Comparison of ImageJ Software Results Between The Growth of *Lemna minor* in the Presence of Sodium Bicarbonate and Without Sodium Bicarbonate

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Abstract. Water quality in the recent years had been a very important aspect in daily life, especially in big cities where they produced many liquid organic waste. One of a way to overcome this problem, a primary biological treatment could be used to transform this liquid organic waste water to become a cleaner water by using certain plant that belong to family of duckweeds, which was *Lemna minor*. *Lemna minor* plant had the benefit from growing on top of polluted water, in addition it helped to induce a better quality of the polluted by the reducing organic matters. The organic matters itself could be dangerous for daily usage, but for the *Lemna minor* it was useful for their growth due to their unique ability. The ability that was used by this *Lemna minor* was called as phytoremediation which defined as the ability to absorb some excess organic matters and transform it to become a nutrients for the plant growth. In this experiment, the phytoremediation effect would be observed by the growth of the plant inside a home scale bioreactors and the result was compared by using ImageJ Software analysis. The comparison are done between two aquariums that were filled with and without sodium bicarbonate. During the experiment days without the sodium bicarbonate, some of the *Lemna minor* could grow very well, but some of the *Lemna minor* also could not grow very well. During the experiment days with sodium bicarbonate was added, the plant growth was more consistent and grown very well due to the effect of the pH stability and alkalinity return properties. The percent coverage area when the *Lemna minor* grown very well, it reached up to 66 %.

Keywords: Template; *Lemna minor*, ImageJ, Bioreactor, Waste water treatment, Sodium bicarbonate

1. INTRODUCTION

Nowadays, the water system in many cities in Indonesia had problems to recycle the water for clean water to be safe for drinking and other activities (Marleni, 2020). Especially in big cities around Indonesia, many people started to produce more wastewater, compared to water supply company that produce healthy water for daily usage (Soedjono, 2018). Most wastes in Indonesia that were produced are usually in form of organic wastes and these organic wastes also had been produced in many urban areas and not just industries that are related with organic materials (Anwar & Riani, 2018). Organic wastes in Indonesia are most likely from animal wastes, household wastes, organic plant wastes, and many others (Tanaka & Chaerul, 2007).

Eutrophication was defined as the overflow of nutrients that leaded to an accelerated growth of algae or plants such that the growth of those living organisms would disturb the surrounding environment. This phenomena was occurred quite frequently and caused some problems to the natural habitat for all living organism (Adams & Lemley, 2018). At the other hand there was a type of plants that was benefitted from this eutrophication phenomena such that it could absorb this excess of nutrients and improve the quality of the water aspects inside the water systems under controlled environment, which were called duckweed (Shaban & Nassar, 2015). Those aspects above had lead to a study of *Lemna minor* growth with a water that was filled with an organic wastes. The growth would be examined by using ImageJ Software analysis by monitored the percentage of green color area that was represented by the *Lemna minor* plants that were growing along the experiment day.

2. LITERATURE REVIEW

Lemna minor was actually an aquatic plant that came from a sub familiy of duckweeds or *lemnaceae* and the main characteristics of this aquatic plant was that this plant had a small type of structure and easy to float everywhere as long as the place was filled with enough water and enough nutrients in the water, then this aquatic plants could grow and distributed easily in natural environment (Verma, 2015). The other main characteristic of *Lemna minor* is that the body of this plant was in form of fronds and for this *Lemna minor* species had formed only one root (Hasan M. R., 2009).

The other important thing from the duckweeds that these plants had is a high nutrient value intake ability, because due to the fact that it was easy to grow in the natural water environment that had diverse nutrients and as long as those nutrients consisted of ammonium, nitrate, urea, and some amino acid, then *Lemna minor* could absorb those nutrients by absorbing the nitrogen from those chemical compounds that residing inside the water (Goopy, 2003). Other than the nitrogen, phosphorus and potassium in a little amount were also required for maximum of *Lemna minor* (McLay, 2006). The way *Lemna minor* phytoremediation itself was categorized as a phytoremediation with a type that was called rhizofiltration. The rhizofiltration itself was the ability for the plants to absorb the excess nutrients from the ponds through their roots such that the polluted water inside the pond could be cleaned by *Lemna minor* with this ability (Landesman, 2010).

