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Potential of Microalgae Growth in Laundry Effluents for Phosphate Phytoremediation

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ABSTRACT

The objective of this study is as a means of developing large-scale technology for phosphate wastewater management. Laundry activities are one of the largest producing phosphate contaminants by the use of detergent. Various contaminants, such as over nutrients of phosphate, chemicals and pathogens which can pollute the environment and endanger human health. The experiment was conducted by batch method by using water in a stationary or non-flowing state. The results showed that combining phytoremediation technology and monitoring microalgae growth phase could reduce TSS, pH, BOD_5, COD and phosphate values in wastewater. The treatment in this study was to combine two species of microalgae. Studies have shown that the optimal pH for microalgae is in the range of 7.5. Providing moderate amounts of aeration and CO_2 promoted algal growth. The decrease in phosphate levels was 27.86% with the best phase observation at the fourth hour of exponential time. Water quality evaluation of BOD, COD and TSS parameters had a decrease of 51.87%, 51.06% and 52%, respectively. Thus, it can be concluded that the combining of two species of microalgaes in the exponential growth phase have been proven to affect and improve the quality of wastewater from laundry waste and meet the quality standards.

Key Words	Wastewater, growth phase, phytoremediation, microalgae, phosphate
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INTRODUCTION

Laundry activities require the use of a lot of water and detergent so it is linear with the waste that will be generated (Lade & Gbagba, 2018; Lu & Leung, 2003; Pakula & Stamminger, 2010). One of the largest waste compositions in detergents is the builder with the compound sodium tripolyphosphate(Bajpai & Tyagi, 2007; Gary K. Morse, Roger Perry, & Lester, 1995). Waste phosphate compounds entering water bodies will result in over-nutrient events that trigger increased aquatic activity and stimulate logarithmic growth of certain plant species (Cunningham W.P, 2018; Fetahi, 2019; Nadia et al., 2007; Nyenje et al., 2010). Many systems have been used for removing phosphate in wastewater, such as coagulant neutralization mechanism (Owodunni et al., 2023; Patel et al., 2022), combination of electrocoagulation and photocatalyst process (Wibowo et al., n.d.), electrocoagulation method (Fayad et al., 2017), constructed wetlands and struvite precipitation (Reddy & Angelo, 1997).

Environmental considerations also underlie the development of biological wastewater treatment, where the adverse environmental impact is much smaller than chemical treatment (Dutta & Bhattacharjee, 2022; Liu et al., 2020). However, compliance with regulatory standards for effluent management often requires considerable cost and sophisticated technology. Therefore, there is an urgent need for practical and cost-effective wastewater treatment methods (Aslam et al., 2017; Saraswati et al., 2021; Waqas et al., 2023). It requires effective and efficient wastewater treatment technologies to overcome this problem (López-Ramírez et al., 2024; Yuan et al., 2019).

One other method that has been reported to reduce surfactant and phosphate levels is bioremediation (Scott & Jones, 2000). Bioremediation is responsible for degrading, removing, transforming, suppressing or detoxifying various physical and chemical wastes (Kulshreshtha et al., 2014). The chosen bioremediation technique is the utilisation of microalgae as a bioremediator or better known as the ficoremediation technique. Microalgae as a phytoremediator of laundry liquid waste with a focus on phosphate compounds because it considers the ability of microalgae to grow by utilising phosphate compounds as an energy source, so that phosphate waste produced in the laundry industry can be minimised by adding microalgae before the waste is released into the waters (Chai et al., 2020; Touliabah et al., 2022).

The utilization of microalgae as a phytoremediator is based on several advantages that make it a potential choice for management on a small scale, including, few raw materials are required, processes can be more easily monitored, natural and renewable material sources, less secondary waste, low carbon footprint, wastewater reclamation and nutrient recovery, allows using plants that accumulate contaminants to be utilized. And it is easy for small industry on aplication for low cost (Ahmad, J., Abdullah, S. R. S., 2017). In line with this, some researches have been conducted the effectiveness of microalgae in decreasing of organic compunds (*Eesa.1995.1064.Pdf*, n.d.; Molinuevo-salces et al., 2019; Plöhn et al., 2021).

