



Effect of Microalgae *Chlorella sp.* As a Phytoremediation Agent in Lowering Wastewater Pollutant Levels

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ABSTRACT

Wastewater contains many organic and inorganic contaminants that can cause severe damage to the environment and human health, therefore, it is necessary to eliminate all or part of the nutrients contained in wastewater and produce water that can be reused or suitable to be returned to its natural nature. One of the environmentally friendly and beneficial waste treatment technologies is biological waste treatment. Biological waste treatment system is still considered the cheapest way when compared to chemical means considering the price of chemicals is relatively expensive. Microalgae based on phytoremediation technique becomes a promising method because of its viability, one of the microalgae that can be used is *Chlorella sp.* *Chlorella sp.* is one of the microalgae chosen as a means of handling wastewater because this algae can multiply rapidly in its growing conditions, produce oxygen through the process of photosynthesis, contain high proteins with the main components of amino acids, and the structure of these algae cell walls are formed from various metric fibers of polysaccharides. In this article the potential of *Chlorella sp.* as a phytoremediation agent is seen from the content value of Phosphorus, COD, Nitrate, BOD, Nitrogen, TSS in wastewater that has been cultivated *Chlorella sp.* The purpose of this article is to describe the potential of *Chlorella sp.* as a phytoremediation agent against wastewater pollutants. *Chlorella sp.* cultivated in domestic wastewater can be grown by utilizing the content of organic matter and other nutrients contained in domestic waste polluted water for its growth nutrients. In adapting to polluted environments, *Chlorella sp.* requires an average adaptation period of 5 days. *Chlorella sp.*, adapts well to high concentrations of nutrients, and shows great potential in removing nutrients from wastewater. It is proven that the content value of Phosphorus, COD, Nitrate, BOD, Nitrogen, TSS contained in wastewater can gradually decrease along with the increase in biomass *Chlorella sp.* It shows the ability of *Chlorella sp.* to synthesize and accumulate it in their cells. Therefore, it can be concluded that the bioremediation of wastewater using *Chlorella sp.* is an effective and environmentally friendly option to improve water quality and recycle valuable nutrients that can be

Keywords: BOD, *Chlorella sp.*, COD, Total Nitrogen, Wastewater

INTRODUCTION

The increase in population in developing countries, especially in large cities is often accompanied by increased waste production. Problems arise when the available sanitation facilities are insufficient so as to have a negative impact on the environment and humans. Domestic waste discharged without treatment into the water can contaminate both surface water and groundwater. One example of water pollution by domestic waste is eutrophication, which can lead to algae blooms which can then be followed by decreased oxygen concentrations and mass deaths of fish in the waters. In addition to polluting the waters, domestic waste discharged without processing also has a negative impact on human health, as it can cause dangerous diseases such as cholera.

Domestic wastewater can be processed anaerobically and aerobically. It is mentioned that COD concentration above 4000 mg L⁻¹ is

no longer suitable for aerobic processing but more appropriate to be processed anaerobically. Some literature mentions that the total COD of black water in several different locations is more than 7 g L⁻¹.

One of the environmentally friendly and beneficial waste treatment technologies is biological waste treatment. Biological waste treatment system is still considered the cheapest way when compared to chemical means considering the price of chemicals is relatively expensive and the volume of wastewater is quite a lot. According to Hadiyanto (2013), the use of microalgae in processing wastewater is one of the alternative biological waste treatment. This is due to the abundant presence of microalgae and easy to obtain. Microalgae or algae are aquatic organisms better known as phytoplankton, which are generally unicellular species that can live solitary and colonize. One type of microalgae that has been widely known by the public is *Chlorella* sp. *Chlorella* sp. Included in the class Chlorophyta, which is a class of algae that has pigment chlorophyll a and chlorophyll b is more dominant than carotene and xanthophyll so that microalgae of this class show a clear green color like high-level plants. Microalgae is one of the photosynthetic microorganisms developed in addition to bacteria in the prevention of wastewater today. *Chlorella* sp is one of the microalgae chosen as a means of handling wastewater because this algae can multiply rapidly in its growing condition, easy in cultivating, producing oxygen through the process of photosynthesis, containing high proteins with the main components of amino acids. *Chlorella* sp does not need a large area when compared to other plants that are also used as phytomediation due to its micro size. Heavy metal remediation capability by algae is very good when compared with some microbes, fungi, because the structure of algae cell walls is formed from various metric fibers of polysaccharides.

