Study of microalgae (Scenedesmus Sp.) utilization as phosphate bioremediator (PO₄³⁻) in domestic wastewater medium

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Abstract. In this research, the use of Scenedesmus sp. as a phosphate bioremediator agent is expected to be applied for lowering the phosphate levels in the water as well as improving the quality of the environment. Scenedesmus sp. used in this research was isolated from Sungai Cideng. Variation of phosphates were tested to see the effect of various types of phosphate found in the waters in the form of a variation of organic phosphates using the Adenosine 5'- monophosphate (AMP), phytic acid and variation of inorganic phosphates using potassium dihydrogen phosphate (KH₂PO₄) and sodium tripolyphosphate (STPP). It was found that the use of monopotassium phosphate (KH₂PO₄) was better in decreasing phosphate levels and increasing the number of Scenedesmus sp. cells with a phosphate decrease percentage of 87.5 %. Phosphate test was performed using UV-Vis spectroscopy instrumentation and the abundance of cell count was performed using a microscope and hemocytometer. The environmental quality test parameters are temperature and pH. Direct application in wastewater from Muara Angke was also found the decrease of phosphate levels and increase the number of Scenedesmus sp. cells. Scenedesmus sp. has the ability to reduce nutrients in wastewater to below wastewater quality standard of 86.0 % from the initial phosphate concentration of 0.2255 ppm to 0.0314 ppm.

Keywords: Scenedesmus Sp., bioremediator, phosphates, wastewater, Muara angke

1. Introduction

Environmental pollution is the result of the production process, which produces waste. Several types of waste contain phosphate compounds in high concentrations before discharged into the water. It is known, that there are several types of microalgae that can live and thrive in environments with high phosphate concentrations. One of the parameters measured in determining the quality of wastewater treatment results is phosphate content. Phosphate levels in the waters still exceed the predetermined quality standard. Phosphate in waste can be organic phosphate, inorganic orthophosphate and polyphosphate complex. High phosphate content in wastewater can cause eutrophication. Bioremediation technology is a technology that plays an important role in achieving the goal of this research in regard to the environmental side. Starts with a passive metabolic process, bioremediation technology generally involves a process of biosorption or absorption in which the amount of contaminants can be removed and eliminated by sorbent in kinetic equilibrium and compounds contained on the cellular surface of sorbent, then the pollutants can be absorbed into the cellular
structure, and finally there is an active metabolic bioaccumulation process from living organisms and requires respiration where absorbing contaminants are transferred to cell [1]. The opportunity to utilize microalgae in the bioremediation process of household waste is very possible. Wastewater is a very good medium and has the potential to be used by microalgae as a growth medium because its effectiveness and also supported by low cost management [2]. So, this research is needed to obtain more detailed scientific facts. Therefore, a research in the form of microalgae simulation is conducted with the aim to know the extent of bioremediation effect using microalgae to increase the quality of household waste especially to decrease the phosphate level. The microalgae used in this study was Scenedesmus sp., which is a species of microalgae that has a high growth rate, cosmopolitan characteristics, and not motile. Microalgae Scenedesmus sp. is also able to take advantage of various minerals contained in the water to the bottom of the water. This fact became the basis for the use of Scenedesmus sp. as an absorbent pollutant contained in the waste. Observation of bioremediation process is conducted on Scenedesmus sp. microalgae with the measurement of phosphate for 18 days and environmental quality parameters, once in every 3 days.

2. Materials and methods

2.1. Materials

The materials used were microalgae culture Scenedesmus sp. from Sungai Cideng as the raw material, ethanol (C₂H₅OH) (Sigma) for sterilization, medium bold basal for medium growth of Scenedesmus sp., potassium dihydrogen phosphate (KH₂PO₄) (Merck), sodium tripolyphosphate (Na₃PO₄·12H₂O, STPP) (Merck) for variation of phosphate, sulfuric acid (H₂SO₄) (Merck), phenolphthalein indicator (C₆H₈O₇·3H₂O, Sigma), ammonium heptamolybdate [(NH₄)₂MoO₄·4H₂O] (Merck), potassium antimonyl tartrate [K(SbO)₂C₄H₄O₆·H₂O] (Sigma) for molibdate reagent, perchloric acid (HClO₄) (Merck) for destruction, and wastewater as the main ingredient from household wastewater in Muara Angke estuary.

2.2. Preparation of Scenedesmus sp. inoculum

In the first step, Scenedesmus sp. was isolated by referring to previous studies [3]. The cultivation of Scenedesmus sp. contained of 490 mL of bold basal medium (BBM) and 210 mL volume of inoculum Scenedesmus sp. at 30 % concentration (Vinoculum/Vmedia) in a HDPE bottle incubator for 8 days, under the following cultivating conditions placed the illumination of light and fixed the aeration rate at 1 L/min of air supplied into the bottle by using an air pump. The working volume for the experiment was 700 mL for each bottle and the remaining space was for the headspace to promote gas exchange. Cell calculations were performed using a microscope by the Improved Neubauer count chamber method.