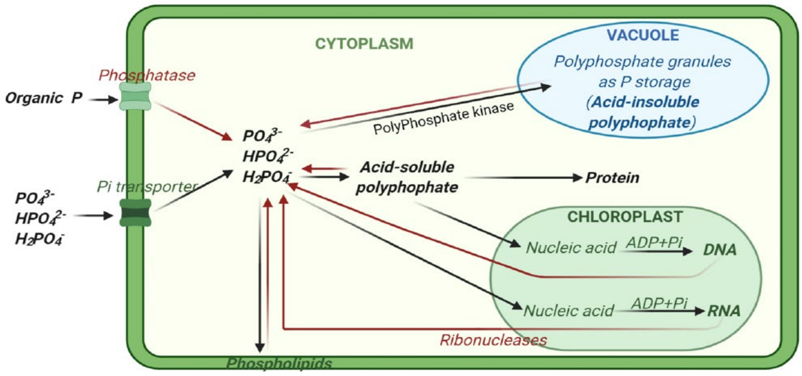

Figure 6. Biochemical Pathway of P in cells

The ultimate goal of utilizing microalgae as bioremediator agents of phosphate compounds in laundry waste is to obtain the results of the suitability of the percentage of phosphate waste contained in wastewater with the provisions of phosphate wastewater quality standards which later when discharged into water bodies will not damage aquatic ecosystems and are safe for use.

TOOLS AND MATERIAL

The research is an experimental study of a wastewater treatment system combining of bioremediation techniques and microalgae growth phase monitoring. The parameters observed are physical parameters, including temperature and total suspension solid; chemical parameters, including pH, BOD_5, COD, and phospate levels. Effluent samples were collected from wastewater discharged by laundry station in Makassar. Sampling of laundry liquid waste directly on the liquid waste disposal hose which will then be collected in a plastic container and brought to the laboratory to be put into a closed container with a capacity of 10 L (420 mm x 270 mm x 150 mm), followed by the preparation of microalgae taken from the breeding centre each put into a 1000 mL volume glass bottle that has been sterilised first.

Samples taken at the specified service are analyzed at the Marine and Fisheries laboratory with proper handling following applicable standards. Then, the samples are tested using the test methods and standards shown in Table 1.

Table 1. Sample Analysis Method

NO	PARAMETERS	SOURCE	METHODS
1	Phosphate	mg/L	Spectrophotometer UV-VIS
2	TSS (Total Suspended Solids)	mg/L	Gravimetry
3	Temperature	°C	Thermometer
4	pH (Degree of Acidity)	-	Potentiometry
5	BOD (Biological Oxygen Demand)	mg/L	Titrimetric/Potentiometry
6	COD (Chemical Oxygen Demand)	mg/L	Spectrophotometer UV-VIS

The phytoremediation process are given four types of treatment, the first treatment with the addition of microalgae *Chlorella vulgaris*, the second treatment with the addition of microalgae *Skeletonema costatum*, the third treatment by mixing the two species of microalgae *Chlorella vulgaris* and *Skeletonema costatum* and the last is the control experiment without the addition of microalgae. The batch method is used in the phytoremediation process by using water in a stationary or non-flowing state (Fahruddin & Effendi, 2019). The treatment was carried out by providing four containers with a capacity of 10 L which had previously been filled with 5 L of laundry wastewater each, given aeration and temperature setting at 25°C. Marked with the numbering P1, P2, P3 and P4. Water sampling was carried out every 2 hours for 10 hours with variations of 2, 4, 6, 8 and 10.

By the process of phytoremediation, microalgae growth populations are monitored. Monitoring of microalgae was carried out every time variation by taking several 1mL of samples and observed by microscope with 400x magnification, cell counting by using hand tally counter with Haemocytometer method.

Microalgae cell density monitoring was calculated using the formula:

$$N = \frac{\Sigma cellxfpx10^4}{4}$$

RESULTS AND DISCUSSION

Wastewater Characteristics

The pre-treatment wastewater exhibited physical characteristics, including physical checking test for odor and a brownish turbid appearance. Laboratory analysis showed that temperature and pH values were within acceptable limits based on regulatory standards, while parameters such as BOD, TSS, COD, and Phosphate exceeded quality standards. The initial characteristics of the wastewater effluent can be seen in Table 2.

Table 2. Characteristics Laundry Wastewater

No.	Parameters	Contents	Unit	Maximum rate*
1	Temperature	28	°C	<30
2	pH	8,17	-	6 - 9
3	BOD_5	187	mg/L	75
4	COD	315	mg/L	160
5	TSS	150	mg/L	60
6	Phosphate	15,29	mg/L	10

