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Detoxification of Glucose, Ammonium and Formaldehyde Using Nitrification and Plant Processes

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ABSTRACT

The purpose of this study was to obtain the removal efficiency values from glucose, ammonium and formaldehyde based on toxicity units. The nitrification process is applied using nitrifying bacteria and the process of plants using water hyacinth. The toxicity tests used were bacterial respiration test and test with batch reactor system for plants. The results show that the EC50 value of formaldehyde was smaller than ammonium and glucose. Formaldehyde was the most toxic substance among these substances, but its detoxification efficiency was high; this may be due to an antagonistic effect (from toxic to less toxic) mixture of substances.

INTRODUCTION

Toxic substances can be either organic or inorganic. These toxic substances have target selectivity, which can affect many tissues and many cells, or biological processes, and the second is that they can affect certain tissues or cells (Hayes 2003). A substance can be tested for its toxicity using exposure to the test biota. In reality, biota is exposed not only to one type of substances, but to be exposed to toxic substances that contain many substances. Negative effects are toxic for living things based on the interaction of many substances in the environment (Di Poi et al. 2018).

In this study 3 types of substances namely ammonium, glucose and formaldehyde were used. Ammonium is a source of nutrition for the growth of plants and bacteria. Glucose is also the main energy source in metabolism, the process of respiration, the growth of new cells, and in high concentrations can stimulate the growth of biota, especially acting as a carbon source for bacteria to grow (Ebel et al. 2007). Formaldehyde, when released into water, will be toxic. Formaldehyde does not last long in the environment, but its continuous release and formation can result in chronic exposure to biota near sources that produce formaldehyde (Mendez et al. 2015).

The existence of water hyacinth and nitrifying bacteria as biota can be used as indicators of certain types of pollution in water bodies and adverse effects on the surrounding ecosystem. With this toxicity test, it is intended to find out the effects of ammonium, glucose and formaldehyde on aquatic biota which can be in the form of effects on growth that results in ecosystem changes. This can show the level of certain pollutions in the environment and their adverse effects on the surrounding ecosystem.

MATERIALS AND METHODS

Initial Data Collection

The preliminary data needed in the study were the characterization of leachate in the solid waste disposal site of Surabaya city, which was located in Benowo. These data are used as the basis for making the concentration of ammonium and organic substances as BOD and COD and specifically, formaldehyde.

Preparation of Test Biota

All water hyacinth (*Eichhornia crassipes*) plants used in toxicity tests must be acclimatized in clean water for 7-14 days. After the propagules revealed that the new shoots have emerged, the latter were used for the test biota. The plants used from the propagation stage are second generation with criteria: number of leaves 3 strands, leaves that are still fresh and not yellowing, plant age is about 20 days and plant height 24-25 cm (Ebel et al. 2007, Hartanti et al. 2014).

Acclimatization was carried out for at least 7-14 days. During this process the plants must get enough sunlight for photosynthesis, the space to grow is large, the water is calm, and the temperature is between 20-30^{o}C. The reactor is placed in greenhouse and the pH for the growth of water hyacinth ranges from 4.5 to 7.5. The microorganism, which was used in this study was *Nitrosomonas europaea*. This bacterium is in the form of liquid culture that is ready to use.

Range Finding Test

In each container 4 plants were placed with a height of about 24-25 cm. The total volume of the solution in the reactor was 4 litres. Variations in toxic concentrations are distinguished in the percentage of toxicity, which are equal to 0% (control); 20%; 40%; 60%; 80% and 100%. For control, it only contains water hyacinth and diluent water without toxicity. Range finding test (RFT) is carried out for 4 days.

In the range finding stage the toxicity test for *Nitrosomonas europaea* was carried out to determine the maximum levels of substances that could be tolerated by the test bacteria. Toxicity tests were carried out using liquid media, namely Nutrient Broth (NB) media commonly used for bacterial culture. Toxicity testing with liquid media was carried out by growing bacteria on NB media, each of which was contaminated with toxins namely ammonium, glucose and formaldehyde. The toxicity concentration used is 0, 20%, 40%, 60%, 80% and 100%. Bacteria inoculated into NB media contaminated with toxicants were then seen to decrease CO_2 gas in the test reactor. The initial number of inoculated bacteria is 10% v/v (William & Dilosi 2018).

