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Bioremediation of Sugar Waste Water Using *Nanochloropsis oculata* to Reduce Pollutant Level and Turbidity

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ABSTRACT

Total sugar production in Indonesia reaches 2.1 million tons annually. The large amount of sugar production in Indonesia causes the extracted waste water to have an impact on the waters due to contamination, deoxygenation by pollutants, and cloudy air. There needs to be an effort to handle sugar waste so as not to harm the environment and endanger living things. This study aims to determine the bioremediation process of Microalgae *N. oculata* as an effort to reduce turbidity and pollutant levels in sugar factory waste water. The method used in this study is an experimental method consisting of five treatments with a ratio of microalgae and sugar waste water in milliliters, namely P0 (0:1000), P1 (300:1000), P2 (300:800), P3 (300:1000). 600), P4 (300:400), and P5 (300:200). The stages of this research were sampling of sugar factory effluent, inoculation of microalgae *N. oculata* into waste for 16 days, and testing of the inoculation results. Parameters of the tests carried out included cell density, Pollution Index, turbidity, TSS, COD, BOD, pH, TP temperature and salinity. The data were analyzed by determining the percentage of decrease in each parameter and calculating the Pollution Index (PI) of the overall parameter. Based on the research, microalgae inoculation was able to reduce turbidity and pollutant levels with the best results determined based on PI calculations, namely P1 and P2 treatments with values of 0.971 and 0.931 where these results were interpreted as research waste according to quality standards.

Keywords: *Bioremediation, Microalgae, Sugar factory, Waste water treatment*

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1. INTRODUCTION

Indonesia has 62 sugar industry units, with details of 50 units managed by BUMN and 12 units managed by the private sector (Kumalaningsih, 2017). Every year, the total sugar production reaches 2.1 million tons. The main contribution of sugar producers in East Java (43.29%), Lampung (25.71%), Central Java (10.07%), and West Java (5.87%)

(Direktorat Jenderal Perkebunan, 2009). The by-product of the sugar industry is liquid waste, which can potentially cause contamination, deoxygenation, cloudy water, and a strong odor in waters caused by biodegradation in the form of hydrogen sulfide gas (Ekawati, 2016). Liquid waste from sugar factories has a pH content of 5.2-6.5, a Biological Oxygen Demand (BOD) content of 1000-4340 mg/L, a Chemical Oxygen Demand (COD) content of 350-2750 mg/L, a Total Suspended Solid content (TSS) of 760-800 mg/L and Volatile Suspended Solids (VSS) content of 163-2190 mg/L (Hofita & Russo, 2019).

Sugar waste water contains various substances, such as sugar cane juice wasted during the sugar production process, detergents, bagasse/bagasse particles, oils and fats used as lubricants, and sugar solids lost during the production process. The sugar industry produces waste water with a high pollution load (Syaifi, 2017). Waste water from sugar factories contains carbohydrates and protein. Sugar waste water also contains oil, alkaline compounds, and acids. Suppose these organic materials are thrown into the water. In that case, they can cause the amount of dissolved oxygen in the water to decrease because aerobic bacteria use them to decompose the organic materials. As a result, fish and other aquatic organisms die because dissolved oxygen is depleted (Siregar, 2005).

As public awareness increases to improve the environment, bioremediation is starting to be applied to overcome pollution caused by hazardous waste (Sjahruland Arifin, 2010). In its development, bioremediation can be used to reduce pollutants in inorganic and organic waste under controlled conditions to reduce and control pollutants from the environment. Microorganisms in bioremediation aim to degrade environmental contamination into a non-toxic form (Hardiani et al., 2016).

Bioremediation of sugar waste water can be carried out using microalgae because it has advantages, one of which is that the principles used in the processing process run naturally so that they do not produce secondary waste. Microalgae were chosen because they reproduce quickly, are abundantly available, are tolerant of toxins, and are non-pathogenic (Hadiyanto et al., 2021). This research uses the microalgae *N. oculata* as a bioremediation agent to reduce turbidity and pollutant levels in sugar factory waste water (Halima, 2019).

N. oculata is a microalga characterized by having non-motile greenish cells; the cells are spherical, have no flagellum, are small in size, and live in the sea, brackish water, and freshwater (Mahdi et al., 2022). *N. oculata* has pigments containing astaxanthin, canthaxanthin, and zeaxanthin and has protein content such as high levels of chlorophyll and carbohydrates (Firgiandini, 2019). *N. oculata* has excellent potential in bioremediation because it has important nutrients such as protein content of 52.11%, Omega 3 HUFAs of 42.7%, lipid content between 31-68% dry weight, and Eicosapentaenoic Acid (EPA) content of 30.5 %. The ability of *N. oculata* to adsorb is relatively high because it has amide, amine, and carboxylic functional groups, which are related to metal ions (Nabilah, 2020).

One alternative that can be done to overcome this is to process sugar waste waterso that the level of turbidity and pollutant levels (BOD et al., pH, salinity) can decrease (Arsyad, 2016). Aeration is helpful for increasing dissolved oxygen levels in water, stirring the water, and increasing the viability of the microalgae *N. oculata* (Ratnawati, 2011).

This research aims to reduce the level of turbidity and pollutant levels in sugar factory liquid waste using *N. oculata* microalgae bioremediation. It is hoped that the results of this research can be used as an alternative to reduce water pollution, which can endanger the aquatic environment and the health of living creatures.

2. METHODS

The method used in this research was an experimental method using five treatments and three repetitions, so that 15 experimental units could be obtained Table 1. Only the sugar waste waterwas changed according to the treatment while the formulation of the microalga *N. oculata* remained the same.

