD content.

b. Hypothesis of the Effects of Aeration Time

Ho: There is no effect of Aeration Time on the COD content.

H1: There is an effect of Aeration Time on the COD content

Based on the ANOVA RAL Factorial table above, for the Aeration Time variable the F-count value > F-table (838.63 > 2.82) can be obtained. Then Ho is rejected and H1 is accepted. Mean Aeration Time affects the COD content

c. Hypothesis of the Interaction Effect of Enzyme Dose With Aeration Time

H0: There is no effect of the interaction of Enzyme Dose and Aeration Time on the COD content.

H1:: There is an effect of the interaction of Enzyme Dose and Aeration Time on the COD content

Based on the ANOVA RAL Factorial table above, the interaction variables for Enzyme Dose and Aeration Time can be calculated > F-table (289.10 > 2.14). Then Ho is rejected and H1 is accepted. It means that the interaction of Enzyme Dose and Aeration Time influences the COD content

**Multiple Linear Regression Analysis**

The results of multiple linear regression analysis of research data are described as follows:

Table 9. Output Variabel Entered / Removed

Variables Entered/Removed <sup>a</sup>			
Model	Variables Entered	Variables Removed	Method
1	Waktu Aerasi (X2), Dosis Enzin (X1) <sup>b</sup>	.	Enter

a. Dependent Variable: Kandungan COD (Y)  
 b. All requested variables entered.

Source: Results of SPSS Data Output Version 25

Based on the table above, explaining the analyzed variables, namely COD Results as the dependent variable and for the independent variable is the Enzyme Dose and Contact Time. Where there are no variables issued.

Coefficients <sup>a</sup>					
Model		Unstandardized Coefficients		Standardized Coefficients	Sig.
		B	Std. Error	Beta	
1	(Constant)	101.363	2.124		.000
	Dosis Enzin (X1)	-.055	.018	-.134	.003
	Waktu Aerasi (X2)	-19.442	.886	-.948	.000

a. Dependent Variable: Kandungan COD (Y)

Table 10. Results of Multiple Linear Regression Tests

Source: Results of SPSS Data Output Version 25

Based on the test results in table 4.9 obtained the results of multiple linear regression equations as follows:

$$Y = 101,303 - 0,055X_1 - 19,442X_2 + e$$

or

$$\text{COD value} = 103,303 - 0,055(\text{Enzyme Dose}) - 19,442(\text{Aeration time}) + e$$

From the results of the equation the constant value is 101.303. These results indicate that:

- Regression coefficient X1 of the enzyme dose of - 0.055 states that for every increase of 1, the yield COD will decrease by 0.055. And conversely, if the contact time decreases by 1, then the COD of the results is also expected to increase by 0.055 with the X2 is fixed by the assumption.
- The regression coefficient X2 of aeration time of -19.444 states that for every increase of 1, the yield COD will decrease by 19.444 with the assumption that X1 is fixed.

**Determination of Coefficient Test**

Results of coefficient of determination with SPSS application version 25 are as follows:

Table 4.10 Determination of Coefficient Test Result

Model Summary				
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.957 <sup>a</sup>	.916	.912	6.860

a. Predictors: (Constant), Waktu Aerasi (X2), Dosis Enzin (X1)

Source: Data Output SPSS

It can be seen that correlation coefficient (R) value is 0.957. This means that the relationship between the independent variable and the dependent variable is known to be 95.7%, meaning that the relationship between the independent variable and the dependent variable is very strong. While determination coefficient value (R<sup>2</sup>) which is equal to 0.916 means that the ability of the independent variable (enzyme dose and aeration time) explain that changing the variation of dependent variable (COD value) is equal to 91.6%, while the remaining 8.4% (100- 91.6%) explained by other factors outside the regression model analyzed.

### Hypothesis test

#### a. Partial Hypothesis Test (t-test)

The results of hypothesis testing with the SPSS software can be seen in the coefficient table sig or significance column, as follows:

Table 11. Partial test result (t-test)

Coefficients <sup>a</sup>						
Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	101.363	2.124		47.731	.000
	Dosis Enzin (X1)	-.055	.018	-.134	-3.115	.003
	Waktu Aerasi (X2)	-19.442	.886	-.948	-21.953	.000

a. Dependent Variable: COD Hasil (Y)

Source : Data Output SPSS

From table 4.11 the t-test results are explained as follows:

- The effect of enzyme dose (X1)

It can be seen the significance value of the Enzyme Dose variable is 0.003, when compared to the significance level ( $\alpha = 0.05$ ) then  $0.003 < 0.05$ . therefore X2 have a significant effect on COD reduction. Or, in the coefficient table of t-value  $>$  t-table ( $-3.115 > 2.010$ ).

- The effect of aeration time (X2)

In table 4.11 it can be seen that the significance value for the contact time variable is 0,000, when compared to the significance level ( $\alpha = 0.05$ ),  $0.000 < 0.05$ . Therefore that aeration time have a significant effect on COD reduction. Or, in the coefficient table t-value  $<$  t-table ( $-21.953 < 2.010$ ) is obtained, so it is said to have a significant effect but has a negative relationship to the decreasing COD content.