Although it seems that duckweeds (*Lemna minor*) could grow on diverse conditions, but for optimal growth in sterile condition there will be some specific range of pH, temperature, light intensity, and nutrients that were needed for the duckweeds growth phase. For *Lemna minor*, it was believed that the optimal temperature growth was around 5.7-29^{o}C and pH with an optimal range of 6.9-7.8 (Priya, 2012). The water system in a high level of acidity with a living plant would usually make the plant stop growing and eventually died out (Alam, 2010). On the other hand when the water system had high level of alkalinity or higher pH would usually benefit the *Lemna minor* itself because those water systems usually had rich ammonium ions, nitrogen, and nitrates which was needed for the *Lemna minor* to grow. The high level of alkalinity in the water systems usually come from detergents and soap based which released many ammonium ions and nitrates which was needed, but sometimes when the pH level kept on increasing and more ammonium ions were released would inhibit the *Lemna minor* growth (Wang & Yang, 2014).

Nitrogen as the main nutrients sometimes come in different forms, the first form was the ammonium ions or ionized ammonia ($NH4^{+}$) and the second type was unionized ammonia (NH3). The first type which was ammonium ions were heavily preferred by *Lemna minor* as the source of the nitrogen with a dependence to the temperature of the water system (Hasan M. R., 2009), but for the unionized ammonia as the source of the nitrogen would have a toxic effect for the *Lemna minor* because these unionized ions of ammonia and high concentration ammonium of ions would inhibit the *Lemna minor* growth due to this unionized ammonia was easily disturbed the cell membrane and disturbed the cell metabolism by easily entered the plant cell (Xu & Zhao, 2012). That was why the amount of the liquid organic waste to the water systems were needed to be carefully examined to know the limits of these excess nutrients.

Nitrogen in form of nitrites were actually achieved by the oxidation of the ammonium ions and the alkalinity would be lost during the process to become the carbon source by nitrifying bacteria (Gerardi, 2002). The undergone chemical reaction was:

$$NH_4^{+} + 1.5O_2 \text{ – Nitrosomonas} \rightarrow 2H^{+} + NO_2^{-} + 2H_2O$$

These alkalinity lost would affect the *Lemna minor* ability to absorb the nutrients, that is why it was essential to add an enough amount of residual buffer of alkalinity to make the pH in the bioreactor tank stable enough and provided the nitrifying bacteria with inorganic carbon (Gerardi, 2002). The chemicals that were needed to be added to neutralizing the nitrite acids that were formed from the nitrification were none other than bicarbonate ($HCO3^{-}$) ions which very useful to provide stable alkalinity by providing inorganic carbon ($C0_2$) for nitrifying bacteria. Although along the process not all the ammonium ions were oxidized to become nitrite ions, but the ammonium ions would be used as a nutrient nitrogen as it was mentioned before and the adding of this bicarbonate ions were used to reduce the nitrites ions developing and provide better stability of alkalinity (Gerardi, 2002).

ImageJ Software would be used to control and examine the Lemna minor growth. The growth of the *Lemna minor* would be examined by using a camera to see the coverage area of the *Lemna minor* every day. ImageJ Software analysis was used mostly to differentiate and quantify images or photos that were related to the life sciences, which was in this case the *Lemna minor* growth could also be observed (Schindelin & Rueden, 2015). The way this ImageJ Software worked was by dissecting the whole picture by either general shape threshold or by color threshold the whole picture to calculate the coverage area of the *Lemna minor* growth day by day throughout the experiment day (Schneider, Eliceiri, & Rasband, 2012). The way the general shape threshold would calculate the coverage area of the *Lemna minor* growth by differentiate the shape of the majority of the whole coverage by the *Lemna minor* which if the majority of the shape had a circle shape. Then it would calculate the only area of the picture that would consist of a circle area and the other shape area would be left out and could not be calculated with the ImageJ Software analysis. For the color threshold itself had different approach to calculate the coverage area of the *Lemna minor* growth, the color threshold would calculate the coverage area based on the color, saturation, and hue of the picture which from those three aspects then the selected majority color coverage would be selected and from those selected majority color, the coverage area with a selected majority color could be calculated by the color threshold method, which in this case the coverage area would be covered as pixels values.

3. METHODOLOGY

The water that was used came from PAM water source that would be the base of the water and the type of the liquid organic fertilizer that was used in the experiment days were Petrovita liquid organic fertilizer which had a concentration of nitrogen (9%), phosphorus (3%), potassium (4%), and other nutrients such as Mn, Cu, Zn, B, and Co that were on the liquid organic fertilizer with a small composition with range of around 3 ppm until 80 ppm value for each of those other nutrients. The nitrogen concentration that were used in the experiments had a concentration of 77,5385 mg/L (56 grams of Liquid Organic Fertilizer were used) of Nitrogen which were calculated based on the nitrogen concentration and the aquariums size.

