Study of *Chlorella vulgaris* Density and Growth Rate at Different Effluent Concentrations

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Abstract

Household wastewater processing at the Communal Wastewater Treatment Plant (WWTP) operated by the Guyub Rukun Community in Tegalsari Hamlet, Salatiga City, Indonesia, is expected to reduce water pollution in the Cengek River. However, effluent testing revealed high concentrations of TP, COD, and BOD. This study aims to conduct laboratory-scale phycoremediation experiments using Chlorella vulgaris with media mixed using Communal WWTP effluent. Samples were taken from the outlet pipe to obtain household wastewater treatment effluent. Sampling was done using the grab sampling method at 2 PM during high sanitation activity. Based on laboratory tests, the highest Chlorella vulgaris density for 0% effluent concentration was 77.75 x 10^4 cells/mL (day 7), 66.13 x 10^4 cells/mL (day 6) in 20%, 126.13 x 10^4 cells/mL (day 7) in 40%, 69.38 x 10^4 cells/mL (day 6) in 60%, 188.88 x 10^4 cells/mL (day 6) in 80%, and 232 x 10^4 cells/mL (day 7) in 100%. The best growth rate at 0% effluent concentration was 3.87 cells/mL/day (day 6), 3.52 cells/mL/day (day 6) in 20%, 4.25 cells/mL/day (day 6) in 40%, 3.56 cells/mL/day (day 5) in 60%, 4.69 cells/mL/day (day 6) in 80%, and 4.99 cells/mL/day (day 7) in 100%. The results showed that Chlorella vulgaris's best density and growth rate was 100% effluent concentration. This indicates that the WWTP effluent's TP, TN, and Nitrate contents can be a good substitute for microalgae chemical fertilizers. This study also demonstrated that Chlorella vulgaris is in effective phycoremediation agent and can grow in high concentrations of WWTP effluent.

Keywords: Wastewater, Microalgae, Nutrient, Phycoremediation, Pollution

INTRODUCTION

A Communal Waste Water Treatment Plant (WWTP) is a government solution to address the problem of high household waste production in residential areas. The Communal WWTP in Tegalsari Hamlet was built in 2017 under the management of the Guyub Rukun Community. This Communal WWTP is a manufactured type built on the Cengek River channel, where the effluent from the Communal WWTP is channeled into this river (Salatiga City Public Works and Spatial Planning Office, 2020). Salatiga City Government (2023) states that the Cengek River has a secondary irrigation network.

The problem arises from effluent discharge in the Cengek River, with some effluent quality parameters exceeding predetermined quality standards. According to the effluent test results, the TP, COD, and BOD concentrations exceed the class II river water quality standards set out in Government Regulation No. 22/2021 (Government of the Republic of Indonesia, 2021). According to Regulation No. 68/2016 (Ministry of Environment and Forestry of the Republic of Indonesia, 2016), the BOD concentrations exceed the domestic wastewater quality standards. In addition, the emergence of unpleasant odors in the effluent is also a post-construction problem that needs to be resolved.

Phycoremediation is emerging as a promising, sustainable biological technology for treating household wastewater. This type of remediation is implemented by utilizing microalgae to remove contaminants in wastewater (Kumari *et al*., 2021). Through photosynthesis, microalgae can absorb various pollutants (Neneng

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et al., 2020), such as Nitrogen, Phosphorus, heavy metals, and other organic compounds, to support their metabolism (Apandi *et al*., 2019). This process not only reduces the pollutant load in waste but also improves its quality. Still, it will also produce biomass from microalgae that can be used as biofuel (Rana & Prajapati, 2021), animal feed (Apandi *et al*., 2019), and other commercial products (Pooja *et al*., 2022). In addition, phycoremediation is also affordable, environmentally friendly, and easy to maintain (Arrojo *et al*., 2022).

Chlorella is a genus of microalgae well known for its rapid growth rate, due to its high nutrient uptake capacity (Arrojo *et al*., 2022). When applied in household waste management, *Chlorella* can absorb and metabolize various macronutrients and micronutrients in wastewater (Kumari *et al*., 2021). Besides being affordable and environmentally friendly, phycoremediation with *Chlorella* is also profitable because it requires less energy than conventional household waste processing systems. *Chlorella's* bio-absorption capacity in carbon fixation during photosynthesis also reduces CO_2, reducing greenhouse gas emissions (Maria *et al*., 2021).