#### b. Simultaneous Test (F-test)

The result SPSS software version 25 simultaneous testing as follows:

Table 12. Simultaneous Test Results (F-test)

ANOVA <sup>a</sup>						
Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	23135.208	2	11567.604	245.817	.000 <sup>b</sup>
	Residual	2117.604	45	47.058		
	Total	25252.813	47			

a. Dependent Variable: COD Hasil (Y)  
 b. Predictors: (Constant), Waktu Aerasi (X2), Dosis Enzin (X1)

Source : Data Output SPSS

A significance value is  $0,000 < 0.05$ , this shows that all independent variables (the enzyme dose (X1) and the aeration time (X2)) simultaneously have a significant effect (together) on the yield COD (Y). Or, in the ANOVA table, the f-value  $> f$ -table ( $245.817 > 2.82$ ) is obtained. Then it can be stated that the enzyme dose and aeration time simultaneously influence the COD results. Therefore that  $H_0$  is rejected and  $H_1$  is accepted, meaning that the enzyme dose and aeration time simultaneously and significantly influence the COD results.

## VI. CONCLUSIONS

Based on the results of research, enzyme doses significantly influence on decreasing COD content. The f-value  $> f$ -table Anova factorial RAL =  $43\ 177.30 > 2.82$  and partial test value (t-test) f-value  $> f$ -table =  $-3.115 > 2.010$  and significant value of  $0.003 < 0.05$ . In addition, the aeration time also has a significant effect on reducing the COD content with the value of f-count  $> f$ -table Anova factorial RAL =  $838.63 > 2.82$  and partial test value (t-test) f-count value  $> f$ -table =  $-21.953 > 2.010$  and significant value of  $0.000 < 0.05$ . Enzyme dosage and contact time has significant effect on decreasing COD content. Multiple Linear Regression is  $Y = 101.303 - 0.055X_1 - 19.444 X_2 + e$ . The correlation coefficient number is 95.7% and the coefficient of determination value is 91.6%. From the research data it can be seen that the dose of the microstart enzyme and the optimum contact time is 100 ppm with a contact time of 2 hours. This is indicated by the decrease in COD content reaching 56.3% which is 45 mg/l which is the lowest point of the research results.

## VII. RECOMMENDATION

Recommendation from this study is that further research needs to determine the effect of enzymes on decreasing the sludge volume in activated sludge. While the company's suggestion is to apply the right dose of the enzyme in order to achieve the maximum function of the enzyme.

## REFERENCES

- [1] Aber, S., Salari, D., & Parsa, M. R. (2010). Employing the Taguchi method to obtain the optimum conditions of coagulation–flocculation process in tannery wastewater treatment. *Chemical Engineering Journal*, 162(1), 127-134.
- [2] Alaerts, G., & Santika, S. S. (1987). Metode penelitian air. *Usaha Nasional*. Surabaya, 309.
- [3] Bandpei, A. M., Mohseni, S. M., Sheikhmohammadi, A., Sardar, M., Sarkhosh, M., Almasian, M., ... & Rezaei, S. (2017). Optimization of arsenite removal by adsorption onto organically modified montmorillonite clay: Experimental & theoretical approaches. *Korean Journal of Chemical Engineering*, 34(2), 376-383.
- [4] Jabarullah, N. H., Jermisittiparsert, K., Melnikov, P. A., Maseleno, A., Hosseinian, A., & Vessally, E. (2019). Methods for the direct synthesis of thioesters from aldehydes: a focus review. *Journal of Sulfur Chemistry*, <https://doi.org/10.1080/17415993.2019.1658764>.
- [5] Ghozali, I. (2018). Aplikasi Analisis Multivariate SPSS 25 Edisi 9. *Semarang: Universitas Diponegoro*.
- [6] López, C., Moreira, M. T., Feijoo, G., & Lema, J. M. (2011). Economic comparison of enzymatic reactors and advanced oxidation processes applied to the degradation of phenol as a model compound. *Biocatalysis and Biotransformation*, 29(6), 344-353.
- [7] Nomor, P. M. L. H. (3). Tahun 2010 Tentang Baku Mutu Air Limbah Bagi Kawasan Industri. *Jakarta: Menteri Negara Lingkungan Hidup*.
- [8] Nuraini, S. (2017). Pengolahan Limbah Air Industri Secara Tepat dan Efisien Menggunakan Lumpur Aktif atau Karbon Aktif. Jurusan Kimia. Institut Teknologi Sepuluh Nopember.
- [9] Nursanti, M., & Fadryani, F. Analisis Varian Dua Faktor Dalam Rancangan Pengamatan Berulang Studi Kasus: Pertumbuhan dan Perkembangan Perkecambahan Kacang Tanah. *Jurnal Ilmiah Matematika dan Terapan*, 13(2).

- [10] Pemerintah, P. P. R. I. P. No. 82 Tahun 2001 Tentang Pengelolaan Kualitas Air Dan Pengendalian Pencemaran Air.
- [11] Raghunathan, T. E., Lepkowski, J. M., Van Hoewyk, J., & Solenberger, P. (2001). A multivariate technique for multiply imputing missing values using a sequence of regression models. *Survey methodology*, 27(1), 85-96.
- [12] Sudjana, P. (1994). Desain dan Analisis Eksperimen, edisi III. Penerbit *TQRSITO Bandung*.
- [13] Sugiyono, P. (2011). Metode penelitian kombinasi (mixed methods). *Bandung: Alfabeta*.
- [14] Ternes, T. A., Kreckel, P., & Mueller, J. (1999). Behaviour and occurrence of estrogens in municipal sewage treatment plants—II. Aerobic batch experiments with activated sludge. *Science of the Total Environment*, 225(12), 91-99