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ANALISIS PERBANDINGAN KEPADATAN SEL *CHLORELLA VULGARIS* DALAM SISTEM KULTUR YANG BERBEDA

Comparative Analysis Of Chlorella Vulgaris Cell Densities In Different Culture Systems

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ABSTRAK

Chlorella vulgaris adalah mikroalga kosmopolitan dengan nilai gizi tinggi, yang banyak digunakan dalam akuakultur, farmasi, dan produksi biofuel. Penelitian ini mengevaluasi kinerja pertumbuhannya di tiga skala kultur (botol laboratorium, carboy, dan tangki beton perantara) di BPBAP Situbondo dari Februari hingga Juni 2024. Penelitian ini bertujuan untuk menganalisis pengaruh skala terhadap produktivitas biomassa dan mengidentifikasi faktor lingkungan yang kritis. Hasil penelitian menunjukkan variasi pertumbuhan yang signifikan di antara skala. Botol laboratorium (5 L) mencapai kepadatan sel tertinggi (54 × 10^6 sel/mL) pada hari ke-11, yang dikaitkan dengan distribusi cahaya yang optimal dan suhu yang stabil (30–33°C). Kultur carboy (10 L) menunjukkan produktivitas sedang (31 × 10^6 sel/mL), sedangkan skala perantara (700–800 L) menghasilkan kepadatan terendah (6,8 × 10^6 sel/mL) karena keterbatasan cahaya dan aerasi yang kurang optimal. Parameter kualitas air tetap stabil di semua sistem, dengan pH 8,0, salinitas 30–34 ppt, dan intensitas cahaya seragam (500 lux). Analisis statistik mengonfirmasi perbedaan signifikan dalam laju pertumbuhan ($p < 0,05$), yang menyoroti hubungan terbalik antara ukuran skala dan produktivitas. Studi ini menyimpulkan bahwa sistem skala kecil menawarkan pengendalian lingkungan yang lebih unggul untuk budidaya C. vulgaris. Untuk produksi massal, perbaikan desain—seperti aerasi yang ditingkatkan, fotobioreaktor tertutup, dan pencahayaan buatan—direkomendasikan untuk meniru efisiensi skala laboratorium. Temuan ini memberikan wawasan yang dapat ditindaklanjuti untuk mengoptimalkan sistem kultur mikroalga dalam produksi pakan akuakultur.

ABSTRACT

Chlorella vulgaris is a cosmopolitan microalga with high nutritional value, widely utilized in aquaculture, pharmaceuticals, and biofuel production. This study evaluated its growth performance across three culture scales (laboratory bottle, carboy, and intermediate concrete tank) at BPBAP Situbondo from February to June 2024. The research aimed to analyze the influence of scale on biomass productivity and identify critical environmental factors. Results demonstrated significant growth variations

among scales. The laboratory bottle (5 L) achieved the highest cell density (54 × 10^6 cells/mL) on day 11, attributed to optimal light distribution and stable temperature (30–33°C). Carboy cultures (10 L) showed moderate productivity (31 × 10^6 cells/mL), while the intermediate scale (700–800 L) yielded the lowest density (6.8 × 10^6 cells/mL) due to light limitation and suboptimal aeration. Water quality parameters remained stable across all systems, with pH 8.0, salinity 30–34 ppt, and uniform light intensity (500 lux). Statistical analysis confirmed significant differences in growth rates ($p < 0.05$), highlighting the inverse relationship between scale size and productivity. The study concludes that small-scale systems offer superior environmental control for *C. vulgaris* cultivation. For mass production, design improvements—such as enhanced aeration, closed photobioreactors, and artificial lighting—are recommended to replicate laboratory-scale efficiency. These findings provide actionable insights for optimizing microalgae culture systems in aquaculture feed production.