Aulia et al suggest that *Chlorella* sp. able to set aside COD and Nitrates on tofu wastewater with consecutive allowance efficiency of 71.56% and 30.03%. In nature setting aside pollutants on microalgae organic wastewater can symbiotic with decomposing bacteria. In this case bacteria and microalgae symbiotic each other in decomposing organic substances present in organic wastewater, where bacteria can utilize the oxygen produced by microalgae and then produce CO₂ that can be reused by microalgae for photosynthesis. This article aims to find out how much influence *Chlorella* has in lowering levels of wastewater pollutants.

POTENTIAL OF *Chlorella* sp. AS A PHYTOREMEDIATION AGENT

Chlorella sp is a unicellular microalgae that is smaller than 10 µm in size and lives cosmopolitanally. *Chlorella* sp. has green leaves (chlorophyll-a), with the availability of H₂O, CO₂ and sunlight capable of photosynthesis to produce energy for cell biosynthesis, cell growth and growth, moving or moving and reproduction. *Chlorella* sp. It will grow normally on media containing at least 19 types of nutrients, which include macro nutrients (nutrients required in large amounts) such as carbon, hydrogen, oxygen, nitrogen, phosphorus, sulfur, sodium, magnesium, calcium. Nitrogen, phosphorus, and potassium as well as micro-nutrients (nutrients required in small amounts) such as magnesium (Mg), iron (Fe), copper (Cu), manganese (Mn), zinc (Zn), silicon (Si), boron (B), molybdenum (Mo), vanadium (V) and cobalt (Co)[1]. *Chlorella* sp. can change its metabolic pathway according to the supply of organic substrates, suggesting that it can sustain heterotrophic growth in addition to common autotrophic growth. *Chlorella* sp [2] is known to the public for its various benefits. In the field of health, *Chlorella* sp has been used as a dietary supplement that can cure a variety of diseases; and in the field of fisheries has long been used as the main food children fish / shrimp in the center / fish seed hall (hatchery).

In addition to utilization directly related to the biomass *Chlorella* sp; the benefits of *Chlorella* sp can also be obtained from metabolic processes, especially in the process of photosynthesis it performs. A chemical reaction that occurs in the process of photosynthesis. The knowledge that *Chlorella* sp will be able to perform photosynthesis to the maximum and efficient when the nutrient is sufficient, then by engineering the direction of reaction balance that occurs; *Chlorella* sp. It can be used to capture carbon dioxide, nutrients and heavy metals dissolved in water bodies, including industrial liquid wastewater bodies [3]. Heavy metal remediation capability by algae is very good when compared with some microbes, fungi, because the structure of algae cell walls is formed from various metric fibers of polysaccharides. The ability of *Chlorella* sp in absorbing heavy metals is supported by the ability to adapt, grow and also economically to be used as a remediation agent in polluted environments. In addition can be used also for bioremediation of heavy metals microalgae *Chlorella* sp can also be used as a precursor biodiesel because it contains 20-50% fat. Examples of wastewater is milk liquid waste, the waste is very easy to decompose / decompose release odors and nutrients (especially N and P) so that if before discharged into public waters are not processed properly will pollute the environment. Considering *Chlorella* sp is of high economic value, and through its photosynthesis is able to absorb nutrients quickly and optimally then the opportunity of utilization of *Chlorella* sp. to reduce the concentration of nutrients in wastewater, while producing biomass needs to be done.

GROWTH AND DENSITY OF CHLORELLA IN WASTEWATER

Nutritional factors are not a barrier to the development of green microalgae, so that cells can grow well. *Chlorella* is cultivated for 15 days in domestic waste polluted water media in containers with a photoperiod of 12 hours of light using 36 watts of TL lamp and 12 hours of dark without the use of lamps with a temperature range of 27.5-28.7°C. Based on the growth curve in the study [4] obtained that on the first observation that the 1st day until entering the 3rd day is a lag phase indicated by the addition of a small amount of biomass. In the second observation to the sixth observation, on the 3rd to the 13th day there is an exponential phase. This is indicated by the increasing amount of *Chlorella* sp. rapidly compared to the beginning of observation. The highest biomass was achieved on day 13. On the seventh observation that enters the 15th day there begins to be a decrease in biomass, so it is

known that the day enters the declination phase. *Chlorella* sp. mortality phase characterized by a considerable decrease in biomass, caused by a higher rate of cell death than the rate of cell growth so that biomass decreases further.