Acute Toxicity Test

The acute toxicity test aims to determine the toxic concentration that can cause a 50% effect of the test biota in a relatively short time. For each different concentration, the test was repeated 3 times. In each reactor in the plant test, 10 water hyacinth plants were kept in 10 litres of water. Toxicants like ammonium, glucose and formaldehyde were mixed and put into the reactor according to the concentration obtained from the RFT results. Negative effect data that occur in plants obtained at 96 hours were used to determine the EC50 value. Variations in toxic concentration were determined in the narrowed range finding test. For control, it only contains water hyacinth and diluent water without toxicity.

Nitrosomonas europaea bacteria was put into a reactor containing NB media contaminated with ammonium, glucose, formaldehyde mixed. Variations in toxic concentration were determined in the narrowed range finding test. For the control, it only contained NB media without toxicity and it was incubated with *Nitrosomonas europaea* as much as 10% v/v. For the bacterial test using respiration test to determine the concentration of toxicity, which caused the inhibition of the function of respiration activity from the bacteria *Nitrosomonas europaea*, can be seen from the volume of air (CO_2 gas), which decreased 50% of the gas volume in the control at the respiration test reactor. Negative effect data on bacteria obtained at 24-hour observation were used to determine the EC50 value. The reactor used is the same as the RFT.

Detoxification Efficiency

The detoxification efficiency of toxicants was determined using toxicity units with the following equation:

$$TU = 1/EC_{50}$$

$$\text{Detoxification efficiency (\%)} = \frac{TU_{in} - TU_{eff}}{TU_{in}} \times 100\%$$

TU_{in} = toxicity unit influent (in this experiment in the reactor of nitrification process using *N. europaea*)

TU_{eff}= toxicity unit effluent (in this experiment in the reactor of plant process using *E. crassipes*)

RESULTS AND DISCUSSION

Characteristics of Leachate

Leachate used came from the Benowo landfill collection pool. Samples were analysed to determine the initial characteristics of leachate. The parameters used were BOD, COD, pH, temperature, turbidity and NH_4. Table 1 shows the characteristics of Benowo landfill leachate.

Table 1 was used as the basis for making artificial waste concentrations. Artificial waste used comes from chemicals that are made from pro analyst or pure. This study did not use original leachate in the study. This is intended because using artificial waste, desired concentration is obtained and the achievement can be close to what we want rather than original waste.

Test Against *N. europaea*

Ammonium solution used was 147 mg/L, 294 mg/L, 441 mg/L, 588 mg/L and 735 mg/L and control solution (without ammonium). The results of the range finding test revealed that the highest ammonium concentration which showed a decrease in the volume of water in the measuring cup was 294 mg/L, which was as much as 25 mL.

The glucose concentration used in the RFT stage was 203.44 mg/L; 406.88 mg/L; 610.32 mg/L, 813.76 mg/L, 1,017.2 mg/L and control solution (without glucose). The results of the range finding test showed that the highest glucose concentration which showed a decrease in the volume

Table 1: Characteristics of Benowo landfill leachate.

No.	Parameter	Unit	Results
1	Temperature	°C	28°C
2	pH	-	8.21
3	Turbidity	NTU	478
4	DO	mg/L	0.8
5	BOD	mg/L	1085
6	COD	mg/L	2560
7	NH_4	mg/L	735

of water in the measuring cup was 1,017.2 mg/L, which was 27 mL.

Formaldehyde concentration used was 0 mg/L as control, 0.24 mg/L, 1.2 mg/L, 2.4 mg/L, 12 mg/L, 24 mg/L, 120 mg/L, 240 mg/L, 480 mg/L, 960 mg/L, 1,440 mg/L, 1,920 mg/L and 2,400 mg/L. The results of the range finding test showed that glucose concentration which showed a decrease in the volume of water in the measuring cup was 0.24 mg/L which is 10 mL.