Table 1 Treatment Formulation

Treatment	Formula		
P0	1000 ml Sugar waste water (Control)		
P1	Mikroalga 300 ml	:	1000 ml Sugar waste water
P2	Mikroalga 300 ml	:	800 ml Sugar waste water
P3	Mikroalga 300 ml	:	600 ml Sugar waste water
P4	Mikroalga 300 ml	:	400 ml Sugar waste water
P5	Mikroalga 300 ml	:	200 ml Sugar waste water

Waste Water Sampling

Sugar waste water is obtained from a sugar processing factory in Kudus City, Central Java. Sampling uses black jerry cans, which prevent light from entering the waste. Before using the jerry can, it is best to clean the inside by rinsing it using the waste water that will be collected. The sample is slowly put into the jerry can. Samples were tested for physical parameters in the form of turbidity, TSS, and temperature, as well as chemical parameters in the form of BOD, COD, Total Phosphate (P), salinity and pH.

Inoculation of Microalgae into Sugar Waste Water

Inoculate the microalgae *N. oculata* into the sugar waste water sample by adding 300 ml of microalgae according to the treatment into the sugar waste water and then leaving it for 16 days.

Waste Water Testing

Sugar liquid waste was tested before and after inoculation on microalgae. The test consists of measuring cell density, physical parameters in the form of turbidity, TSS and temperature, as well as chemical parameters in the form of BOD, COD, Total Phosphate (P), salinity and pH. Measurements on day 1 and day 16 include cell density measurements, physical parameters and chemical parameters. The routine measurements include cell density, temperature, salinity and pH. The measurements of physical and chemical parameters are based on the test methods in Table 2.

Table 2 Testing Methods

Testing	Method	
BOD	SNI 6989.72:2009	Central Java Province Regional Regulation No.5 of 2012
COD	SNI 6989.2:2019	Spektrofotometre
TSS	SNI 6989.3:2019	Gravimetry
Total P	APHA 4500-P C, 2016	Phosphorus
pH	SNI 06-6989.11- 2004	pH meter
Turbidity	SNI 06-6989.25-2005	Nefelometer

Data analysis

The data obtained from bioremediation testing using the microalga *N. oculata* were then collected, averaged and analyzed for percentage reduction in cell density, physical parameters (turbidity, TSS and temperature), chemical parameters (BOD, COD, Total

Phosphate (P), salinity and pH). The chemical and physical parameters are then calculated and the percentage reduction for each parameter is calculated. The percentage reduction equation is presented in the equation:

$$\% \text{ Reduction} = \frac{N_1 \; x \; N_{16}}{N_1} \text{ x } 100\% \quad (1)$$

Where N1 is the parameter value for the first day and N16 is the parameter value for the 16th day. If the percentage shows a negative number, it means that the parameter value has increased.

Water quality status uses the Pollution Index (IP) method based on the Decree of the State Minister for the Environment 115 of 2003 concerning guidelines for determining water quality status. PI calculation using Excel by entering the following equation:

$$PI = \sqrt{\frac{\frac{(C_i}{L_{ij})_M^2}+\frac{(C_i}{L_{ij})_R^2}}{2}} \quad (2)$$

Li: Concentration of water quality parameters in water use quality standards (j)
Ci: Concentration of water quality parameters survey results
PIj: Pollution index for designation (j) (Ci/Lij)M: Maximum Ci/Lij value
(Ci/Lij)R: Average Ci/Lij value

The evaluation of the overall PI value is done by comparing the calculation results with the standard PI below.

$0 \leq PI \leq 1$ = Meets Quality Standards (good condition)
$1 < PI \leq 5$ = lightly polluted
$5 < PI \leq 10$ = moderately polluted
$PI \geq 10$ = heavily polluted

3. RESULTS AND DISCUSSION

Microalgae Density

Microalgae cell density is an important parameter and influences waste water quality parameters. The initial cell density of the microalga *N. oculata* which was inoculated with sugar waste water was 12,000,000 cells/ml. The cell density of the microalgae *N. oculata* was then calculated on days 4, 7, 10, 13 and 16. The results of observations of the density of the microalgae *N. oculata* are presented in Figure 1.

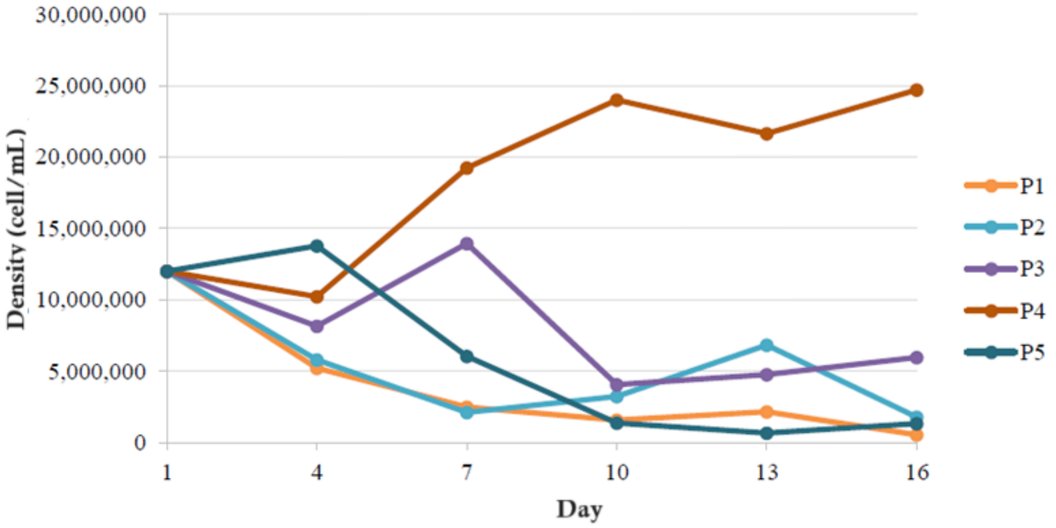

Figure 1. Cell Density of *N. oculat*a with Sugar Liquid Waste Concentration Treatment

Description: P1 (1000 ml of sugar waste water with 300 ml of *N. oculata*), P2 (800 ml of sugar waste water with 300 ml of *N. oculata*), P3 (600 ml of sugar waste water with 300 ml of *N. oculata*), P4 (400 ml of liquid waste sugar with 300 ml *N. oculata*), P5 (200 ml sugar waste water with 300 ml *N. oculata*).