Kata Kunci	*Chlorella vulgaris, Skala Kultur, Produktivitas Biomassa, Kualitas Air, Pakan AKuakultur*
Keywords	*Chlorella vulgaris, Culture Scale, Biomass Productivity, Water Quality, Aquaculture Feed*
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INTRODUCTION

Microalgae are microscopic unicellular organisms that play a pivotal role in aquatic ecosystems. As primary producers, they not only form the foundation of aquatic food chains but also serve as bioindicators of water quality (Dayana et al., 2022; Rahman, 2015; Prayogo & Arifin, 2015). The presence and diversity of microalgae reflect the fertility and environmental conditions of aquatic systems. Their composition and productivity are directly influenced by physicochemical parameters such as temperature, pH, salinity, and nutrient availability. Distinct microalgal groups—including Bacillariophyceae (diatoms), Chlorophyceae (green algae), Chrysophyceae (golden algae), and Cyanophyceae (blue-green algae)—exhibit unique characteristics that determine their ecological distribution and functions.

Among microalgae, *Chlorella vulgaris* (Chlorophyceae) has garnered significant attention due to its cosmopolitan distribution across freshwater, brackish, and marine environments. This species is nutritionally rich, containing essential proteins, lipids, vitamins, and antioxidants, making it valuable for diverse industrial applications such as fish feed, health supplements, cosmetics, and biodiesel production (Octhreeani & Soedarsono, 2014; Novianti et al., 2017; Rihi, 2019; Istirokhatun et al., 2017). Its rapid growth rate and photosynthetic efficiency further render it ideal for large-scale cultivation. However, optimal growth of *C. vulgaris* is contingent upon adequate nutrient supply (particularly nitrates and phosphates) and controlled environmental conditions, including lighting and aeration.

C. vulgaris cultivation typically involves three stages: laboratory-scale, intermediate-scale (semi-mass), and mass-scale (Mufidah et al., 2017). The intermediate stage bridges small-scale research and commercial production, allowing adaptation to

technical-grade fertilizers and evaluation of resilience and productivity before large-scale implementation (Aprilliyanti, 2016; Novianti et al., 2017). However, transitioning from laboratory to production scales often faces technical challenges, including environmental parameter instability, contamination, and reduced cell density due to system inefficiencies. Thus, a comprehensive understanding of *C. vulgaris* growth dynamics across cultivation scales is critical for productivity optimization.

This study was conducted at the Brackish Water Aquaculture Center (BPBAP) in Situbondo, Indonesia, a facility equipped with comprehensive resources for live feed development. The site was selected for its controlled aeration systems, high-quality seawater supply, and technical expertise in microalgal culture. The research aimed to: (1) analyze *C. vulgaris* growth in three distinct cultivation systems (bottles, carboys, and intermediate-scale tanks), and (2) identify critical factors influencing productivity. The findings are expected to provide technical recommendations for enhancing large-scale *C. vulgaris* production, particularly to support sustainable aquaculture feed supplies.

METHODS

Materials and Methods

Study Period and Location

This research was conducted from February 19 to June 21, 2024, at the Brackish Water Aquaculture Center (BPBAP) in Situbondo, East Java. The location was selected based on the availability of aquaculture facilities and technical support for live feed cultivation activities.

Equipment and Materials

Research Equipment

The main equipment included an autoclave for media sterilization, a hemocytometer for cell density counting, and a microscope for morphological observation of *Chlorella vulgaris*. The aeration system consisted of an air pump, airstones, and tubing for oxygen supply. Culture vessels included carboys and Erlenmeyer flasks with capacities ranging from 5-20 liters, while lighting was regulated using UV-C lamps with an intensity of 1500-2000 lux. Additional supporting equipment included measuring cylinders for chemical measurement, an analytical balance, and cleaning tools such as brushes and alcohol for sanitation.

Research Materials

The primary material was a pure culture of *Chlorella vulgaris* as the initial inoculum with a density of 10^5 cells/mL. The culture medium consisted of a mixture of sterilized seawater (salinity 30 ppt) and freshwater. Walne fertilizer was used as the main nutrient source, while thiosulfate was employed to neutralize chlorine residues. Supplementary materials included vitamins and trace metals to optimize microalgal growth.

Culture Technique of *Chlorella vulgaris* at BPBAP Situbondo

Equipment Sterilization

The culture procedure began with the sterilization of equipment and materials to eliminate microbial contaminants. Sterilization was defined as the process of eliminating both pathogenic and non-pathogenic microorganisms, including spores (Hanifah et al., 2021). Equipment such as glass bottles (5 L) and carboys (10 L) were washed with liquid

soap (Sunlight) and freshwater, then dried. Aeration tubing and weights were soaked in a chlorine solution (10 ppm) overnight, followed by washing with soap and drying.