Research by Menció *et al.* (2016) indicates that *Chlorella* can produce enzymes that reduce pollutants through its cellular metabolic processes (Vingiani *et al*., 2019). *Chlorella vulgaris* efficiently removes Nitrogen and Phosphorus content in household waste, where these two pollutants are the main contributors to eutrophication in aquatic ecosystems (Madadi *et al*., 2021). In addition, this microalgae species also has good BOD and COD reduction capabilities within 10 hours to 42 days of culture (Narayanan *et al*., 2021). Testing of Nitrate, COD, BOD, and other parameters in the study by Pooja et al. (2022) showed satisfactory results, with more than 90% reduction. In the study by Madadi et al. (2021), reductions in BOD, COD, Total-N, and Total-P were 30.36%, 10.89%, 69.89%, and 92.59%, respectively.

MATERIAL AND METHODS

This research is a laboratory experiment to test the density and growth rate of *Chlorella vulgaris* grown in effluent from the Communal WWTP treatment. Pure culture of *C. vulgaris was obtained from the Centre for Brackish Water Aquaculture (BBPBAP) in* Jepara, St. Cik Lanang, Bulu, Jepara, Indonesia. Wastewater samples were taken from the effluent coming from the outlet pipe of the Guyub Rukun Communal WWTP, Tegalsari Hamlet, Salatiga City (Figure 1). Sampling was conducted using the grab sampling method at 2 PM, when sanitation activity intensity was high. The household wastewater effluent was collected in a light-tight container for further phycoremediation testing.

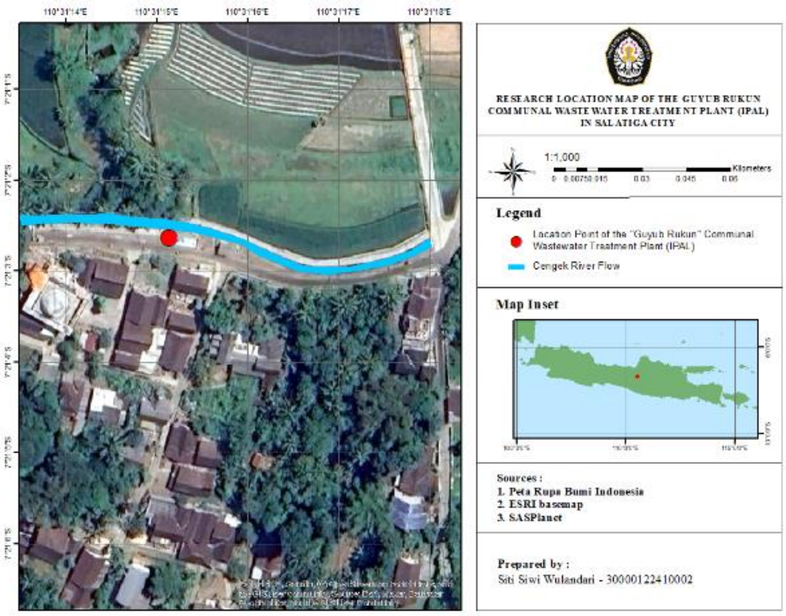

Figure 1. Guyub Rukun Communal WWTP Location

Figure 2. Lab Scale Phycoremediation with *Chlorella vulgaris*

Density and growth rate tests of *C.vulgaris* were conducted at the Ecology and Biosystematics Laboratory, FSM, UNDIP. Cell culture was carried out in the research set with five levels of effluent concentration, namely 20%, 40%, 60%, 80%, 100%, and control (0%) (Figure 2), with two repetitions being carried out for each treatment. The initial density of *C. vulgaris* used was 1 × 10^6 cells/mL; in a 500 mL media volume, 100 mL of microalgae culture was added. The culture was grown in a glass bioreactor with 24 hours of light irradiation for 9 days. Density measurements were taken every noon (12 PM) using the formula. The population of *C. vulgaris was measured using a Haemocytometer in blocks A, C, G, and I,* with 16 boxes in each block (Utermöhl, 1958). The. Cells were measured every day at 12 PM. Density estimation was determined using the following equations. (Khairunnisa *et al*., 2024).

$$D = N \times 10^4$$

Where D is the density in units of cells/mL, and N is the average calculated cells based on the observed blocks.

$$\mu = LnN_t - LnN_0/t_t - t_0$$

The results of population density calculations are used to create density graphs representing cell growth. Density values are also used to calculate specific growth rates (μ) with the above formula (Reynolds, 1984). N_t and N_0 are the cell densities on day t and day 0, while tt and t0 are the observation times on day t and day 0.

