Whole Effluent Toxicity (WET) Test On Microalgae Reactor and Acute Toxicity on *Daphnia* sp.

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Abstract. Processing using alga reactor was made one of the solutions in wastewater treatment. Symbiotic relationship of Algal-bacteria increases the potency of processing wastewater. These capabilities can be enhanced by compounding CO2 in photosynthesis in processing wastewater. WET (Whole Effluent Toxicity) testing is needed to monitor the effects of toxicity of algae reactor. The research was carried out using waste of Water Waste Management Installations in Sewon, Yogyakarta with supplying 0.2 L/min and 0.5 L/min of CO2. This research aims to know the LC50 and analyze the characteristic relationship of waste water as the results of algae reactor process. Acute toxicity test is performed with a static method within 24 hours using animal test *Daphnia* sp. on day 1, 6 and 13. Based on the analysis of the characteristics of waste are known that DO, pH, temperature, light intensity strongly influenced the death of *Daphnia* sp. Based on the results of the analysis of algae-bacteria, the best result came with the addition of CO2 amounting to 0.2 L/min. While the worst occurred at reactor Algae.

1. Introduction

The combination of microalgae and bacteria in wastewater treatment is an alternative technology (Posadas et al, 2013). Algae reactors is used as one of the solutions in wastewater treatment. In addition to photosynthesis, microalgae can absorb nitrogen and phosphorus and produce oxygen that is used by bacteria so that it can reduce wastewater parameters [2].

Testing the toxicity of wastewater in Indonesia are often made. However, it still focuses on one or more of the pollutant content in wastewater. Monitoring overall treatment wastewater to determine the total effect of waste on organisms by the Whole Effluent Toxicity (WET) method [3]. WET testing aims to identify, track and monitor sources of toxicity in domestic or industrial waste streams [4].

The high population growth in Yogyakarta, causing the burden of waste water entering the Sewon WWTP is getting higher. Meanwhile, the capacity of WWTP Sewon is 15,500 m3 / day so special attention is needed. This study aims to analyze the effects of sewon wastewater effluent on aquatic organisms using the WET method. WET testing (Whole Effluent Toxicity) makes it possible to calculate the source of toxicity and show that poisons that expose aquatic organisms may become less or more toxic due to certain characteristics of wastewater [5].

Leachate toxicity testing using *Daphnia* sp was carried out because it has a high reproductive rate and is one of the components in ecology that has a high sensitivity to contaminants [6]. *Daphnia* sp. is a freshwater organism that is associated with major producers and highest trophic organisms. Contaminants that affect *Daphnia* sp. can affect the ecosystem. The use of *Daphnia* sp. in toxicology testing carried out in several countries to monitor the treatment of lymphatic water and determine the quality standards that will be set [7].

2. Method and materials

2.1. Sampling site
Samples were collected from microalgae reactors on days 1, 6, and 13. The microalgae used in this study was chlorella sp. With four reactors such as algae, algae-bacterial, algae bacterial with injection CO2 0.2 L/s and algae bacterial with injection CO2 0.5 L/s. Acute toxicity tests were analyzed at the Biotechnology Laboratory of the Islamic University of Indonesia and algae reactors were located in the Hazardous Waste Room, Sewon Laboratory with open (roofed) conditions exposed to direct sunlight. Samples were collected from April 3, 2017 to April 21, 2017.

2.2. Culture and acclimatization Daphnia sp.
Daphnia sp. obtained from the PASTY Animal Market and cultured for 3 weeks with the aim of producing good broodstock in the number and physical condition of the test animals. Daphnia sp. placed in a beaker measuring 200 ml, with stable environmental conditions, namely at an optimal temperature of 25 ± 1 °C, DO at least 4 mg / l and pH 7 [3]. Feeding is given five times a week with 10 ml of FERMIPAN brand yeast. All experiments are carried out in a place that is not exposed to direct sunlight and the replacement of culture water is carried out every 2 weeks or according to needs/conditions. Subcultures are carried out by removing the parent daphnia sp. ready to reproduce into a 100 ml beaker glass. at this stage of subculture, neonate Daphnia sp. who are less than 24 hours old [8][3].

