

# Utilization *Chlorella* sp. With The Addition Of EM4 As A Phytoremediation Agent On Samples Of Liquid Waste In The Cracker Industry, Indramayu Regency

## ABSTRACT

Liquid waste from the cracker industry which is directly discharged into water bodies without prior treatment will cause water pollution which is characterized by a decrease in water quality. One alternative to reduce pollutant compounds biologically is to use the phytoremediation method. Phytoremediation is plant-mediated uptake of pollutants. This study aims to determine the best concentration of EM4 which is in symbiosis with *Chlorella* sp. in the process of reducing pollutant wastewater from the cracker industry. This study used a completely randomized design with five treatments and three replications. These treatments included P<sub>A</sub> (without adding EM4), P<sub>B</sub> (adding EM4 10 ml), P<sub>C</sub> (adding EM4 15 ml), P<sub>D</sub> (adding EM4 20 ml), P<sub>E</sub> (adding EM4 25 ml). The data obtained were analyzed statistically using Analysis of Variance (ANOVA). If the calculated F is greater than the F table then proceed with the Duncan's Multiple New Range Test (DNMRT) at the 5% level. The results showed that the addition of *Chlorella* sp. 800 ml ( $11,8 \times 10^6$  cells/ml) and the addition of 25 ml of EM4 is the best concentration in reducing the pollutant content of the cracker industrial wastewater. The P<sub>E</sub> treatment (EM4 25 ml) had a percentage decrease in the value of BOD<sub>5</sub> 83.82%, Ammonia of 99.91%, Phosphate of 95.78%, CO<sub>2</sub> of 97.78%, and increased the density of *Chlorella* sp. to  $52.08 \times 10^6$ , DO from 1.7 mg/L to 5.9 mg/L, pH to 8.2, and temperature to 28.8°C. However, the BOD<sub>5</sub> value is still very far above the quality standard so it does not meet the quality standard according to Government Regulation No. 22 of 2021 Class II and Class III concerning the implementation of environmental protection and management for fishing activities.

*Keywords: phytoremediation, cracker industry wastewater, Chlorella sp., EM4*

## 1. INTRODUCTION

Indramayu Regency is the northern coastal area of West Java with a beach length of 114 km, so it has great potential in the fisheries sector [5] potential fish processing industry in Indramayu is the fish/shrimp cracker processing industry. In Indramayu Regency, the production of fish/shrimp cracker processing has the highest production, which is equal to 45.20% of the total production of processed fishery products [11]. One of the villages which is the center of the fish/shrimp cracker processing industry is Sindang District. Cracker Factory X is a cracker factory that has the second largest production among other companies with a total production of 545 tons per year (Indramayu Regency Industry and Trade Cooperative Service, 2010).

The existence of a cracker processing unit in Indramayu Regency has a positive impact, but it also has a negative impact, namely in the form of environmental pollution due to liquid waste that is not treated properly so that the waterways around the location of the cracker industrial center are black and smell bad [1].

The liquid waste generated in the cracker processing process comes from the process of washing fish/shrimp, washing the cracker production equipment, and steaming residual water so that the liquid waste produced by the cracker industry is an organic liquid waste [1]. The liquid waste from the shrimp/fish cracker industry generally contains many nutrients in the form of protein, carbohydrates, fat, crude fiber, mineral salts, calcium, and phosphorus [17].

Judging from the characteristics of the organic compounds from the cracker industrial wastewater, if the waste is left alone without processing it will pollute the environment around the industry. This of course will disturb the aquatic ecosystem causing a decrease in the DO content in the water, so that organisms in the environment lack oxygen which for a certain **period time** will be dangerous.

One alternative to be able to maintain water quality and reduce organic waste compounds in the cracker industry is phytoremediation. Phytoremediation is **the plant-mediated** uptake of pollutants **utilizing** destruction, inactivation, or immobilization of pollutants into harmless forms [4]. This method can be done by using organisms that can reduce organic waste. One of them is the type of microalgae *Chlorella* sp. *Chlorella* sp. is one of the selected microalgae as organisms for handling organic liquid waste because it can reproduce quickly which is very much determined by the availability of nutrients, besides **that *Chlorella* sp. is easy** to cultivate, produces oxygen through photosynthesis, and contains high protein with main component of amino acids [2]. *Chlorella* sp. **needs** light and CO<sub>2</sub> to photosynthesize. CO<sub>2</sub> needs obtained from other microorganisms such as bacteria because they are **can** convert complex carbohydrates into CO<sub>2</sub> so that it can be used by microalgae for photosynthesis. EM4 consists of five groups of microorganisms namely, photosynthetic bacteria, *Lactobacillus* sp., *Streptomyces* sp., yeast, and *Actynomictes* [13]. Apart from being a supplier of CO<sub>2</sub> and N for microalgae, EM4 can also be used to reduce pollutants [12]. The presence of EM4 in wastewater can degrade organic compounds faster than using only natural microorganisms [16].

Growth media *Chlorella* sp. **is not** a land with special water, but sufficient water containing nitrogen and rich in organic matter [9]. Based on these characteristics, the cracker industrial wastewater is one of the possible materials to be used as a growing medium *Chlorella* sp. The addition of EM4 to liquid waste aims to accelerate the pollutant reduction process. So this study aims to reduce the levels of contaminants in the cracker industrial wastewater using *Chlorella* sp. with the addition of EM4

## 2. METHODOLOGY

### 2.1 Time and Place of Research

In-situ observations were carried out at the Cracker Factory X in Indramayu Regency which included measurements of temperature, pH, DO, and CO<sub>2</sub> in cracker liquid waste before treatment. Meanwhile *Ex-situ* observation carried out at the Laboratory of Water Resources Management FPIK Padjadjaran University which includes BOD measurements, Ammonia, Phosphate, and Density *Chlorella* sp.