RESULT AND DISCUSSION

Guyub Rukun Communal WWTP Effluent Condition

Guyub Rukun Communal WWTP is a community-based infrastructure built to treat household waste sourced from residential areas in Tegalsari Hamlet. This Communal WWTP uses microbial processing technology via an anaerobic process (Mladenov *et al*., 2022). Effluent testing is needed to determine the effectiveness of waste treatment in this installation.

The effluent test results show that the temperature, pH, TN, Nitrate, TDS, and TSS values are below the water and waste quality standards. However, the COD, BOD, and TP values are high and exceed the existing quality standards. According to research by Ganji *et al*. (2024), the high values of BOD and COD are due to excessive concentrations of organic and inorganic pollutants, so dissolved oxygen will be focused on breaking down compounds through chemical processes (COD) or biological mechanisms by microorganisms (BOD) (Verma *et al*., 2022).

Meanwhile, Total Phosphate (TP) refers to the concentration of Phosphate in sewage. Using detergents, soaps, and organic waste significantly contributes to TP (Yang *et al*., 2021). TP values exceeding the threshold are feared to increase the risk of eutrophication if the waste flows directly into the river body (Larasati *et al*., 2021). In addition, the disposal of effluents with high TP values in soil can also potentially increase groundwater pollution.

The TP is one of the macronutrients that support cell growth in microalgae, including *C.vulgaris*. This compound plays a vital role in cell metabolism, including protein synthesis, chlorophyll function in photosynthesis, and other important functions (Azam *et al*., 2022). In their research, Arrojo *et al*. (2022) mentioned that using sewage effluent can support the growth of *C.vulgaris*. This is because the effluent contains high TP. The density and growth rate of microalgae cells will be described in the following discussion.

Density and Specific Growth Rate

During the 9-day culture period, the density of *C.vulgaris* showed an increase in population density, which proved the culture's success on media mixed with household waste, although the trend was less consistent. This is similar to previous studies, which showed that waste in different concentrations can cause inconsistent microalgal cell growth (Verma *et al*., 2022).The nutrient composition of different waste concentrations results in longer lag phases and greater variation in population density (Liyana *et al*., 2023). Differences in TP and TN values also cause variations in density, leading to differences in microalgae cell growth (Kumari *et al*., 2021).

The density graph (Fig. 4) is similar to research by Arrojo et al. (2022), showing that the waste treatment density is higher than that of the control. This result shows that household waste can serve as a nutrient source for microalgae (Amaral *et al*., 2023). In the research by Pooja et al. (2022), the highest density of *C. vulgaris* was observed at a 100% waste concentration on day 7 and persisted in the stationary phase. This density value is not optimal because low salinity conditions inhibit the growth of *C.vulgaris* (Aziz *et al*., 2020).

C.vulgaris bioreactor showed the highest density on different days (Figure 3). The highest density for 20% and 60% UW concentration was on day 4, 40% UW concentration on day 6, and 0% and 100% UW concentration on day 7. Nutrient composition consisting of different waste concentrations causes additional time in the lag phase and impacts variations in population density (Liyana *et al*., 2023). Density variations are also caused by differences in TP and TN levels, leading in variations in microalgal cell growth (Kumari *et al*., 2021). At the end of the experiment, the density of *C.vulgaris* was obtained from 100% UW (208.13 x 10^4 cell/ml), followed by 0% UW (66.13 x 10^4 cell/ml), 40% UW (63.75 x 10^4 cell/ml), 80% UW (61.25 x 10^4 cell/ml), 20% UW (22.88 x 10^4 cell/ml), and 60% UW (13.75 x 10^4 cell/ml).

The cell density in this study was categorized as lower compared to the studies of Arrojo *et al*. (2022) and Wang *et al*. (2018) because chemical fertilizers are only given at the beginning of the culture; the rest of the microalgae cells must adapt to the waste and use it as a nutrient source. Density in the study of Arrojo *et al*. (2022) showed the highest value of 65.5 x 10^6 cells/ml, while the

Table 1. Effluent Testing Results of Guyub Rukun Communal WWTP

Parameters	Effluent result (mg/L)	Status
Temperature °C	26.3	Under the Quality Standard*
pH	6.99	
Salinity (ppt)	0	-
TN	11.3	Under the Quality Standard*
Nitrate	0.66	
TDS	375	
TSS	18	
COD	101.5	Over the Quality Standard*/**
TP	51.1	Over the Quality Standard*
BOD	21.7	

*Indonesian Government Regulation 22/2021 on the Implementation of Environmental Protection and Management
**Regulation of the Minister of Environment of the Republic of Indonesia 68/2016 concerning Domestic Wastewater Quality Standards

research of Wang *et al*. (2018) gave the highest density value of 83.5 x 10^6 cells/ml in 100% tofu whey wastewater. pH conditions in the media cause this difference. *Chlorella* sp. can grow in the pH range of 5-10. The investigation results show the lowest density value obtained at pH 5 (Zhang *et al*., 2014). In this study, only a 100% UW bioreactor with a pH value close to the initial pH reported in Wang *et al*. (2018) (pH 9) shows good cell growth. (Cell growth is relatively slow and tends to stagnate in other bioreactors because the microorganisms in them cannot adapt to relatively low pH (pH 5-7).