**Wastewater quality standard*
Source: primary data

Wastewater parameter
1. pH

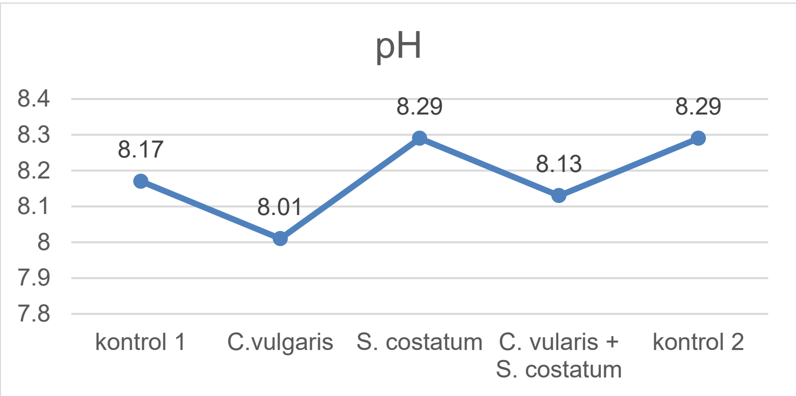

Figure 1. Graphic of pH concentration

All tests have reached the quality standard value of laundry wastewater of 6-9, but there is a decrease in pH in the *Chlorella vulgaris* microalgae test and the mixed test of *Chlorella vulgaris* and *Skeletonema costatum* significantly the pH of wastewater is 8.01 and 8.13, respectively, while the recommended pH based on regulation for hygienic sanitation is 6.5-8.5.

In the test containing microalgae *Chlorella vulgaris* has a lower pH than the control pH due to the amount of dissolved carbon dioxide decreasing due to the photosynthesis process by microalgae cells, microalgae *Chlorella vulgaris* is more capable of growing rapidly in wastewater than other species so as to reduce rapidly the concentration of carbon dioxide in water (Molinuevo-salces et al., 2019).

2. BOD concentration (*Biological Oxygen Demand*)

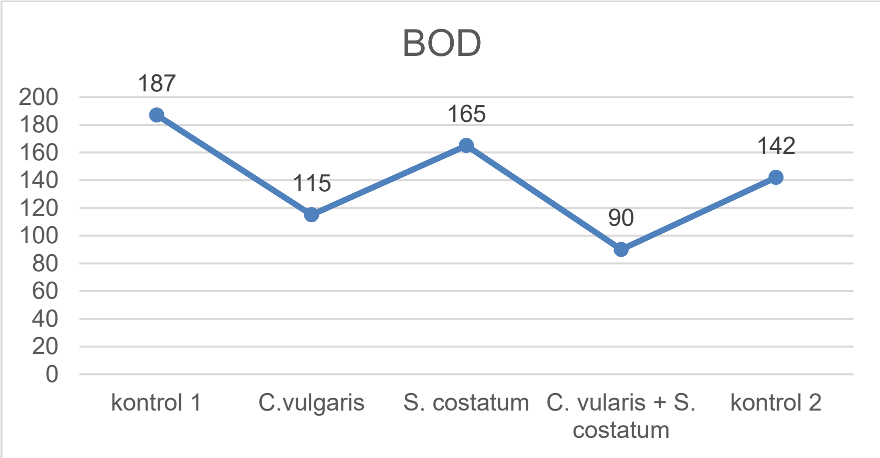

Figure 2. Graphic of BOD

BOD concentrations in all treatments dropped significantly, the initial control concentration was 187 and there was a decrease of 24%, the largest decrease in BOD concentration in the test with a mixture of microalgae *Chlorella vulgaris* and *Skeletonema costatum* by 51.87% from the initial control without the addition of microalgae. The decrease in BOD levels in the microalgae addition treatment was related to the growth in the amount of microalgae biomass, linearly with this, the total organic substances that must be degraded also increased. The decrease in BOD concentration was also shown in the control treatment without the addition of microalgae, this is due to the presence of potential bacterial species that also have the ability to degrade liquid waste, namely Staphyllococcus aureus, Pseudomonas pseudomallei, and Actinobacillus sp. (Fidiastuti et al., 2017).