Microalgae density can be interpreted. Based on the graph in Figure 1, it can be seen that *N. oculata* cell growth was highest at the P4 concentration, while cell growth was lowest at the P1 concentration of liquid sugar waste.

The growth phase of microalgae is characterized by the number of cell densities. The growth phase of microalgae is divided into 4 phases, namely the lag phase, exponential phase (log phase), decreasing log phase, stationary phase and death phase. The lag phase is the adaptation phase of microalgae to sugar waste water media. In the lag phase, microalgae adapt to new environmental conditions, characterized by decreased cell density. Based on Figure 1, the lag phase of the microalgae *N. oculata* for each treatment is different. Treatments P1, P2, P3 and P4 respectively require an adaptation time of 10 days, 7 days, 4 days and 4 days. P5 treatment may experience a short adaptation time between days 1-4. The adaptation phase will be shorter or even invisible if the inoculated cells come from a culture that is in the exponential phase (Registra et al, 2017). The difference in adaptation time for each treatment is due to differences in the volume of sugar waste water.

The exponential phase begins with an increase in cell density for several days until it reaches a peak. Each treatment entered a different exponential phase marked by an increase in cell density after the lag phase. Hadiyanto and Azim (2012) explained that the exponential phase is characterized by an increase in cell density. Based on Figure 1, Treatments P1, P2, P3, P4, and P5 experienced an exponential phase on day 13, day 13, day 7, day 10, and day 4. The differences in exponential phase time are influenced by the different inoculum volume and growth speed of the *N. oculata* microalgae.

The next phase is the stationary phase which is characterized by a decrease in the rate of cell growth. The decline in growth is generally influenced by biomass that has reached the maximum population stage, so that the need for food in the medium is reduced. The decrease in growth rate in the stationary phase is due to the formation of secondary metabolite compounds. Cell metabolism products that accumulate in the culture media can inhibit cell metabolic processes and limit nutrients (Dwilda et al, 2012). Based on Figure 1, Treatments P1, P2, P3, P4 and P4 experienced a decrease in stationary phase (log phase) after day 13, day 13, day 7, day 10 and day 4. However, in treatments P3, P4 and P5 there was an increase in cell density on day 10, day 13 and day 13. The increase in cells is thought to be because there are some microalgae cells that are living/dividing again (Simamora et al., 2017).

The final phase is the death phase which is characterized by a decrease in cell density. In this phase, the number of dead microalgae cells is greater than the number of living cells. This is because the availability of nutrients is becoming increasingly low (even depleted), food reserves in the body's cells are decreasing, and the accumulation of toxins is increasing. Dead microalgae cells can even lyse (break apart) and dissolve into the medium. Based on Figure 1, treatments P1 and P2 experienced a decrease in cell density from day 13 to day 16, approaching zero. A cell density that is close to zero indicates that nutrients derived from sugar waste water can be absorbed optimally, thereby reducing pollutant levels. In addition, the cell density that is close to zero causes the color of the waste resulting from inoculation with microalgae to be clearer than in treatments P3, P4, and P5.

Sugar Waste Water Quality Index

Determining the waste water quality index is used to simplify several types of parameters into certain values that can describe water quality so that they are easy to understand. The method used in this research to determine the waste water quality index is by using the Pollution Index (PI) method. This method refers to the Decree of the Minister of Environment No. 115 of 2003 concerning Guidelines for Determining Water Quality Status where the calculated PI value is divided into 4, namely $0 \leq PI \leq 1.0$ according to quality standards or good, $1.0 < PI \leq 5.$ .0 Lightly polluted, 5.0 value $< PI \leq 10$ Moderately polluted, and PI value > 10 Heavily polluted. The PI value is determined from the result of the maximum value and the average value of the concentration ratio per parameter to the quality standard value. The reference quality standards used are based on waste water quality standards for sugar factories with a capacity of less than 2,500 tonnes of sugar cane as regulated in Central Java Provincial Government Regulation Number 5 of 2012 concerning Waste Water Quality Standards. Water quality status shows the level of water quality conditions by comparing predetermined quality standards. The results of calculating the waste water quality index using the PI method are presented in Figure 2.