A variance test with ANOVA was used to analyze the effect of different effluent concentrations on density and specific growth rate (Katiyar *et al*., 2021). The ANOVA test showed a p-value < 0.05, indicating that the difference in effluent concentration significantly affected density and growth rate. Meanwhile, the Tukey HSD follow-up test showed significant differences in density across three classes and two distinct growth rate classes.

In *C.vulgaris* culture, the lag phase is shown in the curve with a less significant increase in growth from day 0 to day 3, while growth began to increase from day 4 to day 7 and experienced a slowdown in growth until day 5. The highest population was obtained from the bioreactor with 100% effluent content. This indicates that microalgae can not only adapt to environmental conditions high in pollutants but also absorb them and use them as a metabolic substrate to support cell division more optimally.

The specific growth rate graph (Figure 4) shows how far microalgae cells can grow and divide, increasing the population. The mechanism of macronutrient and micronutrient consumption plays a vital role in cell growth and pollutant removal efficiency (Tsirigoti *et al*., 2015). At the end of the *C. vulgaris* phycoremediation experiment, the best growth rate was 100% UW (4.99 cells/ml/day), followed by 80% UW (3.76 cells/ml/day), 40% UW (3.58 cells/ml/day), 0% UW (3.14 cells/ml/day), 20% UW (2.74 cells/ml/day), and 60% UW (2.05 cells/ml/day).

Culture in 100% wastewater also showed the highest growth rate of *Chlorella*, according to Taufikurahman and Istiqomah (2019) in dairy wastewater medium, followed by high Ammonia and Phosphate removal efficiency after 11 days of culture. Similar results were also reported in the research of Singh *et al*. (2017), which showed the highest growth rate of *Parachlorella kessleri-I* in 100% municipal wastewater. The research report of Aravantinou *et al*. (2013) in (Amit *et al*., 2017) stated that marine microalgae have a higher potential growth rate than freshwater microalgae cultured in synthetic wastewater.