3. COD concentration (*Chemical Oxygen Demand*)

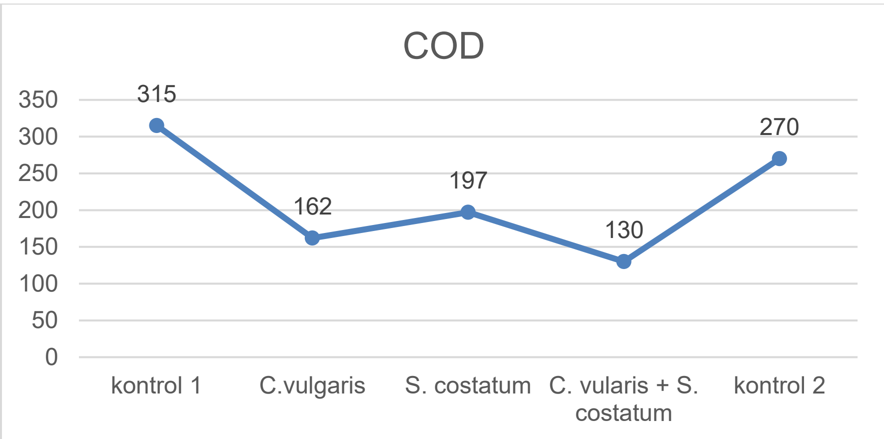

Figure 3. Graphic of COD

The percentage reduction in COD levels in all treatments was 25.53% (control), 48.09% (*Chlorella vulgaris*), 46.91% (*Skeletonema costatum*) and 51.06% (*Chlorella vulgaris* and *Skeletonema costatum*), respectively. According to Reddy and D'Angelo (1997) and Ghaly et al. (2005), there are several things that cause a decrease in COD concentration in the treatment with the addition of microalgae, among others: (i) microalgae are able to degrade nutrients and other organic materials (ii) oxygen present in wastewater is utilised by microorganisms thus increasing the removal of organic materials and (iii) the nutrient content contained in wastewater is consumed by microorganisms (Ghaly et al., 2005; Reddy & Angelo, 1997). The content of organic compounds contained in wastewater acts as nutrients for microorganisms that are eventually degraded into simpler compounds.

4. TSS (Total Suspended Solids)

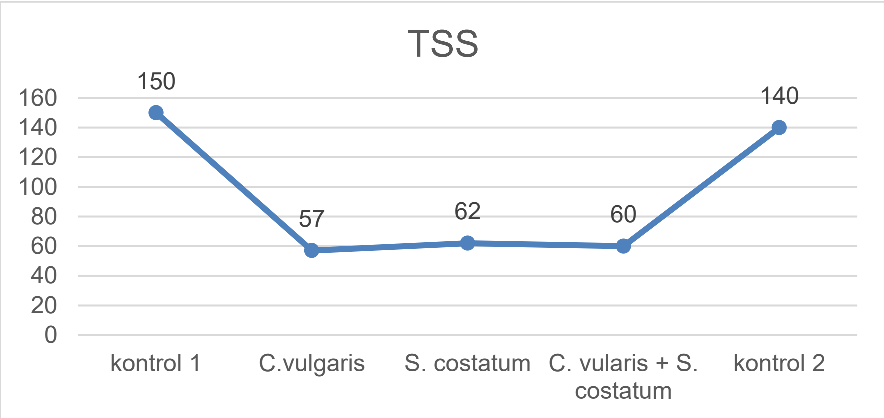

Figure 4. Graphic of TSS

The addition of microalgae can significantly reduce the TSS value by 46.66% (*Chlorella vulgaris*), 43.33% (*Skeletonema costatum*), 52% (*Chlorella vulgaris* and *Skeletonema costatum*), respectively. High values of suspended solids although not directly toxic, but excessive suspended material can increase the value of turbidity which will further inhibit the penetration of light into the water and will ultimately affect the photosynthesis process. However, the amount of suspended solids can also be generated by the amount of dead microalgal biomass that has blocked light penetration so that it must pay attention to the life phase of the microalgae (Terry & Raymond, 1985).

Phytoremediation Treatment

Observations on each treatment were carried out with time variations at the 2nd, 4th, 6th, 8th, and 10th hours. Observations were made by looking at physical changes and the amount of microalgae density during time variations. Time variations were determined in accordance with the theory of microalgae growth phases, namely the adaptation phase, logarithmic phase, growth rate decline phase, stationary phase and death phase (Borowitzka, 1997).

Table 3. Cell density in time

Time	Cell density (x 10^4 sel/mL)		
	P1	P2	P3
2	14,15	9,15	13,475
4	26,40	11,75	21,175
6	15,375	5,925	14,880
8	7,55	2,975	8,37

10	4,45	2,075	4,425

In **Table 3**, the density of the two microalgae seedlings was first calculated before being introduced into the test wastewater for the phytoremediation process. Determination of microalgae seedling density is calculated as hour 0. At the 2nd hour calculated as the microalgae adaptation phase, microalgae P1 experienced an increase in cell density by 8% from the initial cell density, while microalgae P2 experienced a decrease in the number of cells in the adaptation stage by 13.60% and microalgae P3 also experienced an increase in cell density by 13.58%. The density of microalgae P1, *Chlorella vulgaris* in the 2nd hour adaptation stage immediately increased due to the characteristics of this species, microalgae *Chlorella vulgaris* tends to be able to live in extreme conditions (Sabeti et al., 2018), morphologically has mitochondria formed from double membranes, proteins and phospholipids that control all growth processes and maintenance processes (Coronado-reyes et al., 2022). While in P2 microalgae, *Skeletonema costatum* has a physical character in the form of strands of chains or filaments.