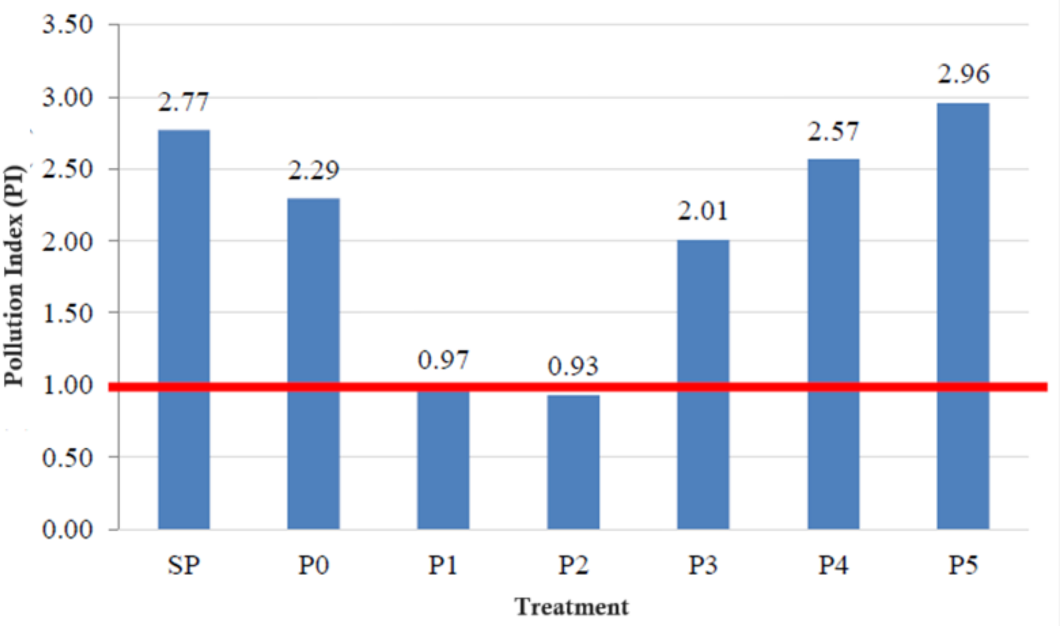

Figure 2. Waste water Quality Index

Based on Figure 2 treatments P1 and P2 have an PI value below 1, which means that treatments P1 and P2 meet the quality standards for sugar waste water in Central Java province number 5 of 2012. This is different from treatments P3 and P4, although they are able to reduce the PI of liquid waste. with the addition of microalgae, treatments P3 and P4 were still lightly polluted. The percentage reduction in PI in treatments P1, P2, P3 and P4 was 64.92%, 66.36%, 27.46% and 7.57%. This is different from treatment P5 which experienced an increase in the PI value of 6.72%. The increase in the PI value in the P5 treatment was due to the greater number of microalgae inoculated in the liquid sugar waste, namely 300 ml with 200 ml of sugar waste. Too many microalgae compared to sugar waste causes the growth of microalgae cells to be less than optimal due to limited nutrients from sugar waste, thus causing an increase in the PI value of the waste due to an increase in waste pollutants such as COD.

Biological Oxygen Demand (BOD)

Biological Oxygen Demand (BOD) is a measurement of the amount of oxygen needed by bacteria to break down almost all dissolved and suspended organic substances. BOD value is an important parameter. Asdak (2018) stated that the greater the BOD index number for a body of water, the greater the level of pollution that occurs, and in general a high BOD number indicates that the concentration of organic matter in the water is also high (Grass and Beach, 2008). The BOD measurement results are presented in Figure 3.

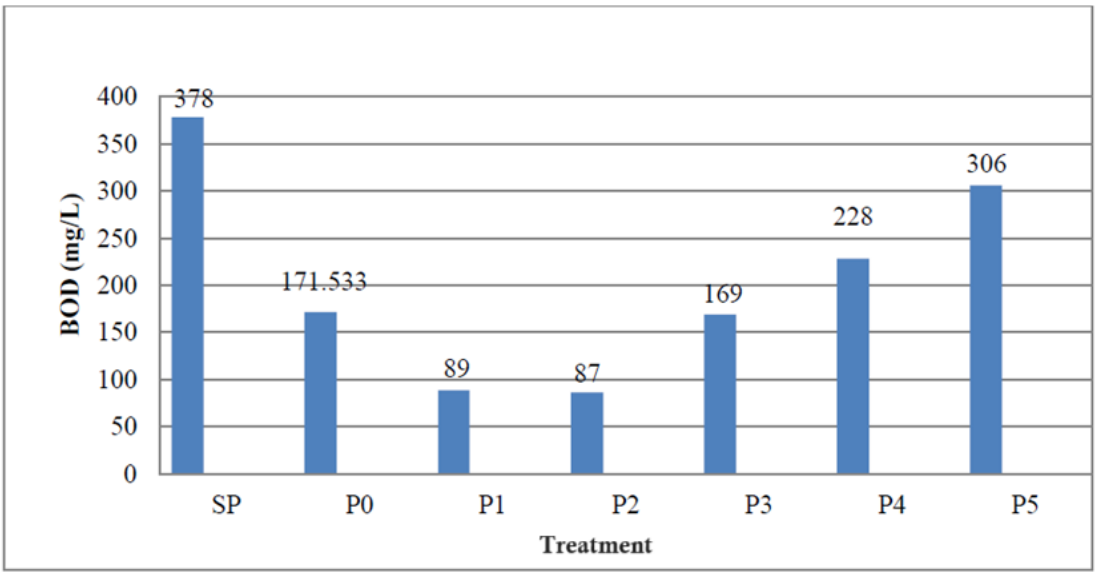

Figure 3. Average of BOD concentration

Based on Figure 3, it is known that the BOD value in sugar waste water before giving *N. oculata* was 378 mg/L. The BOD value exceeds the maximum limit of sugar factory liquid waste quality standards based on Central Java Provincial Regulation number 5 of 2012 which has been set at a maximum of 100 mg/L. Sugar waste water that is discharged directly into water bodies exceeding the maximum quality standards can cause environmental pollution. Giving *N. oculata* is one of the efforts used to reduce the BOD value of liquid sugar waste. The results of waste inoculation with the microalga *N. oculata* at P1 were 89 mg/L with a reduction percentage of 64.919%, P2 became 87 mg/L with a reduction percentage of 64.112%, the 3rd treatment became 169 mg/L with a reduction percentage of 31,854 % and the 4th treatment reduced BOD levels to 228 mg/L with a reduction percentage of 8.064%.

According to Kawaroe (2010), the decrease in BOD after inoculation of the microalgae *N. oculata* was caused by the ability of the microalgae to biologically process factory waste water media and provide nutrient input for growth. The microalga *N. oculata* causes a decrease in the BOD value in waste because the organism absorbs organic compounds in the waste. The growth of *N. oculata* requires organic compounds as nutrients and in the process of absorbing these organic compounds it requires oxygen (O2) for photosynthesis reactions. The results of the photosynthesis reaction are used by *N. oculata* to optimize the decomposition of organic compounds in liquid sugar waste. According to Fardiaz (1992), high photosynthetic activity can produce high levels of oxygen so that the dissolved oxygen contained in liquid waste will increase. The amount of dissolved oxygen in waste water will stimulate the work of microorganisms in breaking down polluting compounds.

The decrease in BOD values was also seen in the control treatment (P0), which experienced a decrease in BOD values of 54.6% even without inoculation of the microalgae *N. oculata* for 16 days. It is suspected that there are decomposing bacteria in sugar waste water that live naturally and when allowed to stand cause evaporation, resulting in a decrease in BOD levels.