### 2.2 Materials

The main ingredients used are samples of cracker industrial wastewater, *Chlorella* sp., EM4, Walne fertilizer, distilled water, O<sub>2</sub> reagent, MnSO<sub>4</sub>, H<sub>2</sub>SO<sub>4</sub>, PP indicator, NaoH, signette, Nessler, SnCl<sub>2</sub> solution. The tools used in this study were jirrigation, pH meter, DO meter, thermometer, 2.5 L jars, aerator, microscope, haemocytometer, 40 Watt TL lamp, winkler bottle, erlenmeyer, incubator, burette, dropper pipette, test tube, spectrophotometer.

### 2.3 Research Methods

#### 2.3.1 Container Preparation

**The container** to be used **was sterilized** by washing, then sprayed with 70% alcohol.

#### 2.3.2 Cultivation *Chlorella* sp.

Initial inoculant *Chlorella* sp. got from one *e-commerce* who came from Depok City. Calculation of stock density is carried out at *haemocytometer* and observed using a microscope with a magnification of 40 times with 3 repetitions. Density calculation formula *Chlorella* sp. method is used *Small block* (Satyantini et al., 2012).

$$D = \frac{nA + nB + nC + nD + nE}{5(4 \times 10^{-6})}$$

D : density *Chlorella* sp. (sel/ml)  
 $nA + nB + nC + nD + nE$  : amount of *Chlorella* sp. in boxes A, B, C, D and E  
5 : number of squares counted  
 $4 \times 10^{-6}$  : small box area A, B, C, D, dan E

According to Ekawati (2005), to determine the volume of stock culture added to the culture media, the following formula can be used:

$$V_1 = \frac{V_2 \times N_2}{N_1}$$

$V_1$  : Inoculant volume for initial stocking (ml)  
 $N_1$  : Inoculant stock density of *Chlorella* sp. (sel/ml)  
 $V_2$  : Volume of culture media to be used (ml)  
 $N_2$  : *Chlorella* sp. inoculant density required (sel/ml)

Cultivation is done by adding *Chlorella* sp. and distilled water whose volume is adjusted to a 1 L sterile container of 12 pieces. Walne fertilizer was added [20]. The culture was carried out until *Chlorella* sp. reach a population peak. This culture must be given light in the form of a 40 watt fluorescent lamp and provided with aeration so that the algae seeds can carry out photosynthesis and continue to reproduce [10].

### **2.3.3 Liquid Waste Sampling**

Samples of cracker industry wastewater were taken from settling ponds at the Cracker Factory X, Indramayu Regency. Liquid waste samples were taken as much as 20 liters using a black jerrycan. In situ measurements of temperature, DO, and pH of the cracker liquid waste samples were carried out. Furthermore, the cracker industry liquid waste was brought to the Aquatic Resources Laboratory of FPIK UNPAD to be analyzed for levels of BOD<sub>5</sub>, Ammonia and Phosphate before adding *Chlorella* sp. and EM4.

### **2.3.4 Treatments**

The research design used was a completely randomized design (CRD) consisting of 5 treatments and 3 replications. As much as 1 liter of cracker industry liquid waste is put into the treatment container. Then addition of *Chlorella* sp. refers to [14] namely by adding *Chlorella* sp. which was harvested at the peak of the population as much as 800 ml with a cell density of  $11.8 \times 10^6$  cells/ml as well as the addition of EM4 according to the treatment into the cracker industrial wastewater. Then left for 7 days. BOD<sub>5</sub>, CO<sub>2</sub>, Ammonia, Phosphate, pH, and Temperature were analyzed [16].

P<sub>A</sub>: *Chlorella* sp. 800 ml (cell density  $11.8 \times 10^6$  cells/ml) and 1L of cracker industry wastewater without EM4

P<sub>B</sub>: *Chlorella* sp. 800 ml (cell density  $11.8 \times 10^6$  cells/ml) and 1L of cracker industrial wastewater + 10 ml of EM4

P<sub>C</sub>: *Chlorella* sp. 800 ml (cell density  $11.8 \times 10^6$  cells/ml) and 1L of cracker industrial wastewater + 15 ml of EM4

P<sub>D</sub>: *Chlorella* sp. 800 ml (cell density  $11.8 \times 10^6$  cells/ml) and 1L of cracker industrial wastewater + 20 ml of EM4

P<sub>E</sub>: *Chlorella* sp. 800 ml (cell density  $11.8 \times 10^6$  cells/ml) and 1L of cracker industrial wastewater + 25 ml of EM4

## **2.4 Parameters Observation**

The remediation process was carried out for 7 days. Parameters measured to analyze water quality are BOD<sub>5</sub>, DO, CO<sub>2</sub>, Ammonia, Phosphate, pH, and Temperature. Parameters DO, CO<sub>2</sub>, pH, and temperature were observed every day for 7 days. While BOD<sub>5</sub>, ammonia, and phosphate were observed on days 0 and 7. Then the biological parameters in the form of calculating the density of *Chlorella* sp. carried out until the 7th day. The formula for calculating the density of *Chlorella* sp. The method used is the Small block method.

## **2.5 Data Analysis**

The test results for BOD<sub>5</sub>, ammonia, phosphate and the density of *Chlorella* sp. in this study were analyzed statistically using Analysis of Variance (ANOVA). If the data obtained is  $F_{count} \geq F_{table}$  then a further test is carried out with the Duncan's New Multiple Range Test (DNMRT) at the 5% level. In addition, water quality data in the form of DO, CO<sub>2</sub>, pH, and temperature were analyzed using descriptive analysis.