Culture Medium Sterilization

The culture medium used seawater filtered through a filter bag to remove coarse particles. For the bottle scale, water was boiled for approximately 45 minutes, poured into sterile jars, and tightly sealed with plastic. For the carboy scale, seawater was filtered and treated with chlorine (300 mL/160 L) for 2 hours with aeration, then neutralized using sodium thiosulfate (5 mL). A chlorine test was used to ensure the medium was chlorine-free (indicator: clear color).

Fertilizer and Vitamin Formulation

Walne fertilizer for laboratory scale was prepared by dissolving $NaNO_3$ (100 g), NaH_2PO_4 (20 g), Na_2EDTA (45 g), and trace metals such as $FeCl_3$ and $MnCl_2$ in 1 L of sterile distilled water. Na_2EDTA was added last to maintain solution stability, which was then stored at 4°C. Vitamins consisted of thiamine (B1: 20 g), cobalamin (B12: 0.1 g), and biotin (0.1 g) dissolved in sterile distilled water with an application dose of 1 mL per liter of culture medium. For the trace metal solution, $ZnCl_2$ (2.1 g), $CoCl_2$ (2 g), and $CuSO_4$ (2 g) were dissolved in distilled water and sterilized using an autoclave at 121°C for 30 minutes.

Culture Process

For the bottle scale, the culture medium was prepared with a seawater to freshwater ratio (6:4), supplemented with 5 mL of Walne fertilizer, and inoculated with *C. vulgaris* inoculum (1 L). Aeration was provided through sterile tubing, and cultures were incubated under 40-watt lamps. For the carboy scale, the medium consisted of 4 L seawater and 2 L freshwater neutralized with thiosulfate, supplemented with Walne fertilizer (10 mL), and inoculated with inoculum from the bottle scale (1 L per carboy). Incubation was conducted in an air-conditioned room with stable lighting. For the intermediate scale, a concrete tank (1 m × 1 m) was sterilized using chlorine (350 mL) and filled with filtered seawater (salinity 30-34 ppt). Inoculum from the carboy scale (5 L) was added to 700-800 L of medium, supplemented with Walne fertilizer (350 mL), and continuously aerated.

Water Quality Measurement

Daily measured parameters included temperature, maintained at 25±1°C, pH within the range of 7.5-8.5 (measured using a pH meter), and salinity between 30-34 ppt (measured with a refractometer).

Cell Density Calculation

C. vulgaris cells were counted using a hemocytometer with the formula: Total cells/mL = (Number of cells counted / Number of squares counted) × 10^4 Samples were observed under a microscope with four counting replicates.

Harvesting

For laboratory scale, cultures were harvested during the logarithmic growth phase, approximately days 7-13, for use as inoculum in subsequent culture stages. For the intermediate scale, cultures were filtered using a 2-3 micrometer mesh sieve, and the harvested product could be dried or directly used as feed for shrimp or fish larvae.

Data Collection Techniques

Primary Data

Primary data were collected through three main approaches: (1) Direct observation of daily culture parameters including pH (7.5-8.5), temperature (25±1°C), and cell density; (2) Structured interviews with BPBAP technicians using validated questionnaires; and (3) Active participation in all culture stages from inoculation to harvesting.

Secondary Data

Supporting data were gathered from various sources, including BPBAP technical reports (2019-2023), journal articles on microalgae cultivation (particularly from *Aquaculture Reports* and *Algal Research*), and standard APHA (2017) protocols for water quality analysis.

Data Analysis

This study employed both quantitative and qualitative analytical approaches. Descriptive analysis was used to present water quality parameters (temperature, pH, salinity) and *Chlorella vulgaris* growth patterns across different culture scales (Maudina & Diamahesa, 2023; Aulia & Diamahesa, 2024; Nisa et al., 2024; Yusrin & Diamahesa, 2024). Cell density was calculated using a haemocytometer with four replicates. Data were validated through triangulation (observation, interviews, and literature) and quality control of equipment.