Before the experiment started, aquariums were filled with water, the aeration source which was the aquariums pumps must be set. Then each of the aquariums would be filled with water that had a size 60 cm * 30 cm * 35 cm. After the water was filled, the liquid fertilizer was added to the water as the liquid organic waste with an amount of 55 grams (+/-5 grams of tolerance) for each of the repetition. For the last two repetition besides the fertilizer NaHCO3 (sodium Bicarbonate from Baking Soda) would be added with an amount of 1/8 of the fertilizer amount as another comparison to the previous experiments. Since the liquid fertilizer had been added, the aquariums would not be directly be filled with *Lemna minor*, but the wastewater was let still for a day to make sure that the liquid organic fertilizer had been distributed all the way in the aquariums. After all of the liquid organic fertilizer had been distributed for a day, then the *Lemna minor* would be added throughout the aquarium with an amount of three times the circular object placement for the *Lemna minor* seeds to grow, then the *Lemna minor* percentage coverage area picture would be captured by the hand phone camera to calculate the percent coverage area day by day.

The cropped picture of *Lemna minor* could undergone color threshold to calculate the coverage area from the highlighted area. The color threshold results from the first cropped picture would be shown below with yellow highlighted area as the *Lemna minor*.

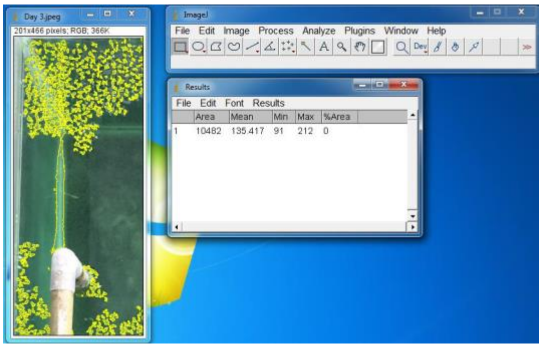

Figure 1. Yellow Highlighted Area of *Lemna minor*

The next picture that would be shown was the red highlighted picture that selected the water coverage area without the *Lemna minor* which would be shown below.

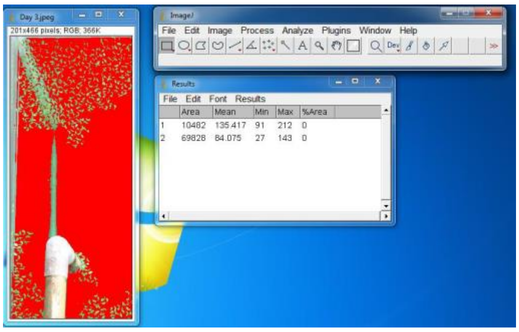

Figure 2. Red Highlighted Coverage Area without *Lemna minor*

After the coverage area of each of the cropped pictures were achieved, then the percent coverage area could be calculated by using a simple formula which was:

$$\frac{(Total\ Yellow\ Highlighted\ Area)}{(Total\ Red\ Higlighted\ Area + Total\ Yellow\ Highlighted\ Area)} * 100\%$$

Figure 3. Percent Coverage Area of *Lemna minor* Growth Formula

4. RESULT

The experiment itself would also depend on the *Lemna minor* abilities to absorb the excess nutrients. Since the liquid organic fertilizer was filled with an organic nitrogen then the phenomena that could happened inside the aquariums that were filled with *Lemna minor* was when the excess nutrients were absorbed, then nitrification of nitrogen could also happen for organic nitrogen could be transformed and absorbed by the *Lemna minor* at the same time (Arias, Ramirez, & Fernandez, 2016). Most of the excess nutrients that were absorbed by the *Lemna minor* were actually the nitrates which also can act as the substitution of ammonium ions for *Lemna minor* nutrient uptake, the reaction would be shown below (Kano, Kitazawa, & Sato, 2021).

$$NH_4^+ + 2HCO_3 + 2O_2 \rightarrow NO_3^- + 2CO_2 + 3H_20$$

From those expression of the chemical reactions, it could be seen that there were two bicarbonate ions to uptake the Nitrate ions. Actually the plant itself was the one that released the bicarbonate ions or hydroxyl ions which needed to be done from the plant to ensure the electrical neutrality across the soil-root interface such that it caused the rhizosphere pH area to increased (Neina, 2019). For all the successful excess nutrients that were absorbed, then the phenomena would apply the above phenomena that had been explained previously, but along the way some of the experiments had some fails and success along the way so the other phenomena that was happened would be explain in the detailed day by day explanation of the ImageJ Software results.