2.3. Culture media
The freshwater solution used in this study contained 80-100 mg CaCO3 / l, 60-70 mg / l alkalinity, pH 7.6 ± 2 and a conductivity of 300 µc / cm [3]. The material needed to make a freshwater solution of one liter is to dissolve 0.096 grams of NaHCO3, 0.06 grams of CaSO4.2H2O, 0.06 grams of MgSO4.H2O and 0.004 grams of KCl into one liter of distilled water. The solvent and all ingredients mixed with freshwater were aerated first using an aerator before being used in test and control solutions, at least 0.5 hours before used.

2.4. Data Analysis
The median lethal concentration (LC50) was calculated for acute test. The associated confidence generally determined with EPA Probit. The toxic unit was calculated according to the US EPA manual; TU=100/LC50.

3. Results and discussion
3.1. Characteristics of Wastewater Treatment for Algae Reactors
Overall results of the analysis of the parameters of wastewater have decreased. A significant reduction occurred in algae-bacterial reactors with a CO2 gas supply of 0.2 L / min. While in reactors without CO2 supply the decrease is not too significant. This is due to poor microalgae growth in reactors without CO2. The CO2 compound is a component that is very influential in the growth and metabolism of microalgae used for photosynthesis [9].

The level of CO2 supply affects the decrease in pH, seen at pH 0.2 L / min in the normal pH range of 6.3 - 7.8 so that it can increase the efficiency of decreasing wastewater. Whereas at a pH of 0.5 L / min 5.8 - 7.3. According to Shintawati (2011)[10] Chlorella sp. has the optimum pH at pH 7. The optimum pH will help photosynthesis and optimum absorption.

| Rektor | 0 | 1 | 4 |
|--------|---|---|---|
| Table 1. Wastewater parameters with algae reactor |   |   |   |
### Table 1. Wastewater parameters with algae reactor

| Reaktor | 0   | 1   | 4   | 10  |
|---------|-----|-----|-----|-----|
|         | B   | C   | p   | D   | B   | C   | p   | D   | B   | C   | p   | D   |
| Amo     | su  | hu  | D   | H   | O   | Amo | su  | hu  | D   | H   | O   |
|         | 6.6 | 33.3| 30.0| 590.3| 710.7| 710.7| 7.9 | 2.0 | 7.48 | 7.6| 6.8 | 7.29 | 6.6 |

| Alga-bakteri | 0.2 L/m | 2.5 | 33.3 | 30.0 | 590.3 | 710.7 | 710.7 | 7.9 | 2.0 | 7.48 | 7.6| 6.8 | 7.29 | 6.6 |

| Alga-bakteri | 0.5 L/m | 2.5 | 33.3 | 30.0 | 590.3 | 710.7 | 710.7 | 7.9 | 2.0 | 7.48 | 7.6| 6.8 | 7.29 | 6.6 |

### 3.2. Temperature, pH and Light Conditions

The pH value is a factor that determines the ability of microalgae to use nutrients. Chlorella sp. able to adapt to pH ranging from 4.5 to 9.3 [11]. According to [10] Chlorella sp. has the optimum pH at pH 7. The optimum pH will help photosynthesis and optimum absorption.

![Figure 1. Corellation LC50 with pH](image)
The graph shows the temperature is always changing, this condition is caused by changing sun exposure to the reactor. Changes in sun exposure caused by weather factors when the data collection process is done. Temperature is important in describing nutrients and the ability of microalgae to process it[12]. Toxic units in daphnia are not greatly affected by temperature because they are still in optimal condition. In the study of [13], at the best temperature around 30 ± 2 °C. However, at temperatures reaching 35 °C causing a decrease in the number of cells until the death of algae [14].

The highest light intensity occurs in algae and algae-bacterial reactors, because the reactor is directly exposed to sunlight. The peak occurred on the first day, but these conditions fluctuate. Increased mortality of Daphnia sp. at Algae and bacterial Algae reactors are caused by a decrease in the number of cells in the reactor. This is because the level of light intensity at the reactor which is high disrupts Chorella's metabolism in carrying out photosynthesis so that photoinhibition will interfere with CO2 absorption and O2 release [15]. This is due to algae metabolism influenced by light and irradiation time thereby increasing algal productivity.