2.3. Preparation of phosphate solution

To examine the effect of phosphate type on bioremediation activity, several phosphate compounds were used as model including sodium Tripolyphosphate (STPP) salt, potassium dihydrogen orthophosphate (KH₂PO₄) which is an inorganic phosphate type and Adenosine 5'-monophosphate (AMP) and Phytic acid (IHP) which is a type of organic phosphate. Preparation of KH₂PO₄, STPP, AMP and IHP from phosphate solution was conducted by dissolving variation of phosphates with distilled water in a measuring flask. Then, 500 mL of phosphate solution was made with concentration of 0; 0.2; 0.4; 0.6; 1 ppm.

2.4. Sampling research

The wastewater was taken from the waters of Muara Angke, Teluk Jakarta. The determination of the waste source was also selected based on the initial survey of the sampling. The location of Muara Angke, Teluk Jakarta is close to the household activities. Surface water was taken from the bridge on the waters of Muara Angke, Teluk Jakarta.

2.5. Bioremediation

Cultivated algae was taken 15 mL then centrifuged at 3000 rpm for 15 minutes to separate the media. The water sample was inserted into a beaker glass 1000 mL, then the water sample was filtered using a Buchner filter to remove the unwanted microalgae as well as suspended material. The same thing was
done on the sample solutions of organic and inorganic phosphate types. Experiments on the study were observed daily until 18th. There was also an addition of solution of 1 ppm Cd metal in wastewater and phosphate compounds to see the effect of 500 mL of metal ions with a mixture of wastewater and 50:50 phosphate compound then the sample was transferred into a plastic gallon.

2.6. Determination of phosphate levels
The data obtained from laboratory test results were analyzed using simple comparative phosphate (PO$_4^{3-}$) levels before and after treatment to know and see the effectiveness of Scenedesmus sp. in lowering phosphate levels (PO$_4^{3-}$). Phosphate measurements used a spectrophotometric method using the principle of molybdate reagent.

3. Results and discussion

3.1. Results of algae cultivation observation
To know the morphology of microalgalae, an observation using microscope with 1000 x magnification was used. Based on the results of green algae, it can be concluded that the green algae is a green algae Scenedesmus sp. Based on the structure morphology obtained in figure 1, it shows that the morphological structure of the green algae is Scenedesmus sp. with the obtained characteristics similar to the reference literature in (e). The cylindrical algae are obtained, the terminals are round and there is spine in the form of fine hair at the edges of each corner, having colonies of 2-4 cells laterally. The results can be seen in figure 1.

Microalga Scenedesmus sp. was taken from the algae stock in Geostech BPPT Serpong derived from the river Cideng as the mother cultivation microalgalae. Cultivation was done among others to obtain a more pure and not polluted algae to multiply the number of microalgae in the sufficient amount for the research. In addition, cultivation was also done to see the growth curve of Scenedesmus sp. in its growth media.

The condition of culturing is obtained from measuring the condition of the media after autoclaving on the preparation of media for the nutritional needs of Scenedesmus sp. microorganisms. The nutrients used are derived from the bold basal medium or hereafter referred to as BBM media. The pH of the media measured using pH-meter is 6.6 and the temperature is 26.9 °C. This is in accordance with the optimum conditions for the growth of algae culture Scenedesmus sp.

3.2. Growth Curve Data
The growth phase of Scenedesmus sp. graph shows an increase in the number of cells during the 18 days of observation as seen in figure 2. The measurement of cell count was performed using a

Figure 1. Observations using a microscope with 1000 x magnification of Scenedesmus sp. (a) without dilution and with dilution (a) 10$^{-4}$ and (b) 10$^{-2}$
hemocytometer to determine the growth of microalgae. For 18 days, there was a qualitative observation corresponding with the color that the growth of microalgal cell counts also increased. The original light green color changed on the first day to a darker color in the next day. The color change from bright green to solid green occurs due to the increase population of *Scenedesmus sp*. Microalgae increased the number of *Scenedesmus sp*. cells indicating the occurrence of utilization of nutrients contained in the fuel medium for microalgae is proliferating. Based on the observation, it was found that the number of cells experiencing a maximum increase is on the 6th day of 1.00 x 10^6 cells/mL indicating that on the 6th day the exponential phase occurs and then undergoes the stationary phase. Thus, the microalgae is taken on the 6th day due to the maximum metabolism of the day and the phase.