## **3. RESULTS AND DISCUSSION**

### **3.1 Characteristics of Cracker Industry Liquid Waste**

The characteristics of the cracker industrial wastewater at the Cracker Factory X before treatment are shown in Table 1.

**Table 1. Characteristics of Cracker Industry Liquid Waste at the Cracker Factory X**

Observation Parameters	Test Results	Quality standards*	Unit
BOD <sub>5</sub>	910	3*	mg/L
DO	1,7	4*	mg/L
CO <sub>2</sub>	176	< 5**	mg/L
Amonia	12,97	0,2*	mg/L
Phospate	1,58	0,2*	mg/L
pH	6	6-9*	
Temperature	29,3	DEV 3*	°C

\*PP RI No. 22 of 2021 concerning the implementation of environmental protection and management in class II for fishing activities

\*\* Effendi 2003 for the benefit of fisheries

From Table 1. it can be seen that the high pollutant content of the cracker industrial wastewater, there are 5 parameters that show that it is not in accordance with the quality standards. The results of the characterization of the cracker industrial wastewater at the Cracker Factory X, Indramayu Regency, indicates that the liquid waste is not feasible if it is discharged directly into the waters because it can pollute the waters.

### 3.2 The Density of *Chlorella* sp.

Fig 1. Shows that the average cell density increased during the 7 days of observation. The increase in cells indicates that the microalgae *Chlorella* sp. can adapt and grow in the cracker industry liquid waste. According to Afifah et al., (2020) microalgae need nutrients such as N-ammonia, N-nitrate, N-nitrite, phosphate and other inorganic nutrients in the culture media used for growth.

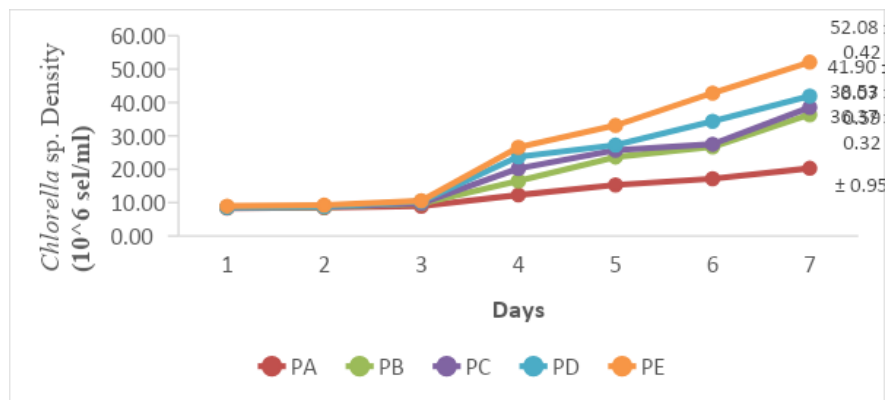


Fig 1. Daily Density *Chlorella* sp.

Cell density on P<sub>A</sub> (Control) showed an increase in cells that were not as much as in the treatment with the addition of EM4. The treatment of P<sub>E</sub> (EM4 25 ml) with the addition of more EM4 concentrations will help degrade organic matter into inorganic materials, which can then be utilized by *Chlorella* sp. for cell development. The more the number of bacteria, the more simple inorganic compounds such as carbon, nitrogen and phosphorus are produced from bacterial activity which will then be directly utilized by microalgae. So that increasing the number of bacteria will also increase the number of microalgae [15]. In addition, the microorganisms contained in EM4 are able to inhibit the growth of pathogenic microorganisms by releasing antibiotic compounds.

Based on the results of the Anova test, it was found that F count (1305.34) > F table (3.48), then H<sub>0</sub> was rejected and H<sub>1</sub> was accepted. This shows that the addition of different EM4 has a significantly different effect on the density of *Chlorella* sp. In this study, the treatment of P<sub>E</sub> (EM4 25 ml) is the best treatment in increasing the cell density *Chlorella* sp. because the number of cells on the last day of observation on the 7th day resulted in the highest density of 52.08 ± 0.42 x 10<sup>6</sup> sel/ml.