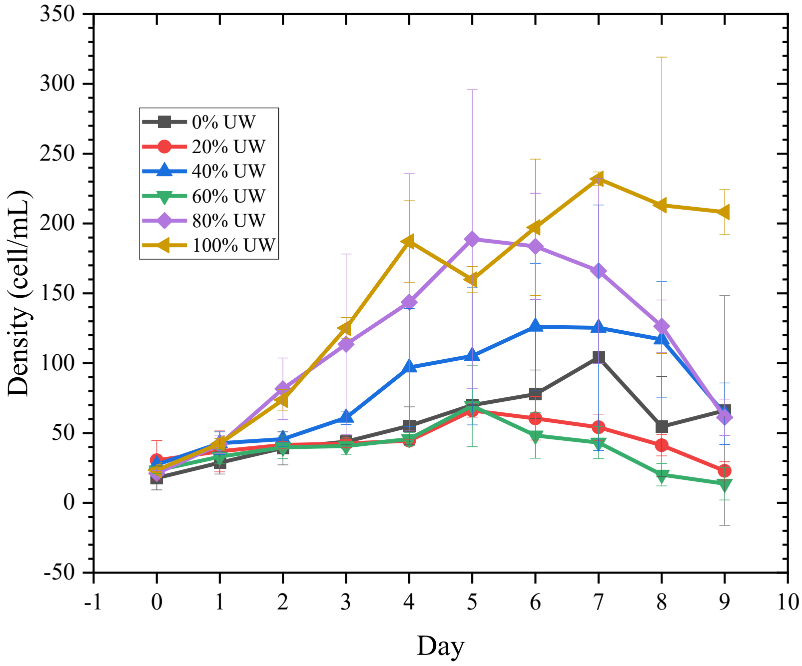

Figure 3. The Density of *C.vulgaris* During Experiment

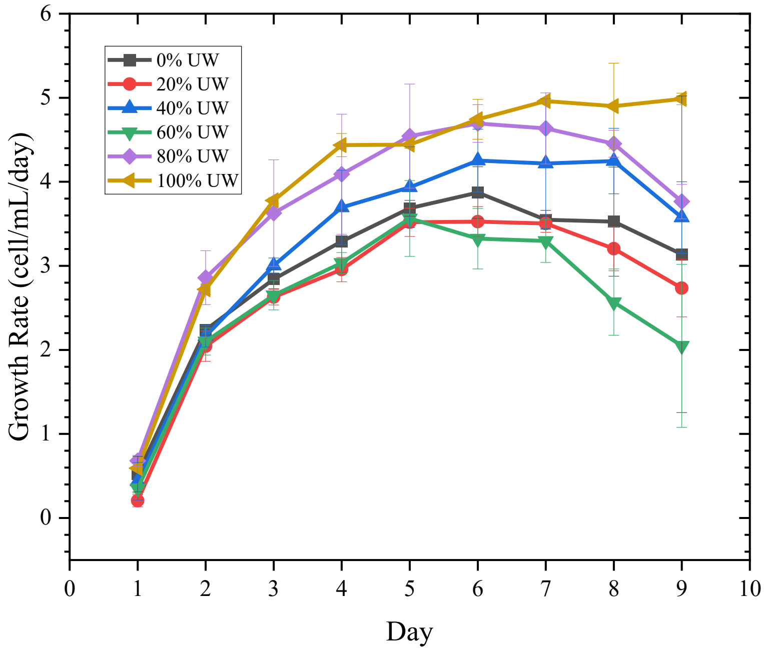

Figure 4. Specific Growth Rate of *C.vulgaris* During Experiment

Similar to the cell density results, the specific growth rate also showed lower values than reported in the literature. The highest growth rate at 100% UW only showed a value of 4.99 cells/ml/day (doubling time of 0.138/day). This result differs from Wang et al. (2018), which reported the best growth rate of 0.76/day (doubling time) on the last day of the culture period (day 9), demonstrating that microalgae can utilize nutrients from tofu whey wastewater. The specific growth rate is also higher in other studies by Moradi et al. (2024), with a value of 5.3 cells/mL/day (day 1) for microalgae grown in 80% synthetic wastewater media. Interestingly, this study illustrated that the best growth rate was on the first day of the sowing period. Specific growth rates also continued to decline significantly, indicating that microalgae are under environmental stress from chemicals in the waste.

Temperature and pH are critical environmental factors in increasing growth rate and cell density (Tan *et al*., 2021). The availability of Nitrogen, Phosphorus, and other macronutrients plays a role in supplying nutrients for cell growth. Nutrient concentrations in optimal levels can increase the activity of the RuBisCo enzyme in photosynthesis (Katiyar *et al*., 2021). In addition, light is also a necessity in photosynthesis because low light can limit growth, while too much light can inhibit photosynthesis (Amit *et al*., 2017). The photosynthesis process influences increase in temperature and pH through an oxidation mechanism (Mao et al., 2023), with an optimal temperature range for *Chlorella* growth of 20-30°C and pH >7 (Huang *et al*., 2022).

CONCLUSION

The laboratory testing results showed that the effluent from the Communal WWTP, which treats household waste, can be used as a fertilizer supporting the growth of C. vulgaris. Based on laboratory tests, the highest *C.vulgaris* density in the 0% effluent concentration bioreactor was 77.75 x10^4 cells/mL (day 7), 20% concentration at 66.13 x 10^4 cells/mL (day 6), 40% concentration at 126.13 x 10^4 cells/mL (day 7), 60% concentration at 69.38 x 10^4 cells/mL (day 6), 80% concentration at 188.88 x 10^4 cells/mL (day 6), and 100% concentration at 232 x 10^4cells/mL (day 8). The best growth rate at 0% effluent concentration was 3.87 cells/mL/day (day 6), 20% concentration at 3.52 cells/mL/day (day 6), 40% concentration at 4.25 cells/mL/day (day 6), 60% concentration at 3.56 cells/mL/day (day 5), 80% concentration at 4.69 cells/mL/day (day 6), and 100% concentration at 4.99 cells/mL/day (day 7). The culture results showed that the best density and growth rate of *C. vulgaris were at 100% effluent concentration, followed by 80%, 40%, 0%, 60%, and 20%*. This result proves that *C.vulgaris* can grow in media with high pollutant content and is good at phycoremediation. Nutrients contained in WWTP effluent can be substituted for chemical fertilizers for microalgae growth. These nutrients include Phosphate, Nitrogen, Nitrate, and others. The result will undoubtedly benefit future knowledge development because it can use microalgae in waste remediation and reduce chemical fertilizers in microalgae breeding.

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