Chemical Oxygen Demand (COD)

COD levels indicate the total amount of oxygen needed to chemically oxidize organic materials into CO2 and H2O. The COD value reflects the organic matter content of waste water, including organic matter that cannot be biodegraded (Mahida, 1993). Increasing COD content can affect the pH value, brightness level and dissolved oxygen content (Apriadi, 2005).

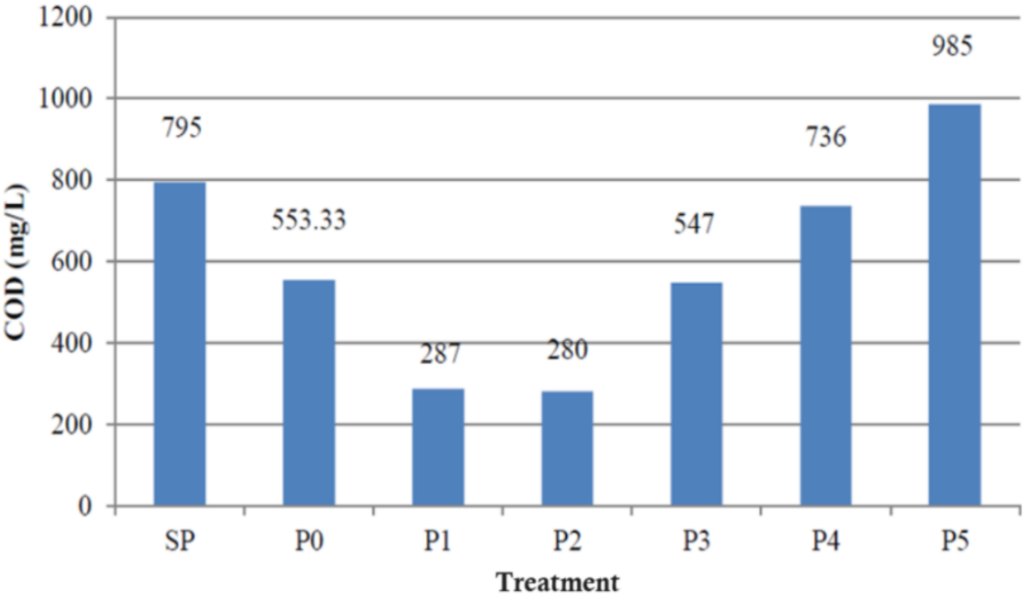

Figure 4 Average of COD concentration

Observation results in Figure 4 show COD levels ranging from 280 to 985 mg/L. COD levels in sugar waste water before giving *N. oculata* were 354 mg/L then decreased by 63.9%, 64.7%, 31.24% and 7.48% in treatments P1, P2, P3 and P4 after inoculation *N. oculata* with sugar liquid waste. The reduction in COD levels in this treatment was due to *N. oculata* being able to absorb organic and inorganic compounds contained in liquid sugar waste. These organic compounds are nutrients needed by *N. oculata* for its growth. During treatment, organic and inorganic compounds will decompose into proteins and amino acids naturally by bacterial activity which breaks down complex compounds into simple ions which are ready to be used as nutrition for the microalga *N. oculata* (Septory and Triyatmo, 2016). During the nutrient absorption process, microalgae need oxygen to absorb organic compounds in sugar waste water as well as an effort to reduce waste. This ability to grow can be seen from the positive trend in the amount of dissolved oxygen as an effect of optimal photosynthesis processes.

The increase in COD levels in P5 by 23.9% was possible because the number of microalgae was greater than that of liquid sugar waste. This causes microalgae to experience a fairly long adaptation period. The adaptability of microalgae is influenced by organic and inorganic compounds in the media used as a source of nutrition in this research, namely liquid sugar waste. If there is none or the amount is too large, a reduction process of organic compounds will occur so that the growth of microalgae is hampered, which is marked by an increase in COD levels due to the production of dissolved organic carbon (DOC) originating from hydrocarbon compounds originating from sugar factories, namely carbohydrates, saccharose, fructose, or derivatives. other disaccharides (Rusdiana et al., 2020). DOC production in water occurs due to the release of intracellular compounds (Isnadia, 2013). The process of releasing intracellular compounds is called lysis, whether experienced by algae or bacteria (Puspitasari et al., 2014). This intracellular compound easily dissolves in water, thereby increasing the COD concentration in the water.

Total Suspended Solids (TSS)

Total Suspended Solid (TSS) is the amount of weight in mg/L of dry sludge in waste water after being filtered with a 0.45 micro membrane (Sugiharto, 1987). High Total Suspended Solids (TSS) will prevent sunlight from entering the water, thereby disrupting the photosynthesis process which causes a decrease in dissolved oxygen released into the water by microalgae, thereby disrupting the aquatic ecosystem. In addition, if this amount of suspended material settles, the formation of mud can disrupt the flow and cause shallowing (Soemirat, 2004). Conversely, the lower the TSS level, the easier it is for sunlight to penetrate water bodies so that microalgae can carry out the photosynthesis process.