### 3.3 Biochemical Oxygen Demand (BOD<sub>5</sub>)

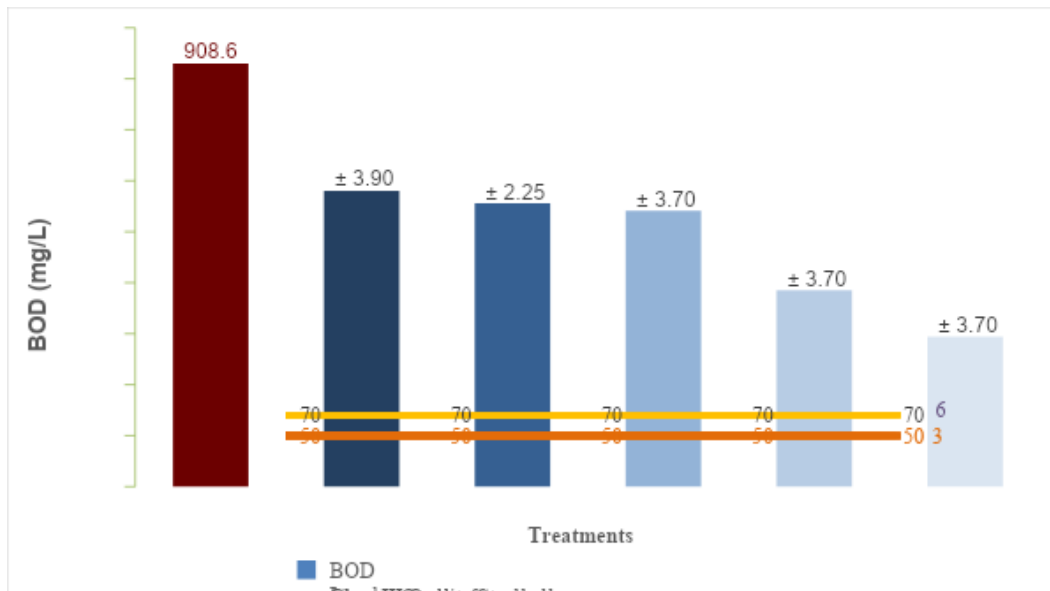


Fig 2. BOD<sub>5</sub> Value

Fig 2. shows that the value of BOD<sub>5</sub> will be lower with the addition of more and more EM4. In the treatment of P<sub>A</sub> (Control) BOD value<sub>5</sub> can be reduced although not as much as the treatment with the addition of EM4, this is due to the lack of microorganisms that can decompose the cracker industrial wastewater. According to [21], microorganisms in liquid waste use oxygen molecules to oxidize organic matter. The process of photosynthesis is carried out *Chlorella* sp. will produce oxygen that can be used by microorganisms in EM4 to help the decomposition process of organic matter. There is a cooperation between *Lactobacillus* sp. *Saccharomyces* sp. contained in EM4 can ferment organic matter into lactic acid compounds [18]. In addition, the help of protease enzymes from various types of microbes found in EM4 plays a role in the process of breaking down proteins into ammonia, nitrites, nitrates, and CO<sub>2</sub> [18]. BOD value<sub>5</sub> in each treatment is still high and still very far from the quality standard

Based on the results of the Anova test, it was found that F count (959.11) > F table (3.48), then H<sub>0</sub> was rejected and H<sub>1</sub> was accepted. This shows that the addition of different EM4 has a significantly different effect on the reduction of BOD<sub>5</sub>. In this study, the treatment of P<sub>E</sub> (EM4 25 ml) is the best treatment in reducing the BOD<sub>5</sub> content in the cracker industry wastewater due to a decrease in BOD values<sub>5</sub> produced has the greatest percentage of 83.81% with a value BOD<sub>5</sub> 147 ± 3.70 mg/L, although it still does not meet the quality standard.

### 3.3 Dissolved Oxygen (DO)

Table 2. Average Daily DO During the Study

The increase in DO value is thought to be due to the large supply of oxygen from the photosynthesis process *Chlorella* sp.. Table 2. shows that the more EM4 is added, the resulting DO value will increase. The highest DO value was found in the P treatment<sub>AND</sub> with a DO content of 5.9 ± 0.1 mg/L.

Treatments	DO (mg/L)						
	D+1	D+2	D+3	D+4	D+5	D+6	D+7
P <sub>A</sub> (Control)	1,8 ± 0,1	1,9 ± 0,0	2 ± 0,1	2,2 ± 0,1	2,3 ± 0,0	2,5 ± 0,0	3,1 ± 0,1
P <sub>B</sub> (EM4 10 ml)	1,9 ± 0,0	2,4 ± 0,0	2,7 ± 0,1	3 ± 0,0	3,1 ± 0,1	3,6 ± 0,1	3,8 ± 0,0
P <sub>C</sub> (EM4 15 ml)	2 ± 0,0	2,8 ± 0,1	3,5 ± 0,0	3,6 ± 0,1	4,1 ± 0,0	4,3 ± 0,1	4,8 ± 0,0
P <sub>D</sub> (EM4 20 ml)	2,2 ± 0,0	3,2 ± 0,1	3,9 ± 0,0	4,1 ± 0,1	4,5 ± 0,0	4,9 ± 0,0	5,3 ± 0,0
P <sub>E</sub> (EM4 25 ml)	2,4 ± 0,0	3,7 ± 0,1	4 ± 0,0	4,2 ± 0,0	4,8 ± 0,1	5,3 ± 0,0	5,9 ± 0,1

Photosynthesis process *Chlorella* sp. assisted by bacteria on EM4. The important role of bacteria contained in EM4 can produce simple compounds such as CO<sub>2</sub>, nitrogen and phosphorus that are easily absorbed by *Chlorella* sp.. In line with [16], the more CO<sub>2</sub> The resulting product can support the process of photosynthesis *Chlorella* sp. the end product is oxygen. This is what causes the DO value to increase from the beginning of the liquid waste test until the last observation on the 7th day which indicates that the water quality is getting better.

### 3.4 Carbondioxide (CO<sub>2</sub>)

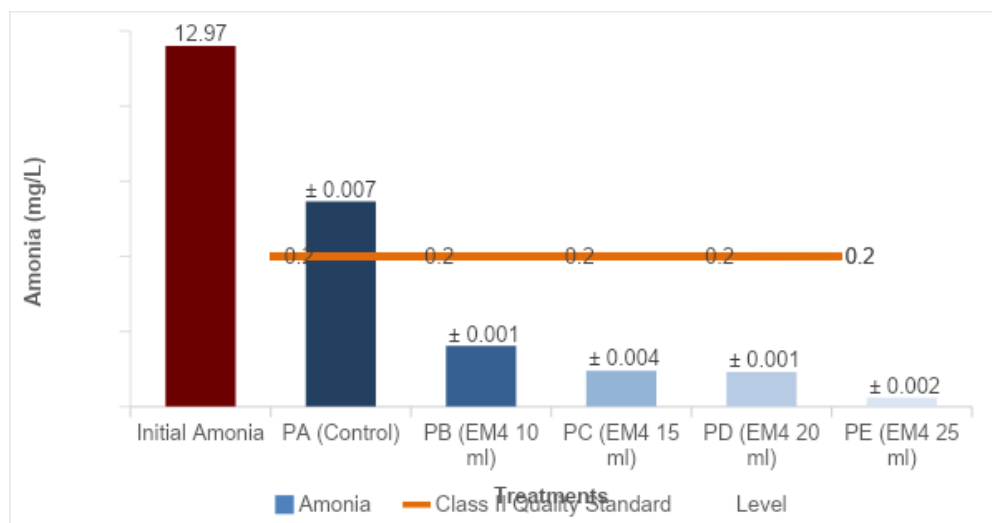
**Table 3. Average Daily CO<sub>2</sub> During the Study**