RESULTS AND DISCUSSION

Result

Density of *Chlorella vulgaris* at Different Scales

The density of *Chlorella vulgaris* at the laboratory scale was calculated using three cultivation scales: jars, intermediate and carboys.

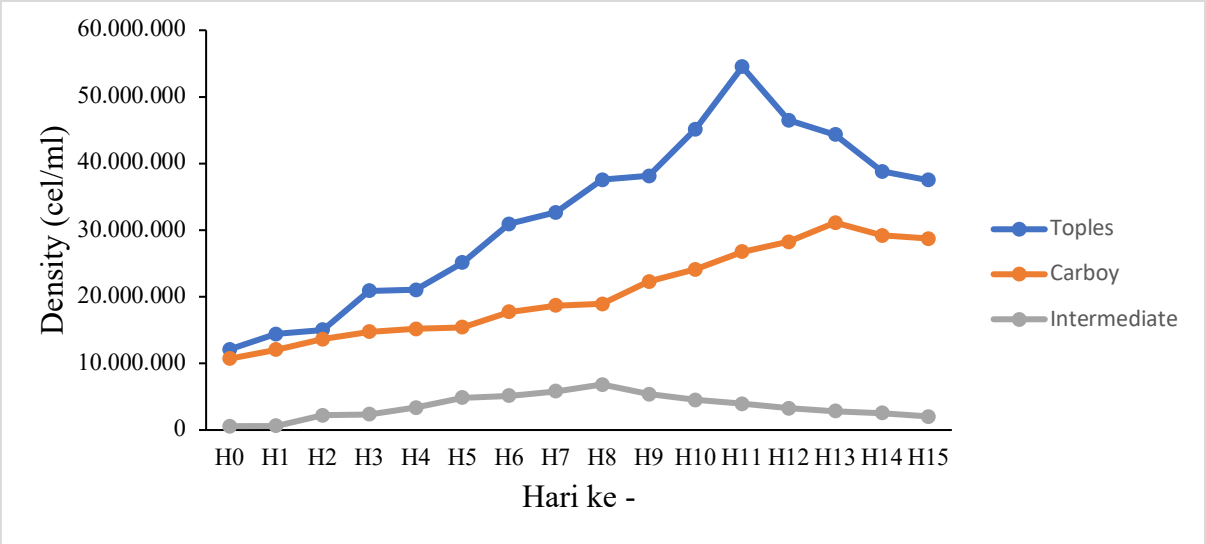

Figure 1. Growth curve of *Chlorella* sp. cultured at different scales

The growth of *Chlorella vulgaris* varied significantly across vessels. The jar yielded the highest cell density (~54 × 10^6 cells/mL) on day 11, indicating that smaller-scale environments provide more optimal growth conditions. The carboy showed moderate

density (~31 × 10^6 cells/mL) and remained suitable for intermediate-scale cultivation. In contrast, the intermediate-scale vessel produced the lowest density (~6.8 × 10^6 cells/mL), likely due to light limitations and reduced circulation in larger-scale systems.

Water Quality Parameters

The temperature conditions recorded during the laboratory and intermediate-scale cultivation of *Chlorella vulgaris* are presented in Figure 2**.**