At the 4th hour the growth of microalgae P1 increased exponentially to almost double by 46.40%, while in microalgae P2 there was an increase with a percentage of 22.12% from previous growth, microalgae P3 also experienced an increase in growth of 36.36% but the increase in the amount of growth was still lower than the initial control value. Significant decrease in microalgae cell density concentration began to occur at the 6th hour in all variations of microalgae species, maximum biomass productivity has been achieved in this phase. Microalgae P1 decreased by 41.76%, microalgae P2 by 49.78% and microalgae P3 also decreased by 29.73%.

In this phase all types of treatments of microalgae species experience competition between cells for total nutrients in wastewater, so that with a maximum density at the 4th hour there is an extreme decrease in the number of cells due to reduced nutrient availability, decreased phosphate levels in wastewater also affect carbon fixation by microalgae (Lee et al., 2014). Until entering the death phase, the number of cell densities continues to decline, one other factor causing low light penetration which is covered by the amount of cell biomass that has been died (Prayitno, 2016).

Table 4. Percentage of cell decrease

Microalga	Cell density percentage of death
P1	65.50 %
P2	80.03 %
P1 + P2	62 %

P1 : *Chlorella vulgaris*
P2 : *Skeletonema costatum*

Effect of microalgae in phosphate phytoremediation

Phosphate is used by microalgae for growth and metabolic processes in cells, so phosphate concentration is a limiting factor for growth, lipid production, fatty acid yield, energy transfer, signal transduction, and photosynthesis (Chu et al., 2013; Solovchenko et al., n.d.). Microalgae can absorb phosphorus in the form of polyphosphate or orthophosphate to enhance growth and nutrient content in the cell (Procházková & Brányiková, 2013; Radin et al., 2017).

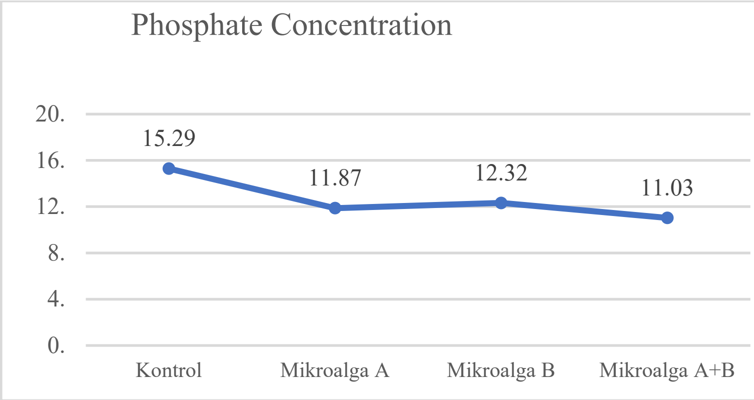

Figure 5. Phosphate concentration

After the phytoremediation process with the addition of microalgae *Chlorella vulgaris* and *Skeletonema costatum*, there was a decrease in all variations of phytoremediation treatments. A significant decrease in phosphate concentration occurred in wastewater with the addition of *Chlorella vulgaris* microalgae and in the variation of mixing two types of microalgae species, respectively 11.87 mg/L and 11.03 mg/L. The percentage reduction in phosphate levels in phytoremediation by *Chlorella vulgaris* was 22.36% and 27.86% in the mixing of two types of microalgae. It was also seen that there was a decrease in phosphate concentration in phytoremediation by *Skeletonema costatum* microalgae by 12.32 or 19.42%. The loss of phosphate during the phytoremediation process is caused by the use of phosphorus for growth (Rao et al., 2011). The decrease in phosphate levels in the environment is utilised by microalgae during the metabolic process, so that in microalgae cells, phosphate is stored as accumulated phosphorus and will be reused by microalgae when in a critical state for survival (Acid & An, 2021).

All types of treatments reached the optimum value at time-4 with each being; *Chlorella vulgaris* 26.40%, *Skeletonema costatum* 11.75% and a mixture of both types of microalgae at 21.175%. *Skeletonema costatum* microalgae tend to be more difficult to develop well due to one of them because the morphological structure of this microalgae species is spiral-shaped with inter-cell connecting chains, the existence of this chain will be damaged and cause cell death due to several factors, some of which are the presence of heavy metal nutrients and bacteria and parasites (Ayodhya D Kshirsagar, 2013).

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