After obtaining the concentration range in each test solution, namely the concentration where *Nitrosomonas europaea* is able to produce CO_2 gas reduction seen from the decrease in water volume in the reverse measuring cup, the three test solutions, namely NH_4Cl, glucose and formaldehyde were mixed to determine the effect that occurs when these substances interact. In this main test, NB media was mixed with toxic substances, NH_4Cl, glucose and formaldehyde, then the bacterium was added to the reactor by 10% of the volume of the test media.

Test Against *E. crassipes*

At this stage it will be known that what maximum toxic concentration that can be received by *E. crassipes*. Definition of toxic effects of exposure to NH_4 solution on water hyacinth is that which does not appear to produce new shoots in plant tissues due to pollution (Mangkoedihardjo & Samudro 2014).

The results of the range finding test that will be selected are ammonium concentrations that do not have a negative effect on plants, namely the concentration that makes water hyacinth show new shoots and remain fresh. In this case NH_4 inhibits the growth of shoots, but not the leaves and stems so they remain green. In the first stage, namely with NH_4 until the fourth day, so the concentration of NH_4 used is at 40%, namely the concentration of NH_4 294 mg/L in plants of *E. crassipes*, because at concentrations of 60%, 80% and 100% all do not show new shoots. The highest concentration of ammonium which is toxic or inhibits growth is unknown, but water hyacinth begins to experience stunted growth at concentrations of NH_4 in water at 370 mg/L (Qin et al. 2016).

In glucose solutions, water hyacinth is able to show new shoots in all the toxic concentrations within 2 days. In RFT glucose solution using a concentration of 100%, which is 1.017 mg/L for the main test. This is because each plant produces glucose in the photosynthesis process which is used as an energy source by these plants. Therefore, organic matter, namely glucose, is a carbon source that is non-toxic, so it does not have a toxic effect on all the concentrations above (Lastdrager et al. 2014). The temperature and pH during the study were 29-30°C and 6.9-7 respectively, which allowed water hyacinth to grow.

In formaldehyde, water hyacinth is able to show new shoots at a concentration of 1% which is 24 mg/L within 3 days, while concentrations above 1% some do not show new shoots. Formaldehyde includes non-biodegradable organic substances which are toxic. Formaldehyde is a toxic organic matter, and with the increasing level of toxicity it can cause the biota to die.

Concentration where water hyacinth is able to live 100% or show new shoots, the three test solutions, namely NH_4Cl, glucose and formaldehyde were mixed to determine the effects that occur when these substances interact. In the main test 10 hyacinth plants were put into each reactor containing toxicant and diluted water with a total volume of 10 L. The mixture of ammonium, glucose and formaldehyde has a 50% toxic effect on biota at the concentration of each substance when mixed, i.e. 360 mg/L, 1300 mg/L and 40 mg/L, respectively.

EC_{50} and Detoxification Efficiency

The EC_{50} value of non-biodegradable organic substance, namely formaldehyde, is smaller than ammonium solution and biodegradable organic substances, namely glucose (Table 2). This shows that formaldehyde is more toxic than glucose and ammonium. In addition, plant processes can reduce the level of substance toxicity.

Table 2: EC_{50}-96h (mg/L) for the test toxicants.

Toxicants	*N. europaea*	*E. crassipes*
Glucose	1066.2	1307.12
Ammonium	329.07	361.31
Formaldehyde	0.34	0.4033

Based on Table 2 data, the detoxification efficiency can be calculated with the following results: glucose (16%) > formaldehyde (15%) > ammonium (7%). Formaldehyde is the most toxic substance among these substances, but its efficiency is high, this may be due to an antagonistic effect (from toxic to less toxic) mixture of substances.

CONCLUSION

The results of the study of the effects of glucose, ammonium and formaldehyde concentration on *N. europaea* bacterium and plant *E. crassipes* showed that the most toxic substance was formaldehyde. However, the efficiency of detoxifying mixed substances indicates the possibility of an antagonistic effect of the mixture of substances. And, that possibility requires further research.

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