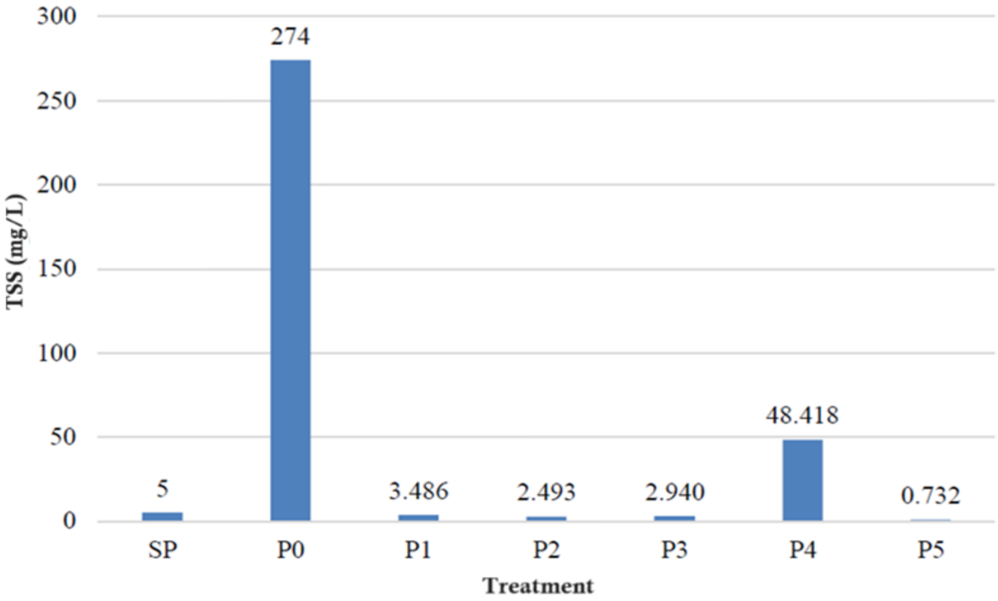

Figure 5. Total Suspended Solid Levels

Based on the research results in Figure 5, TSS levels before treatment were 5.00 mg/L and after inoculation with *N. oculata* for 16 days were able to reduce TSS in treatments P1, P2, P3, and P5 each with a percentage reduction of 30. .23%, 50.13%, 41.19% and 85.37%. The decrease in TSS levels is due to microalgae being able to absorb pollutants as a source of nutrition, or indirectly providing a growing medium for microalgae which is able to break down pollutants and supply oxygen in the aerobic decomposition process (Sunanisari, 2008).

The control treatment without the addition of *N. oculata* (P0) after 16 days showed a percentage increase in TSS levels of 5380%. This is due to the absence of the addition of the microalga *N. oculata* so that suspended solids in the form of organic materials will decompose and the solid materials will float due to the pressure of gas which causes a foul smell and floating dirt.

P4 treatment had a percentage increase in TSS levels of 868%. An increase in TSS levels in P4 allows for a very significant increase in cell density, causing high TSS levels. The amount of solid substances suspended in waste is influenced by the process of absorbing nutrients by the microalga *N. oculata.* This result is in accordance with the total phosphate test where the P4 treatment has the smallest total phosphate compared to other treatments, namely 0.001 mg/L.

Total Phosphate (TP)

Phosphate (P) is a macronutrient needed by microalgae for cell growth, photosynthesis, energy transformation and chlorophyll formation (Kabinawa, 2001). Phosphate levels influence the growth of the microalgae *N. oculata* cells in the bioremediation process. Based on the research results in Figure 6, the microalga *N. oculata* can absorb phosphate (as total P) in sugar factory liquid waste in quite large quantities within 16 days.

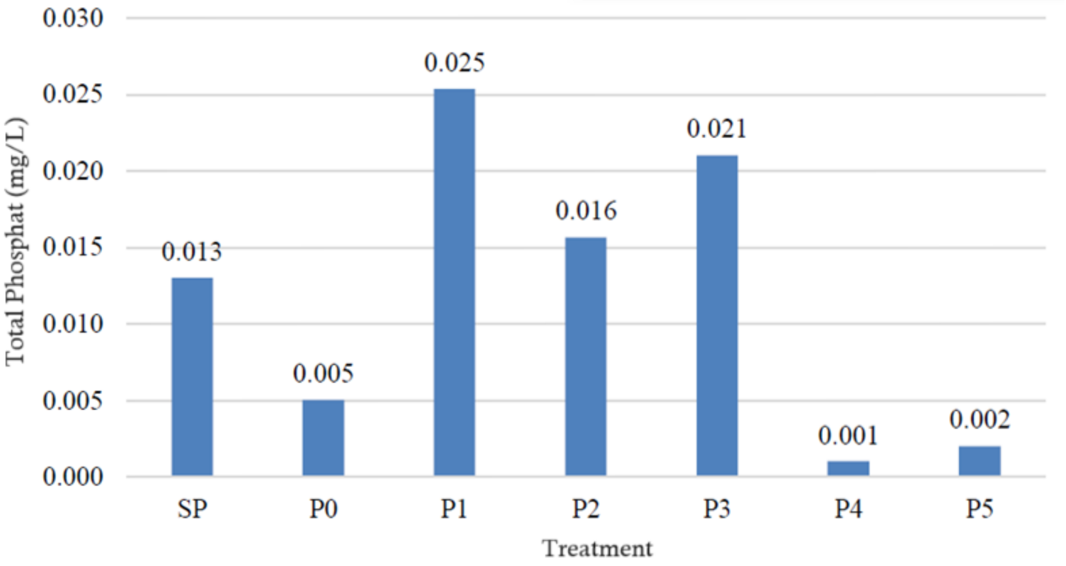

Figure 6. Total Phosphate

The absorption of phosphate levels is characterized by a decrease in phosphate levels after the waste is inoculated by microalgae. Based on the research results in Figure 6, the initial TP content of sugar factory waste water was 0.013 mg/L and after inoculation with *N. oculata* for 16 days the percentage decrease in treatments P4 and P5 was 92.28% and 84.56%. The decrease in phosphate levels was caused by the microalga *N. oculata* utilizing phosphate for cell growth so that the TP levels in the waste were reduced. The largest percentage reduction in TP levels in the P4 treatment reached 92.28%, comparable to the increase in cell density of the microalgae *N. oculata* in the P4 treatment which was 105.9%.

The percentage increase in phosphate levels after microalgae grafting was found in treatments P1, P3 and P2 at 94.89%, 61.59% and 20.56%. The increase in phosphate levels is also proportional to the cell density of the microalgae *N. oculata* which is relatively close to zero. An increase in the value of phosphate levels also indicates that pollution is occurring (Hardyanti and Rahayu, 2007). The increase in phosphate levels is due to sugar waste water containing sugar and organic acids which can be used as a source of nutrition. Microalgae secrete organic acids which can form complex compounds that are difficult to dissolve, causing decreased phosphate fixation and increased phosphate (Fitriatin et al., 2009).