CO<sub>2</sub> concentration experienced a decrease in value in all treatments. Table 3. shows that the value of CO<sub>2</sub> will be lower with the addition of higher EM4. CO<sub>2</sub> value on the treatment of P<sub>D</sub> (EM4 20 ml) and P<sub>E</sub> (EM4 25 ml) on the last day of observation, namely 4.9 ± 0.2 mg/L and 3.9 ± 0.7 mg/L, so that it is in accordance with the levels of carbon dioxide (CO<sub>2</sub>) which is intended for fishing activities which should be less than 5 mg/L [6]. If more than that is very dangerous because it inhibits the binding of oxygen.

Perlakuan	CO <sub>2</sub> (mg/L)						
	H+1	H+2	H+3	H+4	H+5	H+6	H+7
P <sub>A</sub> (Kontrol)	170,1 ± 5,1	105,6 ± 0,0	88 ± 0,0	43,4 ± 1,0	33,7 ± 2,5	17,6 ± 0,0	10 ± 0,0
P <sub>B</sub> (EM4 10 ml)	132 ± 0,0	100,3 ± 1,6	61,6 ± 0,0	41,1 ± 5,1	20,8 ± 1,0	13,2 ± 0,0	8,6 ± 0,0
P <sub>C</sub> (EM4 15 ml)	108,5 ± 5,1	88 ± 0,0	48,4 ± 4,4	26,4 ± 0,0	19,6 ± 2,2	8,8 ± 0,0	6,1 ± 0,0
P <sub>D</sub> (EM4 20 ml)	99,7 ± 5,1	83,6 ± 4,4	36,7 ± 2,5	22,9 ± 1,5	7,9 ± 0,0	7 ± 0,0	4,9 ± 0,2
P <sub>E</sub> (EM4 25 ml)	88 ± 0,0	73,3 ± 5,1	41,3 ± 1,5	23,5 ± 2,5	8,8 ± 0,0	5 ± 0,3	3,9 ± 0,7

Reduction of CO<sub>2</sub> in each treatment occurs because *Chlorella* sp. uses CO<sub>2</sub> as the main carbon source to synthesize new cells and release O<sub>2</sub> through the mechanism of photosynthesis. In treatment with the addition of EM4 can reduce CO<sub>2</sub> more than without the addition of EM4 because there is a symbiosis between *Chlorella* sp. and EM4. Dissolved oxygen is generated by *Chlorella* sp. from the photosynthesis process will be used by bacteria in EM4 to oxidize organic matter in the cracker industrial wastewater to produce simple compounds such as CO<sub>2</sub> which can support the process of photosynthesis *Chlorella* sp., this will speed up the process of decomposing pollutants in waste [16]. [15] that increasing the number of bacteria will also increase the density of microalgae which affects the reduction of CO<sub>2</sub>.

### 3.5 Ammonia (NH<sub>3</sub>)



**Fig 3. Ammonia Value**

$P_A$  treatment (Control) without the addition of EM4 can still reduce the value of ammonia because ammonia compounds are needed by microalgae for growth. In accordance with Mawaddah (2016), ammonia is the main source of nitrogen compounds needed by microalgae for metabolic processes and the formation of new cells. Based on Fig 3. that by administering EM4 at various doses is able to reduce ammonia in wastewater and the more EM4 added, the greater the decrease in ammonia levels. The collaboration of *Lactobacillus* sp. with *Saccharomyces* sp. can ferment organic matter into simpler compounds so that it tends to decompose organic matter more quickly.

In addition, the decreased ammonia levels occurred due to the presence of *Rhodopseudomonas* bacteria on EM4. [8] suggested that in addition to being able to carry out photosynthetic processes, *Rhodopseudomonas* bacteria also use ammonia as an energy source and form cells in the nitrification process. The increase in dissolved oxygen obtained from the photosynthesis of *Rhodopseudomonas* and *Chlorella* sp. will increase the oxidation process of ammonia thereby lowering ammonia levels. Lower ammonia levels are followed by reduced odor in liquid waste due to the activity of *Rhodopseudomonas* bacteria [7].

Based on the results of the Anova test, it was found that F count (2413.00) > F table (3.48), then  $H_0$  was rejected and  $H_1$  was accepted. This shows that the addition of different EM4 has a significantly different effect on the reduction of ammonia. In this study, the  $P_E$  treatment (EM4 25 ml) was the best treatment in reducing the ammonia content in the cracker industrial wastewater because the reduction in the value of ammonia produced had the greatest value of 99.91% with an ammonia value of  $0.012 \pm 0.002$  mg/L.