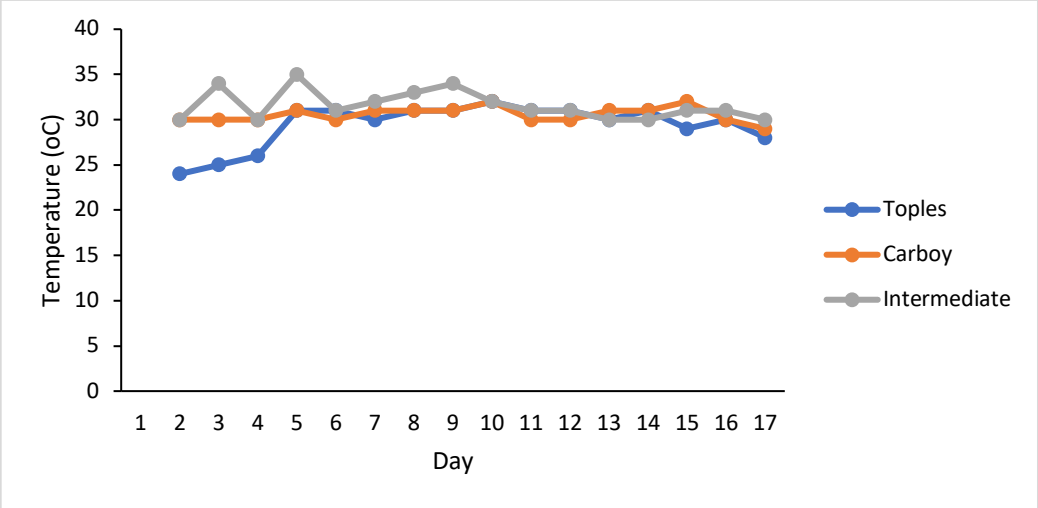

Figure 2. Temperature (°C) during *Chlorella vulgaris* cultivation in different vessels

The temperature range throughout the culture period was approximately 27°C to 36°C. In jar-scale cultivation, the initial temperature was lower (~27–30°C) but later increased and stabilized between 30–33°C. Conversely, in carboy and intermediate-scale systems, temperature fluctuations were minimal, remaining stable at 30–33°C.

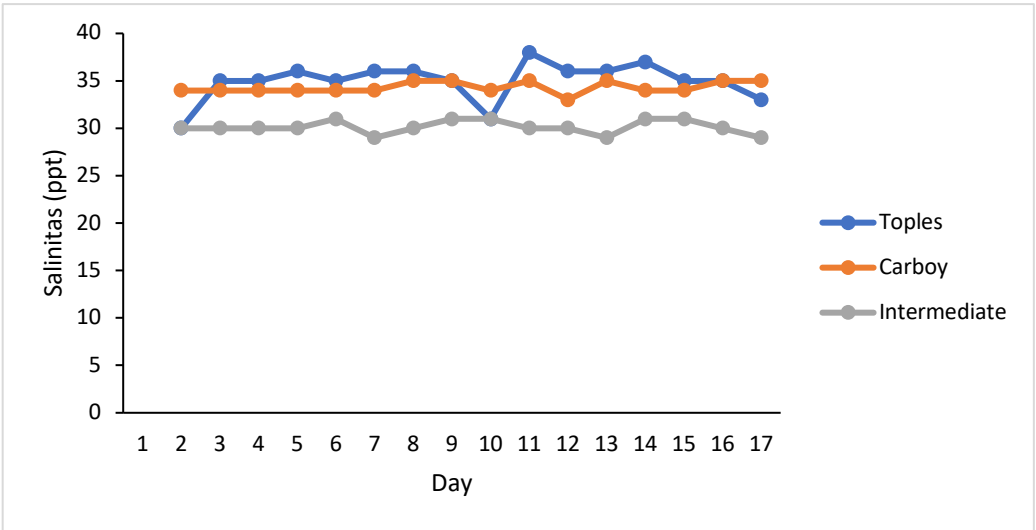

Figure 3. Salinity (ppt) of *Chlorella vulgaris* cultures in different vessels

The measured pH and light intensity across different cultivation vessels are summarized below.

Table 7. pH and light intensity measurements in different cultivation vessels

pH			Light Intensity (Unit)		
Jar	Carboy	Intermediate	Jar	Carboy	Intermediate
8	8	8	500	500	500
8	8	8	500	500	500
8	8	8	500	500	500
8	8	8	500	500	500
8	8	8	500	500	500
8	8	8	500	500	500
8	8	8	500	500	500
8	8	8	500	500	500
8	8	8	500	500	500
8	8	8	500	500	500
8	8	8	500	500	500
8	8	8	500	500	500
8	8	8	500	500	500
8	8	8	500	500	500
8	8	8	500	500	500
8	8	8	500	500	500
8	8	8	500	500	500

The pH remained stable at 8.0 across all treatments, supporting photosynthesis and the growth of *Chlorella***.** The light intensity was uniform (500 lux), indicating that it was not a limiting factor across the different culture scales.

Discussion

The growth of *Chlorella vulgaris* across three cultivation scales—jar, carboy, and intermediate—followed a pattern consistent with the typical microalgal growth curve, comprising the lag phase, exponential (log) phase, stationary phase, and decline phase. Each phase was influenced by environmental factors such as temperature, pH, light intensity, nutrient availability, and the design of the cultivation system.