Then mentioned also in the study [5] which observed Chl-a and density that in the medium of milk waste, chl-a concentration at the beginning of the study decreased to 3 µg Chl-a per liter on the 7th day only then increased by following the tendency of density of *Chlorella* sp. Please note that Chl-a is one of the pigments in the Chlorophyta microalgae that plays a key role in the photosynthesis process. The presence of Chl-a in microalgae is determined by the dissolved nutrients in the growth medium; therefore the content of Chl-a per microalgae individual indicates also the productivity of the microalgae living media. This phenomenon occurred allegedly because at the beginning of the study, the increase in density of *Chlorella* sp was greater influenced by nutrients stored in the congenital cells of the initial seeding medium than the influence of nutrients in the initial LS media. Due to increased density by stored nutrients, the resulting individuals are less able to develop early LS media which is very minimal nutrients. Furthermore, after the 7th day it seems that the condition of *chlorella* sp population has become normal and grows under the influence of nutrients resulting from degradation of milk waste, so that changes in density, Chl-a and Chl-a content of each individual become aligned. Changes in density of *Chlorella* sp. in LSP media i.e. nitrogen enriched milk waste shows that during research the density of *Chlorella* sp tends to increase continuously. This indicates that at the beginning of the study, *Chlorella* sp could grow well due to the availability of nitrogen. Furthermore, the density of *Chlorella* sp in LSP media continues to rise until the end of the study. Chl-a concentration in LSP media, during the study increased until the 10th day with the highest concentration of 738 µg Chl-a per liter. Chl-a concentration in LSP media after the 10th day decreased, which is thought to be because the nutrient decomposition result of milk waste is no longer able to provide enough nutrients to meet the needs of the high density population of *Chlorella* sp. [5]

From the density can be concluded that the increase in the density of microalgae cells coupled with a decrease in the concentration of nutrients in the medium of growth, so that the concentration of nutrients as nutrients for microalgae is not proportional to the number of cell densities that exist. This causes the rate of increase in cell density to be reduced. Kabinawa explained that the number of cells decreases geometrically influenced by several factors, one of which is the availability of nutrients. The longer remediation time causes nutrient levels in wastewater to also decrease, thus not meeting the needs of *Chlorella* sp. togrow. The efficient growth of microalgae in wastewater is dependent on many reasons such as the types and the sources of wastewater [6]

REMOVAL OF NUTRIENTS IN WASTEWATER

Phosphorus

Phosphorus is used by microalgae for the synthesis of cellular constituents such as phospholipids, nucleic acids synthesis and associated reactions with cell division. According to the data, an initial phosphorus concentration of 2.51 mg/L was obtained. Along days, this concentration was progressively decreased to a final concentration of 0.02 ± 0.01 mg/L. So, after 9 days of culture, the removal efficiency of *Chlorella* sp. microalgae in synthetic wastewater was 99.2%. [7]

The gradual reduction in phosphorus levels of culture medium is due to the fact that this nutrient has been absorbed of wastewater by *Chlorella* microalgae, a nutrient necessary for its growth. Thus, phosphorus concentration in the medium is directly related to the growth of the microalga. Furthermore, it can be said that phosphorus concentration is often a limiting nutrient in microalgae growth and the cells can assimilate and store this nutrient diminishing the amount of phosphate in the wastewater.

COD

COD is an indicator of the presence of organic matter in wastewater [8]. So, in the experiments carried out, initial COD concentration in culture medium was 960 ± 1.5 mgO₂/L whereas 9 days later, this concentration was reduced at 277.3 ± 48.9 mgO₂/L. Thus, removal percentage efficiency at the end of the culture was 71.1%. On the basis of the obtained results, it can be concluded that *Chlorella* sp. microalga was able to reduce more than half of the chemical oxygen demand in the synthetic wastewater studied. The obtained results indicate that the levels of phosphorous and COD decreased rapidly due to the fast assimilation by *Chlorella* sp. microalgae in nine days of culture in wastewater. Some studies have demonstrated that this microalga can grow faster in the presence of organic acids or glucose that function directly as essential organic nutrients. It is mentioned that the provision of COD content in wastewater to be most effectively carried out by microalgae is at a concentration of 30%. This is because at this concentration the composition between microalgae and nutrients is ideal. When wastewater concentrations were increased to 40%, cod allowance efficiency decreased. The maximum value of percent cod removal occurs on the 14th day which is 77.8% [9]

Ammonia

NH₃-N content in wastewater was successfully reduced by 54.9% and 49%. The decrease in NH₃-N caused ammonia in waste to react to form ammonium [10]

Nitrate

In reducing pollution in marine water polluted by domestic waste, has a percentage decrease in the value of Nitrates by 91.76% and Ammonia by 76.17% so that the levels of pollutants in the media used are in accordance with quality standards for its designation [11]. Nitrates contained in household wastewater serves as a source of microalgae nitrogen in its growth [12]. Microalgae can reduce nitrogen compounds by 90% in domestic or household wastewater [4]