### 3.6 Phosphate (PO4)

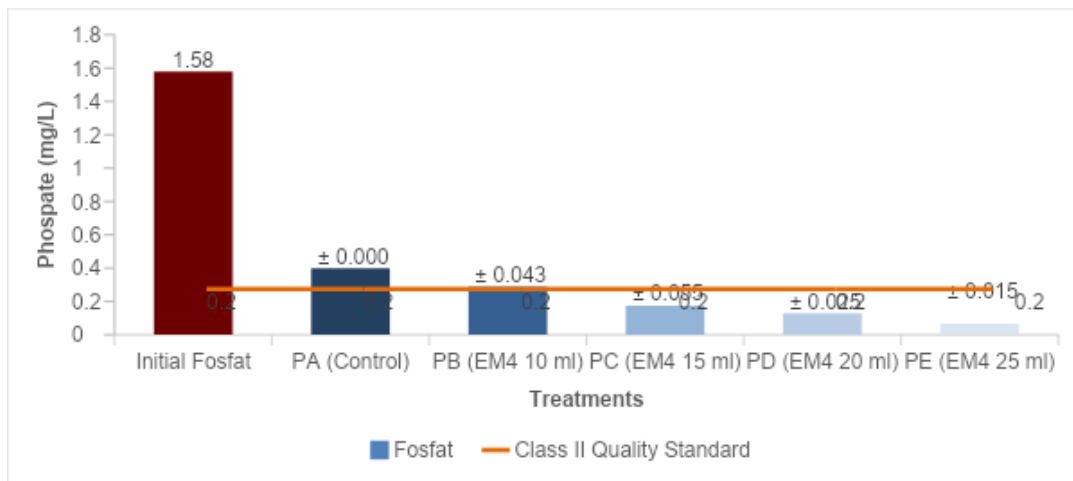


Fig. 4 Phosphate Value

Based on the measurement of phosphate after 7 days the phytoremediation process decreased in value in all treatments. Fig 4. shows that the phosphate value will be lower with the addition of higher EM4. In the treatment with the addition of EM4, the decrease in phosphate concentration in the cracker industrial wastewater occurred due to the mutualistic symbiosis of *Chlorella* sp. with bacteria on EM4. [21] the presence of EM4 which contains phosphate solubilizing bacteria will help the process of dissolving organic phosphate so that it can produce higher levels of phosphorus so that it is easily absorbed by microalgae. The high level of absorption of phosphate levels in all treatments was followed by a high population of *Chlorella* sp. According to [3] phosphate is often considered a limiting factor because phosphate is very much needed in the transfer of P within the cells of organisms for cell division and formation of biomass.

Based on the results of the Anova test, it was found that F count (27.47) > F table (3.48), then  $H_0$  was rejected and  $H_1$  was accepted. This shows that the addition of different EM4 has a significantly different effect on the decrease in phosphate values. In this study, the  $P_E$  treatment (25 ml EM4) was the best treatment in reducing the phosphate content in the cracker industrial wastewater because the reduction in the resulting phosphate value had the greatest percentage of 95.76% with a phosphate value of  $0.067 \pm 0.015$  mg/L.

### 3.7 Potential of Hydrogen (pH)

Table 4. Average Daily pH During the Study

The pH value in each treatment has increased and the pH value is still within the quality standard. The average final pH value on the 7th day was 7.0 - 8.2. The degree of acidity (pH) in each treatment increased in line with the number of additions of EM4.

Treatments	pH						
	D+1	D+2	D+3	D+4	D+5	D+6	D+7
P <sub>A</sub> (Control)	6 ± 0,0	6,5 ± 0,0	6,5 ± 0,1	6,6 ± 0,0	6,7 ± 0,1	6,7 ± 0,0	7 ± 0,1
P <sub>B</sub> (EM4 10 ml)	6,5 ± 0,0	6,6 ± 0,0	6,7 ± 0,0	6,9 ± 0,0	7 ± 0,1	7,1 ± 0,0	7,3 ± 0,1
P <sub>C</sub> (EM4 15 ml)	6,6 ± 0,0	6,7 ± 0,0	6,8 ± 0,1	6,9 ± 0,0	7,1 ± 0,1	7,2 ± 0,0	7,6 ± 0,2
P <sub>D</sub> (EM4 20 ml)	6,7 ± 0,1	6,8 ± 0,0	6,9 ± 0,0	7,1 ± 0,0	7,2 ± 0,0	7,3 ± 0,1	7,8 ± 0,1
P <sub>E</sub> (EM4 25 ml)	7,1 ± 0,6	6,9 ± 0,0	7,2 ± 0,1	7,4 ± 0,0	7,5 ± 0,0	7,7 ± 0,0	8,2 ± 0,3

The pH value in the P<sub>A</sub> without the addition of EM4 also increases, this proves that the process of photosynthesis *Chlorella* sp. can increase the pH value of liquid waste. In the treatment with the addition of EM4, the pH experienced a higher increase. The more organic matter that is broken down by bacteria, the more CO<sub>2</sub> is generated and then used *Chlorella* sp. for the process of photosynthesis. CO<sub>2</sub> absorption by microalgae causes a decrease in CO<sub>2</sub> concentration and results in an increase in the pH value [16].

### 3.7 Temperature

After the phytoremediation treatment was carried out for 7 days, there was no significant change in temperature in all treatments. Optimum temperature for the growth of *Chlorella* sp. which ranges from 25-30°C [2]. An increase of every 1°C from the ideal range can reduce processing efficiency, resulting in an increase in the toxicity of pollutants to aquatic organisms.