3.3. Experimental treatment of variation of inorganic phosphate solution
The resulting cell count, phosphate concentration, temperature and pH can be seen in figure 3. In figure 3b, it can be seen that abundance of *Scenedesmus sp*. in KH.PO inorganic phosphate solution shows an increase every day. From the curve, it can be seen that the number of cells *Scenedesmus sp*. continues to increase with the highest abundance in the solution in a concentration of 1 ppm of 4.88 x 10^5 cells/mL of the initial abundance of 4.8 x 10^4 cells/mL with a decrease in phosphate levels of 28.2% in the initial concentration of phosphate 1.0057 ppm decreases to 0.7215 ppm. The increase in growth shows the microalgae is experiencing a logarithmic phase caused by the availability of nutrients especially phosphate. The increase in the number of cells is thought to be caused by inorganic phosphate in the form of KH.PO, which can be well used to support the growth of cells as indicated by curves that tend to rise from the first day until the last day of observation. The increasing number of *Scenedesmus sp*. was also followed by a decrease in phosphate levels in the medium. After the phosphate measurements, it can be seen in figure 3a it was found that the decrease in phosphate content with the highest decrease of 87.5% in the solution with the initial phosphate concentration of 0.2234 ppm decreased to 0.0278 ppm with the result of the concentration of negative value due to the measured value has exceeded the existing standard curve of exceeding the limit of measurement detection. Furthermore, a decrease of 62.2% indicated in solution with initial phosphate concentration of 0.4244 ppm decreased to 0.1600 ppm, while the reduction of 50.9% in the solution with the initial phosphate concentration of 0.6111 ppm decreased to 0.3000 ppm.

Based on figure 4, it shows the ratio of inorganic phosphate absorption also used phosphate source solution in the form of sodium tripolyphosphate (STPP). In figure 4b, the number of cells *Scenedesmus sp*. shows a daily increase with the highest abundance in a solution in a concentration of 1 ppm of 3.68x10^5 cells/mL from an initial abundance of 4.8x10^4 cells/mL which also correlated positively with a decrease in phosphate levels with decreases in the initial concentration phosphate of 1.79% at a concentration of 1.0057 ppm decreased to 0.9876 ppm. The increasing growth is due to the availability of nutrients especially phosphate but the amount of abundance is found to be lower than that of KH.PO, so it is suspected that *Scenedesmus sp*. can use KH.PO inorganic phosphate solution.

3.4. Experimental treatment of variation of organic phosphate solution
The organic phosphate solution used in this experiment was Adenosine Monophosphate (AMP) and Myo-Inositol Hexakis Phosphate (IP6). Observation results of the influence of abundance of cell *Scenedesmus sp*. to organic phosphate solution as a cultivation medium is presented in figure 5.
Figure 3. Bioremediation using inorganic phosphate KH$_2$PO$_4$ (a) decreased phosphate levels, (b) growth of Scenedesmus sp. (c) effect of pH and (d) effect of temperature.

Figure 4. Bioremediation using inorganic phosphate STPP (a) decreased phosphate levels, (b) growth of Scenedesmus sp. (c) effect of pH and (d) effect of temperature.

On the visible curves, the number of cells Scenedesmus sp. continues to rise each day with the highest abundance in a solution in a concentration of 1 ppm of $3.48 \times 10^5$ cells/mL from the initial abundance of $5.2 \times 10^4$ cells/mL from the first day to the last day, indicated by a curve that tends to rise. On the curve, it is visible the number of cells Scenedesmus sp. continues to increase each day.
Figure 5. Bioremediation using inorganic phosphate AMP (a) decreased phosphate levels, (b) growth of Scenedesmus sp., (c) effect of pH and (d) effect of temperature

with the highest abundance in a solution with concentration of 1 ppm of $3.48 \times 10^5$ cells/mL from an initial abundance of $5.2 \times 10^4$ cells/mL from the first day to the last day, indicated by an upwardly rising curve. It is also demonstrated and correlated with a decrease in phosphate levels that the Scenedesmus sp. absorbed the organic phosphate nutrient content. The result of the decrease of phosphate content was obtained with decrease in the solution of 34.3 \% with initial concentration of phosphate AMP 0.1815 ppm decreased to 0.1191 ppm. Furthermore, 45.4 \% with initial phosphate AMP concentration 0.4216 ppm decreased to 0.2300 ppm. Then, in a solution of 57.4 \% with initial concentration of AMP phosphate of 0.6016 ppm decreased to 0.2560 ppm and a decrease of 13.17 \% with initial concentration of AMP phosphate of 0.9898 ppm decreased to 0.8594 ppm.