Bod

Biochemical Oxygen Demand (BOD) is the amount of oxygen (O₂) that must be used by microorganisms to oxidize organic substances present in the wastewater. High BOD content in industrial wastewater can cause water oxygen drop, anaerobic state (without oxygen) so as to kill fish and cause foul smell. *Chlorella* sp can also lower BOD levels, decrease in BOD value due to the process of photosynthesis of *Chlorella* sp [13]. The addition of EM4 further decreases the value of BOD, this is due to the symbiotic process of mutualism between microalgae and EM4. In contrast to the treatment without the addition of EM4, BOD dropped not as much as the treatment with the addition of EM4, this is due to the lack of activity of microorganisms that can decompose wastewater [14]. The decrease in COD and BOD concentration with 4 x 8 watt lighting intensity and 8-hour lighting cycle looks better than without lighting. The decrease in BOD and COD indicates the retrieval of organic materials by microalgae [15]

Nitrogen

Decrease in nitrogen concentrations occurs with the length of contact time. Total N level allowance deficiency with the addition of bacteria on the 7th to 13th day tends to be more significant when compared to the decrease in total N from day 0 to day 7. This is because at the beginning to the 7th day of the processing process, nitrification bacteria contained in Bioprisma play an active role in forming nitrate compounds as a source of nutrients for microalgae *Chlorella* sp., but microalgae that can utilize these elements are still in the process of adaptation and only grow in small quantities [16]. This is indicated by an insignificant increase in the number of microalgae cells at the beginning of the processing process until the 7th day. On the 7th day until the end of the processing process there is a significant increase in the number of microalgae cells due to the utilization of nitrogen content in wastewater tofu by microalgae so that the total N content in wastewater tofu decreases. Total N allowance efficiency continues to improve with contact time. According to Bahsan (2010), along with the length of time algae cells contact with wastewater, there is an increase in cell density so that nitrogen allowance is higher.[17]

TSS (Total Suspended Solid)

Total Suspended Solid (TSS) are suspended materials (diameter >1 µm) held on a millipore sieve with a pore diameter of 0.45 µm [18]. TSS consists of mud and fine sand and bodies of rhyzes mainly caused by soil erosion or erosion carried into the body of water. TSS parameters also play an important role in waste quality standards. From the value of TSS will be able to know if there has been pollution in the water, if the value increases significantly enough the water will appear murky and dirty so that of course reduce the usefulness of the water [19]

Heavy Metals

From research [1] obtained that the highest absorption of heavy metals by *Chlorella* sp in a row is Cr which is 33%, Cu by 29%, Cd by 15% and Zn by 8%. This indicates that *Chlorella* sp is able to adapt to polluted environments, adaptation period in polluted environments, on average requires 5 days. The rapid decrease in chrome levels indicates that the absorption capability of metals by *Chlorella* sp. very good at the beginning of contact [20]. A drastic decrease in heavy metals at initial exposure due to differences in affinity between metal ions and function groups on the surface of microalgae cells, resulting in tensile forces until a bond between the two (physical absorption) is formed that tends to take place in a short period of time

pH

The occurrence of increased pH during the study is assumed to be in line with the increase in algae density, where increasing algae density means increasing metabolism in breeding culture. Algae is able to use carbon dioxide as the primary carbon source for new cell synthesis and release oxygen through photosynthesis mechanisms. In addition to oxygen coming from the air, the largest oxygen supply is obtained from photosynthesis by microalgae [20]. Chemical reactions to dissociated carbonate and bicarbonate ions support the consumption of algae-bound CO₂ so that OH⁻terakumulasi and pH tend to increase [19]

CONCLUSION

Chlorella sp. cultivated in domestic wastewater can be grown by utilizing the content of organic matter and other nutrients contained in domestic waste polluted water for its growth nutrients. In adapting to polluted environments, *Chlorella* sp requires an average adaptation period of 5 days. *Chlorella* sp, adapts well to high concentrations of nutrients, and shows great potential in removing nutrients from wastewater. It is proven that the content value of Phosphorus, COD, Nitrate, BOD, Nitrogen, TSS contained in wastewater can gradually decrease along with the increase in biomass *Chlorella* sp. It shows the ability of *Chlorella* sp. to synthesize and accumulate it in their cells Therefore, it can be concluded that the bioremediation of wastewater using *Chlorella* sp. is an effective and environmentally friendly option to improve water quality and recycle valuable nutrients that can be used.

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