In the second experiment, the *Lemna minor* seeds were distributed along every corner of the aquarium and from those distributed seeds the picture, some pictures were taken to calculate the percent coverage area. The issues of the experiment that *Lemna minor* had a very long time of acclimatization which turned to shock state and due to those long stage hence some of the *Lemna minor* had died and some decomposed their parental fronds or daughter fronds as organic matters. Although some of the fronds still absorbed some of the excess nutrients, but the majority of the fronds had already decomposed. The acclimatization stage for each plants had many variation, some plants needed many time to adapt with the new environment which could take weeks or sometimes it did not need those long time and only needed a few days (Yuliansyah & Prasetya, 2018).

ImageJ Software analysis results indicated that the results of the area that was covered with *Lemna minor* without the addition of sodium bicarbonate had decreased significantly. The percent coverage area of the *Lemna minor* also changed from where the percent coverage for the last trial was decreasing greatly where the value was 11,2523 %. From the rest of the days the trend would be shown in graph and the trend graph would be presented below.

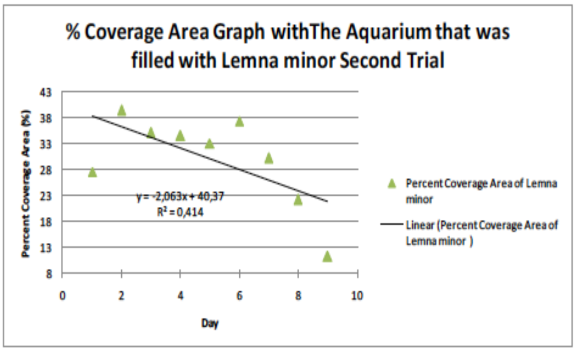

Figure 4. Precentage Coverage Area Chart of *Lemna minor* Without Sodium Bicarbonate

From the chart itself, it could be seen that from ImageJ software analysis that the percentage coverage area of *Lemna minor* was decreasing from the middle of the week and towards the end of the experiment. This indicate that the phytoremediation ability from *Lemna minor* to grow was now affective due to long acclimatization process that made most of the parental and daughter fronds decomposed at the same time.

In the fourth experiment sedum bicarbonate was added into the aquarium and the experiment had a very significant changes compared to the previous second experiment. In this experiment the *Lemna minor* plant seeds had grown and multiplied in a very great numbers of percent area coverage that was filled with *Lemna minor*. Since the adaptation period was quite fast then the absorption rate of the *Lemna minor* itself also affected greatly and it also influenced the amount of daughter fronds that were produced from the parental fronds vary greatly according to (Leng, 2004) which could be proven from the ImageJ results where the percent coverage area in the last day also proven had a value of 36,5666 %. The graphic trends also could be shown below for the overall growth of the fourth experiment.

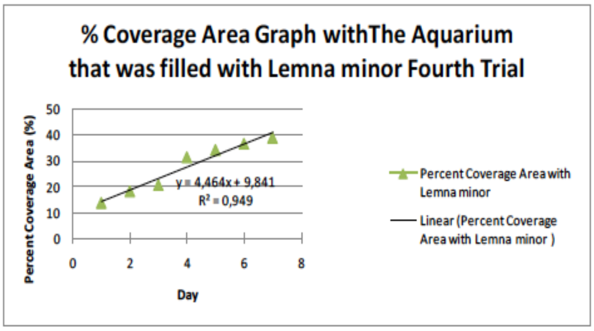

Figure 5. Percentage Coverage Area Chart of *Lemna minor* With Sodium Bicarbonate

From the above chart, it could be seen that the trend of the growth rate kept increasing until the last day of the experiment. It was also shown from the equation that the amount of percentage that increasing each day was around 4.464%. The phytoremediation in this experiment could also be stated quite successful because the acclimatization period of the *Lemna minor* was not too long and most *Lemna minor* did not died in the initial days of experiment. Hence the daughter fronds could be reproduced to replace the old fronds and absorb the excess nutrients that were filled in the aquariums (Jujea, Munteanu, & Varcolici, 2019).