During the initial cultivation period (days 0–1), all scales exhibited a lag phase, characterized by slow cell growth and relatively low cell density. For instance, in jar-scale cultures, the initial density was recorded at approximately 10.70×10^4 cells/mL, increasing gradually to 12.04×10^4 cells/mL. This suggests that the microalgae were adapting to new environmental conditions, including media salinity, temperature, pH, and nutrient availability. According to Tsany (2016), cells in this phase undergo physiological adjustments without active division, initiating protein synthesis as part of early metabolic activity.

From days 3–11, the cultures entered the exponential growth phase, marked by a sharp increase in cell division and a significant rise in cell density. In jar-scale cultures, density peaked at 54.60×10^4 cells/mL, indicating optimal environmental conditions for algal metabolism and photosynthesis. Risna et al. (2022) noted that the exponential phase is characterized by constant cell division rates, periodic biomass doubling, and

peak metabolic activity. During this phase, chlorophyll pigment accumulation was high due to sufficient light intensity, CO_2 availability, and nutrient supply.

The stationary phase (days 12–14) showed slowed growth, attributed to nutrient depletion in the unrefreshed media. At this stage, cell growth and death rates equilibrated, stabilizing population density temporarily. This suggests environmental stress, such as declining media quality and metabolic byproduct accumulation. By day 15, cultures entered the decline phase, with cell counts dropping due to nutrient exhaustion and escalating environmental stress. Visual indicators included clearer media and foam formation.

All scales exhibited similar growth trends but differed markedly in final cell density. The jar scale yielded the highest density, followed by the carboy, while the intermediate scale had the lowest. These differences were driven by variations in light distribution, mixing efficiency, and environmental stability. Small-scale jars benefited from uniform lighting and stable temperatures, whereas intermediate-scale systems faced challenges in maintaining consistent parameters due to direct sunlight exposure and limited temperature/pH control.

Temperature played a critical role, with the cultivation period ranging **from** 27°C to 36°C**. T**he optimal growth temperature for *Chlorella vulgaris* is 25–32°C (Rosyadi et al., 2023). Jar-scale cultures began at lower temperatures (~27–30°C) before stabilizing at 30–33°C**,** while carboy and intermediate scales maintained more stable temperatures (30–33°C). This highlights the impact of vessel material and volume on thermal regulation—e.g., plastic carboys offered better insulation than glass or open systems.

pH remained stable at 8.0 across all treatments, aligning with the optimal range (6–9**)** for *C. vulgaris* (Rosyadi et al., 2023). This stability supported efficient photosynthesis and nutrient uptake, with no extreme acid/base accumulation observed.

Light intensity was maintained uniformly at 500 lux, a minimal but sufficient level for effective photosynthesis. Inadequate light can limit chlorophyll synthesis and reduce photosynthetic rates, but no significant light-related constraints were observed. Boroh et al. (2019) emphasized that appropriate light intensity and wavelength are crucial for microalgal productivity.

Notably, high cell density during the exponential phase did not trigger significant temperature fluctuations, suggesting that respiration and evaporation rates remained below thresholds for media temperature alteration. Carboys, with intermediate volumes, provided better temperature stability than jars, while intermediate-scale systems were more susceptible to external temperature variations due to larger sizes and direct sunlight exposure.

In summary, this study demonstrates that *C. vulgaris* cultivation success depends on the interplay of environmental and technical factors. Small- and intermediate-scale systems (jars and carboys) outperformed large-scale setups. Thus, for mass production, optimized cultivation systems—such as enclosed designs with artificial lighting and controlled aeration—are essential to replicate small-scale efficiencies.

CONCLUSION

The results confirm that cultivation vessel scale significantly impacts *Chlorella vulgaris* biomass yield**.** Small-volume cultures (e.g., jars) achieved higher cell densities due to superior light distribution and environmental control. In contrast, intermediate-scale systems faced technical challenges, including uneven lighting and limited circulation. For large-scale *C. vulgaris* production, system design improvements—such as

enhanced lighting, aeration, and mixing mechanisms—are necessary to emulate small-scale optimal conditions.

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