**Table 5. Average Daily Temperature During the Study**

Treatments	Temperature (°C)						
	D+1	D+2	D+3	D+4	D+5	D+6	D+7
P <sub>A</sub> (Control)	27,5 ± 0,2	27,8 ± 0,1	28 ± 0,0	28 ± 0,2	28,1 ± 0,1	28,1 ± 0,1	28,2 ± 0,1
P <sub>B</sub> (10 ml)	28,2 ± 0,2	28,3 ± 0,1	28,2 ± 0,2	28,2 ± 0,3	28,5 ± 0,1	28,5 ± 0,1	28,6 ± 0,0
P <sub>C</sub> (15 ml)	28,2 ± 0,1	28,4 ± 0,1	28,3 ± 0,1	28,3 ± 0,1	28,5 ± 0,1	28,5 ± 0,1	28,6 ± 0,1
P <sub>D</sub> (20 ml)	28,4 ± 0,2	28,5 ± 0,2	28,6 ± 0,2	28,4 ± 0,3	28,5 ± 0,4	28,5 ± 0,3	28,5 ± 0,2
P <sub>E</sub> (25 ml)	28,6 ± 0,2	28,6 ± 0,3	28,5 ± 0,3	28,5 ± 0,3	28,6 ± 0,3	28,5 ± 0,1	28,8 ± 0,1

Daily temperature in this study ranged from 27.5 – 28.8 °C, which means that the temperature in each treatment was included in the ideal temperature for the phytoremediation process, which was no more than 30°C. Therefore, microalgae and bacteria can reproduce well so that they can carry out the process of photosynthesis and are able to absorb contaminants properly

### 3.8 Best Treatments

Cracker industry liquid waste that has been treated with a phytoremediation process for 7 days using *Chlorella* sp. 800 ml (cell density 11.8 x 10<sup>6</sup> cells/ml) and EM4 at various doses, there was an improvement in water quality in all parameters. The results of the recapitulation of changes in all parameters in each treatment are presented in Table 5.

**Table 6. Results of recapitulation of changes in all parameters in each treatment**

Parameter	Initial Value	quality standard	Treatments				
			P <sub>A</sub>	P <sub>B</sub>	P <sub>C</sub>	P <sub>D</sub>	P <sub>E</sub>
Kepadatan	11,8	-	22,28 ±	36,37 ± 0,32	38,53 ± 0,59	41,90 ±	52,08 ±

<i>Chlorella</i> sp. (10 <sup>6</sup> sel/ml)			<b>0,95</b>			<b>0,07</b>	<b>0,42</b>
BOD <sub>5</sub> (mg/L)	910	3*	290,1 ± 3,9	277,8 ± 2,25	270,5 ± 3,7	192,5 ± 3,7	147,07 ± 3,7
DO (mg/L)	1,7	4*	3,1 ± 0,1	3,8 ± 0,0	<b>4,8 ± 0,0</b>	<b>5,3 ± 0,0</b>	<b>5,9 ± 0,1</b>
CO <sub>2</sub> (mg/L)	176	< 5**	10 ± 0,0	8,6 ± 0,0	6,1 ± 0,0	<b>4,9 ± 0,2</b>	<b>3,9 ± 0,7</b>
Amonia (mg/L)	12,97	0,2*	0,273 ± 0,007	<b>0,081 ± 0,001</b>	<b>0,048 ± 0,004</b>	<b>0,046 ± 0,001</b>	<b>0,012 ± 0,002</b>
Fosfat (mg/L)	1,58	0,2*	0,4 ± 0,0	0,288 ± 0,043	<b>0,175 ± 0,055</b>	<b>0,128 ± 0,025</b>	<b>0,067 ± 0,015</b>
pH	6	6-9*	<b>7 ± 0,1</b>	<b>7,3 ± 0,1</b>	<b>7,6 ± 0,2</b>	<b>7,8 ± 0,1</b>	<b>8,2 ± 0,3</b>
Suhu (°C)	29,3	DEV 3*	<b>28,2 ± 0,1</b>	<b>28,6 ± 0,0</b>	<b>28,6 ± 0,1</b>	<b>28,5 ± 0,2</b>	<b>28,8 ± 0,1</b>

The number in bold indicates meeting the quality standard

\*PP RI No. 22 of 2021 concerning the implementation of environmental protection and management in class II for fishing activities

\*\* Effendi 2003 for the benefit of fisheries

Table 6. Shows that only the BOD<sub>5</sub> whose value is still very far above the quality standard in all treatments, while for other parameters the value already meets the set quality standard. The more EM4 added, the more pollutant levels in the waste will be reduced. P<sub>E</sub>(EM4 25 ml) is the best treatment because it can reduce the highest pollutant levels.

#### 4. CONCLUSION

Based on the research results, it can be concluded that the addition of *Chlorella* sp. 800 ml (11,8 x 10<sup>6</sup> cells/ml) with the addition of 25 ml of EM4 is the best concentration in reducing the pollutant content of the cracker industrial wastewater. P<sub>E</sub> treatment (EM4 25 ml) has a percentage decrease in BOD values<sub>5</sub> of 83.82%, Ammonia of 99.91%, Phosphate of 95.78%, CO<sub>2</sub> by 97.78%, and an increase the density of *Chlorella* sp. to 52.08 x 10<sup>6</sup>, DO from 1.7 mg/L to 5.9 mg/L, pH to 8.2, and temperature to 28.8°C. But the BOD<sub>5</sub> is still very far above the quality standard so that it does not meet the quality standard according to Government Regulation No. 22 of 2021 Class II and Class III concerning the implementation of environmental protection and management for fishing activities.

#### ACKNOWLEDGEMENTS

The author expresses deep gratitude to Prof. Dr. Ir. Zahidah., M.S, Dr. Asep Agus Handaka Suryana, S.Pi., MT., and Heti Herawati, S.Pi., MP. who have helped and guided in this research so that it can provide benefits to all parties.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

#### REFERENCES

1. Abdulgani, H., & Izzati, M. (2014). *The Ability of Typha Angustifolia Plants in the Subsurface Flow Constructed Wetland System for Processing Liquid Waste for the Cracker Industry (Case Study of Liquid Waste from the Cracker Industry Sentra, Kenanga Village, Sindang District, Indramayu Regency, West Java)*. 16(1), 90–101.
2. Aprilliyanti, S., Soeprobawati, R. T., & Yulianto, B. (2016). Relationship of *Chlorella* sp. Abundance With the Quality of the Aquatic Environment on a Semi Mass Scale at BBBPBAP Jepara. *Journal of Environmental Science*, 14(2), 77–81.
3. Buwono, N. R., & Nurhasanah, R. Q. (2018). Population Growth Study of *Spirulina* sp. at Different Culture Scales. *Fisheries and Maritime Scientific Journal*. 10(1), 1–8. <https://doi.org/10.20473/jipk.v10i1.8202>
4. Channey, R., SL, B., YM, L., JS, A., C, H., & Green. (1995). Potential use of metal hyperaccumulators (Vol. 3, Issue 3). *Mining Environmental Management*.