Salinity, pH and Temperature

Salinity, pH and temperature are important parameters that influence the life and growth of aquatic organisms directly or indirectly. Microalgae have different adaptability according to changes in salinity and the type of their native habitat. The high difference in salinity with its native habitat results in difficult adaptations for microalgae and vice versa. The severity of the adaptation process affects the growth and reproduction of microalgae which is disrupted. *N. oculata* can grow at a salinity of 0-35 ppt, the optimum value of salinity that supports its growth is 20-25 ppt (Munandar, 2016). Figure 7 shows the salinity values for each treatment.

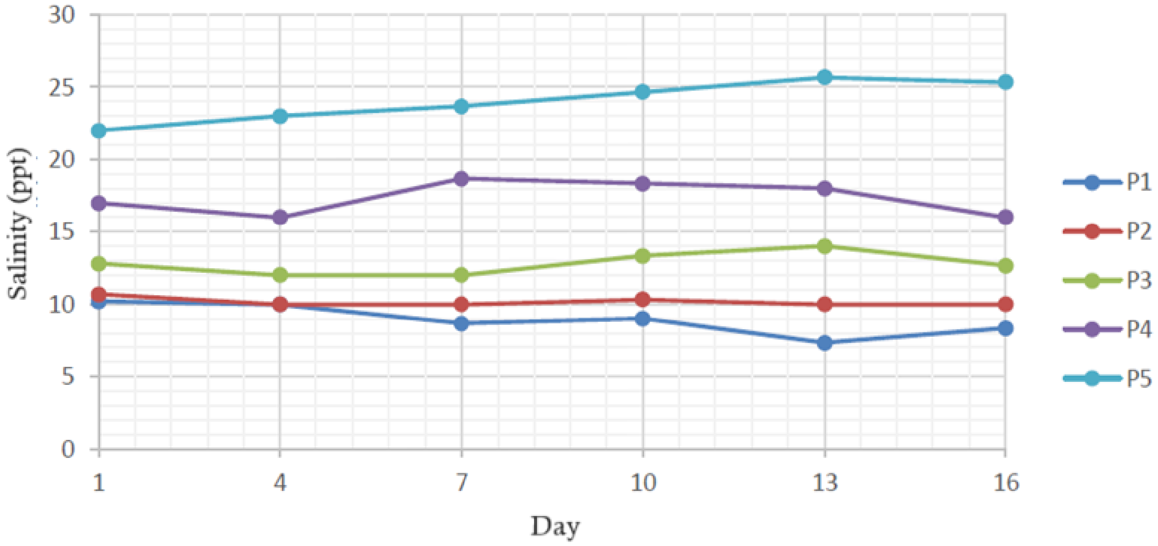

Figure 7. Salinity in Sugar Waste Water Treatment

Sugar waste has a salinity of 0 ppt and the salinity of the microalga *N. oculata* is 29 ppt. If the microalgae *N. oculata* is inoculated in sugar waste, the salinity of the mixture decreases. Based on Figure 7, salinity in all treatments is almost stable. The amount of salinity is influenced by the volume of sugar waste added to the microalgae *N. oculata*. The greater the volume of sugar waste used, the smaller the salinity will be. Optimum salinity for the growth of *N. oculata* ranges from 25 to 35 ppt (Hardiani et al., 2016). Salinity that is too high causes a decrease in cell growth, inhibits the respiration process, photosynthesis process and cell formation in the photosynthesis process (Murwani, 2016). Salinity has an effect on maintaining osmotic pressure with the environment. If the salinity is appropriate, there will be a balance in osmotic pressure between the *N. oculata* cells and the sugar waste watermedia so that the growth and development of *N. oculata* is not disturbed. The salinity in this study had an almost constant average for 16 days and there were no drastic increases or decreases. The rise and fall of salinity causes a decrease in population due to osmotic pressure and osmoregulatory mechanisms affecting metabolic processes.

The degree of acidity (pH) has very big role in reducing liquid waste pollutants, because bacteria grow well at neutral or alkaline pH, while fungi like acidic pH. The sugar waste water formulation added with 300 mL of microalgae has an influence on pH.