3.3. Whole Effluent Toxicity (WET) test
On the first day observations showed that the death of Daphnia sp. the highest is in the AB 0.2 reactor. This is because the pH of the AB 0.2 reactor reaches 5.4. Daphnia habitat water quality can vary. Daphnia can adapt to pH between 6.5 and 9.5, and optimal pH between 7.2 to 8.5[16]. On the 6th day observations, overall each reactor experienced an increase in mortality. Increased mortality of Daphnia sp. in the Algae and Alga bacterial reactors caused by a decrease in the number of Chorella cells in the reactor. This is because the level of light intensity at the reactor which is high disrupts Chorella's metabolism in carrying out
photosynthesis so that photoinhibition will interfere with CO₂ absorption and O₂ release[15]. This is due to algae metabolism influenced by light and irradiation time thereby increasing algal productivity [10].

On observation on the 13th day a decrease in the number of mortality occurred at the AB reactor 0.2 L / min. Increasing the removal efficiency of the reactor with a supply of 0.2 L / min because with a CO₂ rate of 0.2 L / min can make the conditions of pH, temperature and chlorophyll-a reach the optimum conditions to remove ammonia, COD and BOD in wastewater. So it can be concluded processing with the addition of 0.2 L / minute CO₂ is better than other reactors.

| Perlakuan | Days | 1 | 6 | 13 |
|-----------|------|---|---|----|
|           | Level of toxicity | LC₅₀ | TU | Level of toxicity | LC₅₀ | TU | Level of toxicity | LC₅₀ | TU |
| Alga      | SA       | 57,62 | 1,74 | SA       | 9,8  | 10,20 | HA       | 2,3  | 43,4 |
| AB 0.5    | SA       | 99,16 | 1,01 | SA       | 59,27| 1,69  | SA       | 14,352| 6,97 |
| AB 0.2    | HA       | 35,35 | 2,83 | SA       | 26,06| 3,84  | SA       | 19,94 | 5,02 |
| NA= No acute toxicity; SA= Significant Acute toxicity; HA= High Acute toxicity; VH= Very High acute toxicity. Classification taken from [17].

![Figure 4. LC₅₀ at reactor algae](image)
The table above shows that the increase in toxicity is higher. On the first day the algae and bacterial algae reactors showed TU values of 1.74 and 1.01 and in reactors with a CO2 supply of 0.5 L / min around 2.83 can be categorized as Significant Acute toxicity. In the supply of 0.2 L CO2 / minute around 7.41 classified as High acute toxicity. The situation is thought to be an increase in ammonia and a decrease in pH and DO significantly cause an increase in mortality in Daphnia sp.

Increasing the value of TU occurred on day 6 of each reactor except in the reactor with CO2 supply of 0.2 L / min. This condition can explain that by supplying CO2 0.2 L / min can increase the removal of COD, BOD and ammonia. Reactor algae and algae bacteria increase is due to an increase in the intensity of light and temperature which causes a decrease in the level of the number of cells and the production of antibacterial substances on Chlorella sp.

It can be concluded that, on the first day of each reactor into the still in Significant Acute toxicity except the CO2 reactor with 0.2 L / min relatively high acute toxicity. The effect of adding CO2 and algae on wastewater treatment results in the final result of the reactor with a CO2 supply of 0.2 L / min, a decline in the value of 2.53 TU can be classified as Significant Acute toxicity.

4. Conclusion
Algae reactor with 0.2 L / min CO2 supply will produce removal efficiency in wastewater parameters such as ammonia, COD and BOD. The Toxic Unit results in the 0.2 L / min CO2 supply are the best among other reactors. Parameters DO, pH, temperature, light intensity affects the ability of algae reactor. Some suggestions to support the future development of this research: to apply the toxicity testing with other wastes and Testing with other test animals.

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