5. Department of Fisheries and Maritime Affairs of Indramayu Regency. (2010). Fisheries Production in Indramayu Regency
6. Effendi, H. (2003). *Water Quality Review*. Yogyakarta: Kanisius.
7. Firmaniar, E. (2017). *The Effect of Giving a Mixture of EM4, Sugarcane Drops and Tofu Liquid Waste as Liquid Organic Fertilizer on the Growth of Red Spinach Plants*. Sanata Dharma University.
8. Higa, T., & Parr, J. F. (1997). *Effective Microorganism (EM-4) untuk Pertanian dan Lingkungan yang Berkelanjutan. Indonesian Kyusei Nature Farming Societies*, Jakarta.
9. Istirokhatun, T., Aulia, M., & Utomo, S. (2017). Potential *Chlorella* Sp. to Remove COD and Nitrate in Tofu Liquid Waste. *Journal of Precipitation: Communication Media and Environmental Engineering Development*, 14(2), 88–96.
10. Jusadi, D. (2003). *Freshwater Natural Feed Cultivation: Chlorella sp Cultivation (I. Mokoginta, Ed.; Vol. 6)*. Directorate of Education. Directorate General of Primary and Secondary Education Ministry of National Education.
11. Kusrina, R. (2011). *Feasibility Analysis of Cracker Processing Business, Cap Dua Gajah Cracker Company, Indramayu, West Java*. in Thesis. Bogor Agricultural Institute.
12. Lananan, F., Abdul Hamid, S. H., Din, W. N. S., Ali, N., Khatoon, H., Jusoh, A., & Endut, A. (2014). Symbiotic bioremediation of aquaculture wastewater in reducing ammonia and phosphorus utilizing Effective Microorganism (EM-1) and microalgae (*Chlorella* sp.). *International Biodeterioration and Biodegradation*, 95, 127–134.
13. Meriatna, M., Suryati, S., & Fahri, A. (2018) Effect of Fermentation Time and Volume of EM4 (Effective Microorganism) Bio Activator on the Production of Liquid Organic Fertilizer (POC) from Fruit Waste. *Unimal Chemical Technology Journal*, 7(1), 13–29. <https://doi.org/10.29103/jtku.v7i1.1172>
14. Pasaribu, J., Restuhadi, F., & Zalfiatri, Y. (2018). Symbiosis of Mutualism of Microalge *Chlorella* sp with B-DECO3 Decomposing Bacteria in Reducing Pollutant Levels of Sago Liquid Waste *Chlorella* sp. *JOM Faperta*, 5(1).
15. Putri, R., Slamet, A., & Hermana, J. (2014). The Effect of Adding Glucose as a Co-substrate in Diesel Fuel Oil Wastewater Treatment Using a High Rate Algae Reactor System. *Journal of Engineering Pomits, Jurnal Teknik Pomits*, 3(2), 94–97.
16. Restuhadi, F., Zalfiatri, Y., & Aji Pringgondani, D. (2017). Symbiotic Mutualism of Starbact® and Microalgae *Chlorella* sp. in Reducing Pollution from Sago Milling Effluent. *Journal of Environmental Science*, 11(2), 140–153.
17. Saputra, A. D., Haeruddin, & Widyorini, N. (2016). The Effectiveness of the Combination of Microorganisms and Aquatic Plants *Lemna Minor* as a Bioremediator in Reducing Ammonia, Nitrite, and Nitrate Compounds in Fish Washing Waste. *MAQUARES*, 5(3), 80–90.
18. Sari, K. L., Ali As, Z., & Hardiono. (2017). Penurunan Kadar BOD, COD dan TSS Pada Limbah Tahu Menggunakan Effective Microorganism-4 (EM4) Secara Aerob. *Jurnal Kesehatan Lingkungan*, 14(1), 450–458.
19. Sari, S. A., Nurhayati, & Sunaryanto, R. (2022). THE EFFECT OF THE ADDITION OF EFFECTIVE MICROORGANISMS (EM-4) ON THE QUALITY OF TOFU LIQUID WASTE USING AERATION TECHNIQUE. *Metrics Serial Technology And Science*, 3(1), 36–41.
20. Simatupang, D., Restuhadi, F., & Dahril, T. (2017). Utilization of *Chlorella* sp and EM4 Microalgae Symbiosis to Reduce Pollutant Levels of Sago Liquid Waste. *Jom FAPERTA*, 4(1), 1–13.
21. Widjaja, T. (2012). *Industrial Waste Treatment (Biological Process)*.
22. Widyabudiningsih, D., Troskialina, L., Fauziah, S., Siti Djenar, N., Hulupi, M., Indrawati, L., Fauzan, A., & Abdilah, F. (2021). *Production and Testing of Liquid Organic Fertilizer from Fruit Peel Waste with the Addition of EM4 Bioactivator and Variation of Fermentation Time*. *ind. J. Chem. Anal*, 04(01), 30–39. <https://doi.org/10.20885/ijca.vol4.iss1.art4>