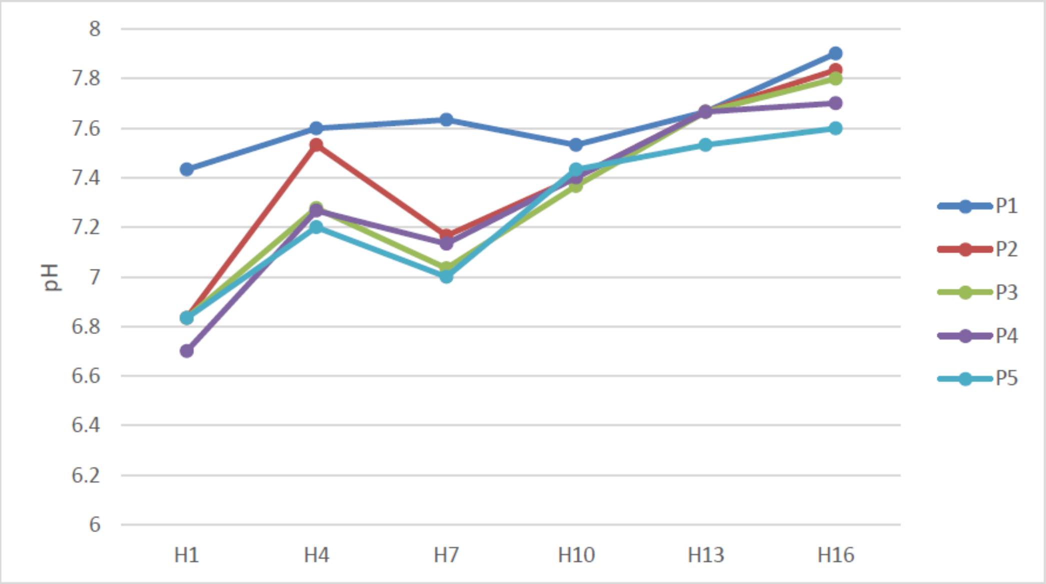

Figure 8. pH in Sugar Waste Water Treatment

Based on Figure 8, the pH value in each treatment of sugar waste wateradded with the microalgae *N. oculata* increased during the processing process. The results of optimal bioremediation in this experiment show that the final pH of the waste is around 7.9, as it is known that at a pH of 6 - 9, biota life in waters can occur normally, both animal and aquatic plant life (Yusuf, 2008). The increase in pH from acidic to alkaline in sugar waste water is estimated by the activity of microorganisms either found in sugar waste water or occurs due to the help of the microalga *N. oculata*. The pH value increases due to the interaction between CO_2 dissolved in water. The higher the concentration of liquid waste provided, the higher the pH value can be seen, in treatments P1 to P5 it meets the quality standards set by Central Java Provincial Regulation Number 05 of 2012 (Hartini et al., 2017).

The high water temperature is caused by the intensity of sunlight entering the water body which is quite high because the sample measurement location is an open area which is directly exposed to sunlight. The intensity of exposure to solar radiation entering the sugar waste water grafting site.

The temperature measurement results in Figure 8 range between 26 – 33°C. According to (Purwanti et al., 2012), the optimum temperature for microalgae productivity ranges from 25 - 33°C. The temperature value of *N. oculata* on a semi-mass scale tends to change, especially on day 4, it has a temperature value that is relatively higher than the other treatments, because the research location is exposed to direct sunlight, but is still able to support phytoplankton life. (Purwanti et al., 2012) stated that the growth rate of microalgae or density decreased at maximum cell concentration due to an increase in temperature above 33 °C.

Turbidity

The level of water clarity (turbidity) is an indicator of the physical properties of water pollution. Sutrisno (2006) explains changes in the physical properties of water with the appearance of color and turbidity related to the mixing of water with organic materials. Sugar liquid waste produces liquid waste that contains a lot of organic substances because the raw materials for the sugar production process use organic materials. Organic substances that dissolve in water will experience decomposition and decay so that oxygen levels in the water drop drastically and cause aquatic biota to die. In addition, organic substances can be used as food for bacteria to support the growth process. This bacteria is a suspended substance so its addition can increase the turbidity of the water. Likewise, microalgae reproduce due to the presence of nutrients in the form of phosphate (P) and nitrogen

(N) so that it can increase the level of turbidity. The results of turbidity testing for each treatment are presented in Figure 9.

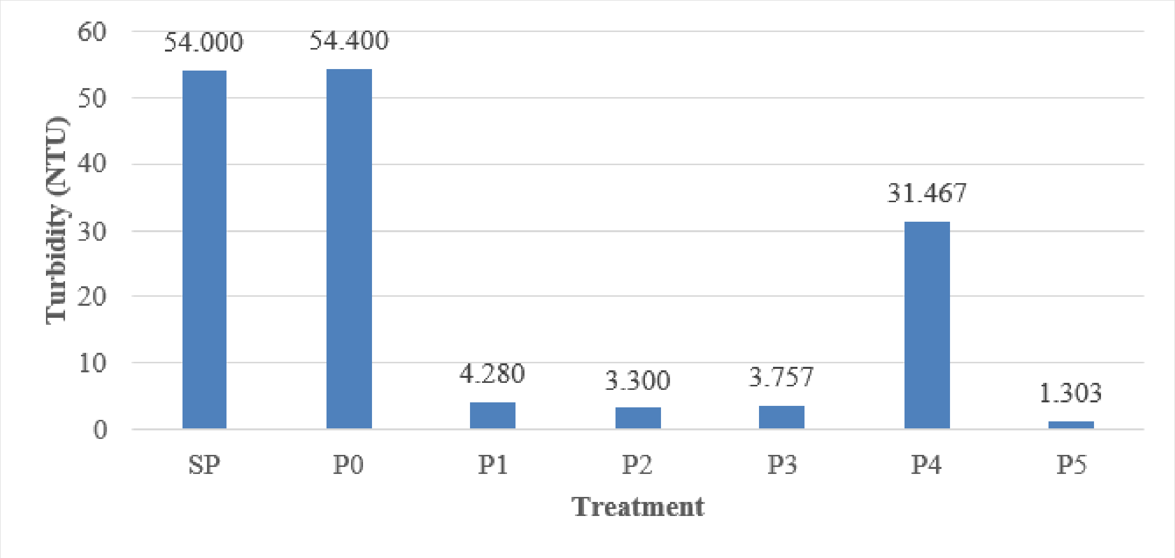

Figure 9. Average Turbidity

Based on Figure 9, it is known that the turbidity value in sugar waste water decreased after adding the microalga *N. oculata*. The percentage reduction in turbidity in treatments P1, P2, P3, P4 and P5 was 92.074%, 93.889%, 93.043%, 41.728% and 97.586%. The significant reduction percentage reaching an average of 94.148% proves that *N. oculata* has the ability to reduce turbidity levels and is very useful in the sugar factory waste water treatment process. These results are in accordance with research by Gupta (2020) that microalgae bioremediation can reduce turbidity levels by up to 78% with the smallest turbidity level value being 12.5 NTU. However, the concentration reduction percentage was less than optimal in the P4 treatment. This is thought to be because the cell density in the P4 treatment is very high, thereby increasing the turbidity of the sugar waste water.

4. CONCLUSIONS

Bioremediation using *N. oculata* can reduce turbidity and pollutant levels in sugar waste water. The results of calculations using the PI method show that treatments P1 and P2 have values of 0.971 and 0.931. These results show that treatments P1 and P2 comply with the quality standards that have been determined with a percentage reduction in PI in treatments P1 and P2 of 64.98% and 66.45%.

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