Characterization using organic phosphate phytic acid can be seen in figure 6. Based on figure 6, it shows the suspected Scenedesmus sp. microalgae. It may be better to use an organic AMP phosphate solution due to the complex phytic acid (IP6) compound form and the unavailability of the fitase enzyme to metabolize the compound. Figure 6 shows a decrease in phytic acid phosphate levels obtained with a decrease of 4.2 \% in a solution with an initial phosphate concentration of 0.9946 ppm decreased to 0.9525 ppm. Furthermore, in solution with initial concentration 0.1893 ppm decreased to 0.1688 ppm with percent decrease of 10.82 \%. Continued in a solution of initial concentration of 0.4109 ppm decreased to 0.3833 ppm with a decrease percentage of 6.71 \%, and in a solution with an initial concentration of phytic acid of 0.6133 ppm decreased to 0.5560 ppm with a decrease percentage of 9.34 \%. Number of cells Scenedesmus sp. in IP6 organic phosphate solution showed an increase every day as shown in figure 6b. Number of cells Scenedesmus sp. continues to increase daily with the highest abundance at 1 ppm concentration of $3.20 \times 10^5$ cells/mL of an initial abundance of $4.8 \times 10^4$ cells/mL. The availability of nutrients, especially phosphates, leads to an increase in the growth of microalgae cells.

3.5. Results of bioremediation of wastewater

Observation results of the influence of abundance cells Scenedesmus sp. to household wastewater as a cultivation medium is presented in figure 7 with varying abundance data in each treatment each day. The calculation of abundance is intended to see how far the Scenedesmus sp. microalgae can utilize the existing content in the culture medium. The abundance curve shows that in the wastewater the
Figure 6. Bioremediation using inorganic phosphate IP6 (a) decreased phosphate levels, (b) growth of *Scenedesmus* sp. (c) effect of pH and (d) effect of temperature.
	number of cells *Scenedesmus* sp. keeps increasing every day at the same time the phosphate decreases. The pattern of growth and reduction of nutrients can be seen in figure 7. The abundance is thought to be due to the amount of nutrient derived from the amount of phosphate used to support cell growth and growth from day one to the last day indicated by a curve that tends to rise, but on the 9th day onwards.

Figure 7. Bioremediation using wastewater (a) decreased phosphate levels, (b) growth of *Scenedesmus* sp., (c) effect of pH and (d) effect of temperature.
the cell growth begins to show a stationary phase indicating that the amount of nutrients available in the media began to decline and not enough for its further growth. The cells have utilized the nutrients for their metabolism. There was a growth of cell number from 4.8 x 10^5 cells/mL to 3.2 x 10^6 cells/mL while the decrease of phosphate concentration of 86.0 % from the initial phosphate 10^5 cells/mL while the decrease of phosphate concentration of 86.0 % from the initial phosphate concentration of 0.2255 ppm decreased to 0.0314 ppm. Thus, it is shown that *Scenedesmus sp.* has the ability to reduce nutrients in the form of phosphate in wastewater and use it for growth.

Based on wastewater quality standards according to the Regulation of the Ministry of Environment of the Republic of Indonesia No. 5 2014, the phosphate concentration is 2 mg/L and according to Government Regulation No. 82 of 2001 on Water Quality Management and Water Pollution Control phosphate criteria as total P reaches class I & II with maximum limit of 0.2 mg/L and reaches class III with number maximum limit of 1 mg/L. So, the use of *Scenedesmus sp.* as bioremediator agent of wastewater has fulfilled the wastewater quality standard that is 0.0314 ppm. The optimum temperature for *Scenedesmus sp.* is 26–32 °C, at temperature 34–36 °C the *Scenedesmus sp.* will stop growing and inhibit the metabolism process to death. So, the temperature obtained indicates that the wastewater is still in the optimum temperature for the growth of *Scenedesmus sp.* The abundance curves tend to increase along with the pH of the medium, which tends to be alkaline.

4. Conclusions

Based on the results of research, *Scenedesmus sp.* can use variation of phosphate types, both inorganic phosphate and organic phosphate for the necessities of life. The use of inorganic phosphate type in the form of KH₂PO₄ is better than STPP. While on the variation of organic phosphate type, the AMP is better than phytic acid. The abundance of *Scenedesmus sp.*, which is applied directly by using wastewater, is increased daily and at the same time the phosphate levels are decreased. So, it can be concluded that *Scenedesmus sp.* has the ability to reduce nutrients in wastewater by 86.0 %. This phosphate decrease is caused by *Scenedesmus sp.*, which can use organic or inorganic phosphate contained in wastewater for the purpose of life metabolism because phosphate is a macro element for the necessities of life. After bioremediation, water quality in the environment is observed from the environmental wastewater. So, the bioremediation showed to be in accordance with the temperature and pH in the threshold of the quality standard of environmental wastewater. So, the bioremediation by using *Scenedesmus sp.* can be said to last well.

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