In the fifth experiment, the aquariums that were filled with *Lemna minor* plant seeds were actually added by sodium bicarbonate. The effect of the sodium bicarbonate was actually providing pH stability along the experiment days (Gerardi, 2002). The adding of the sodium bicarbonate was occurred to help the problems of the previous experiment such as the pH that kept increasing due to the nitrate absorption from the *Lemna minor* which came from the nitrification reduction product (Burton & Stensel, 2004). Along with that phenomena, the sodium bicarbonate also added to wastewater to give back the alkalinity that was lost due the nitrous acid formation that removed the alkalinity from the wastewater according to (Gerardi, 2002) that was filled in the aquariums from the previous equipment because the pH kept that kept rising could not be guaranteed that the alkalinity was still resided inside the wastewater (Burton & Stensel, 2004).

Alkalinity that was returned inside the aquarium was very important due to some effect from the denitrification that took place at the same time. Denitrification itself was defined as the reduction of nitrates to nitric oxide, nitrous oxide, and nitrogen gas. The reaction of denitrification would be shown below:

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$$

Based on the reaction above, it would cause the pH value would be elevated in the anoxic area that had lower dissolved oxygen concentration and some of the alkalinity that was destroyed in the nitrification process to return, but in this case of the experiment these alkalinity returned not have a sufficient effect and only adding a high number read of pH value which later would disturbed the *Lemna minor* growth (Han & Lu, 2019).

Alkalinity was a very important factor to determine the growth rate of the *Lemna minor* which was proven from the coverage area which was kept increasing until the last day and had been calculated by ImageJ Software analysis. The percent coverage value that had been calculated was 65,8925 %. This prove that if the alkalinity was not returned in time of the nitrification like in experiment two then when the pH kept rising and not suitable with the *Lemna minor* condition hence that the phytoremediation effect could not work properly according to (Hasan & Saeed, 2018) and the nitrates had been reduced with denitrification process and the amount of the alkalinity returned was already too late and only added the pH value to kept on rising uncontrolled (Burton & Stensel, 2004).

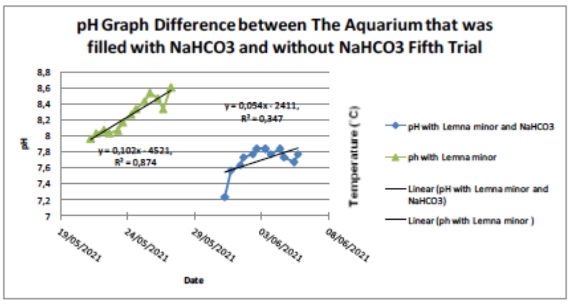

Figure 6. pH Chart Comparison Between *Lemna minor* Growth With and Without Sodium Bicarbonate

From the chart comparison, it could be seen that the pH chart with sodium bicarbonate was gradually increased and stable throughout the experiment days compared to the fourth experiment that kept increasing significantly until the last day of the experiment. In this case the stability could be seen from the coefficient changes of the pH from the fifth experiment compared to the fourth experiment had smaller coefficient changes with 0.054 compared to 0.102 changes in terms of pH value. From this chart also answered the capability of the sodium bicarbonate that was used as the pH stability and alkalinity source at this experiment according to (Burton & Stensel, 2004) which was crucial for the *Lemna minor* growth and absorption ability to absorb the excess nutrients (Halim & Hanafiah, 2020).

5. CONCLUSION

The coverage area that was filled with *Lemna minor* and area that was filled without *Lemna minor* was able to be calculated by the ImageJ Software analysis with the help of color threshold method which was very useful to select and differentiate the color of the *Lemna minor* with the rest of the areas. The percent coverage area was also able to be calculated due to the coverage area was achieved and percent coverage area was able to be examined from day to day throughout the experiment.

The sodium carbonate in this experiment played a very huge role as a source of alkalinity due to the lost of alkalinity during the previous experiments and pH stability substances. This was proven from the second experiments that without the sodium bicarbonate, the experiment had some ups and lows. When all of the external factors were achieved and liked by the *Lemna minor*, it was quite successful for the *Lemna minor* growth and absorption of excess nutrients from the liquid organic fertilizer. Since not all the repetitions of the experiment had the same achieved external factors, so some of the experiments made the *Lemna minor* growth not optimal and made the *Lemna minor* died along the way. That was why the bicarbonate ions played determining factor for the experiments. In the fourth experiment where sodium bicarbonate was added, the growth of *Lemna minor* was more stable.

pH comparison between aquarium with and without sodium bicarbonate also stated the importance of alkalinity towards *Lemna growth* minor. With addition of sodium bicarbonate, pH is more stable hence *Lemna minor* have shorter acclimatation time in comparison without the addition of sodium bicarbonate.

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