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GROWTH PHASE OF *ISOCHRYSIS GALBANA* NATURAL FEED WITH DIFFERENT CULTURE MEDIA VOLUMES ON LABORATORY SCALE

Fase Pertumbuhan Pakan Alami *Isochrysyis Galbana* Dengan Volume Media Kultur Yang Berbeda Pada Skala Laboratorium

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ABSTRACT

Isochrysis galbana is a microalgae belonging to the Chrysophyta group and is often used as natural feed in the aquaculture industry. This microalgae is known for its high nutritional content, including protein, fat, carbohydrates, vitamins, and important minerals. Isochrysis galbana is also one of the microalgae that has high potential as natural feed in fisheries cultivation. One of the causes of low growth and survival in the early development of larvae that still rely on food from natural feed is the availability of feed. The purpose of this study was to analyze the percentage level of increase in the density of the amount of natural feed cultured in Erlenmeyer media with different volumes of culture media. Completely Randomized Design with 3 treatments and 5 replications. This study was conducted for 21 days in the Laboratory at the Sekotong Cultivation Center. The treatments used were culture media volumes of 500 ml, 1000 ml and 5000 ml. The results of the study showed that the 500 ml volume culture media produced a 20% increase in density, then for the 1000 ml volume culture media produced a 16% increase in density and for the 5000 ml volume culture media produced an 8% increase in density. So with a 500 ml volume culture media can effectively increase the density of I. galbana. These findings are expected to provide useful information in the development of natural feed cultivation to increase fisheries productivity. Further research is needed to explore other factors that can affect the growth of Isochrysis galbana, including temperature, light, and the type of nutrients provided.

Key word : growth phase, I. galbana, culture, natural feed

ABSTRAK

Isochrysis galbana adalah mikroalga yang termasuk dalam kelompok Chrysophyta dan sering digunakan sebagai pakan alami dalam industri akuakultur. Mikroalga ini dikenal karena kandungan nutrisinya yang tinggi, termasuk protein, lemak, karbohidrat, vitamin, serta mineral yang penting. Isochrysis galbana juga termasuk salah satu mikroalga yang memiliki potensi tinggi sebagai pakan alami dalam budidaya perikanan.Salah satu penyebab rendahnya pertumbuhan dan kelulushidupan pada perkembangan awal larva yang masih mengandalkan makanan dari pakan alami adalah ketersediaan pakan. Tujuan penelitian ini adalah untuk menganalisis tingkat presentase kenaikan kepadatan jumlah pakan alami yang di kultur pada media erlenmeyer dengan volume media kultur yang berbeda. Rancangan Acak Lengkap dengan 3 perlakuan dan 5 kali ulangan. Penelitian ini dilakukan selama 21 hari di Laboratorium di Balai Budidaya Sekotong. Perlakuan yang dilakukan menggunakan volume media kultur 500

ml, 1000 ml dan 5000 ml. Hasil penelitian meunjukkan pada media kultur volume 500 ml menghasilkan kenaikan kepadatan sebesar 20%, kemudian untuk media kultur volume 1000 ml menghasilkan kenaikan kepadatan sebesar 16% dan untuk media kultur volume 5000 ml menghasilkan kenaikan kepadatan sebesar 8%. Sehingga dengan media kultur volume 500 ml dapat efektif memiliki kenaikan kepadatan I. galbana. Temuan ini diharapkan dapat memberikan informasi yang berguna dalam pengembangan budidaya pakan alami untuk meningkatkan produktivitas perikanan. Penelitian lebih lanjut diperlukan untuk mengeksplorasi faktor-faktor lain yang dapat mempengaruhi pertumbuhan Isochrysis galbana, termasuk suhu, cahaya, dan jenis nutrisi yang diberikan.
Kata kunci : *fase pertumbuhan, I. galbana, kultur, pakan alami*

INTRODUCTION

Microalgae are widely used in the aquaculture industry as live food and feed additives in the commercial rearing of many aquaculture species (Mata *et al*. 2010). Microalgae can be consumed directly such as for molluscs and peneid shrimp or consumed indirectly as food for live prey such as rotifers and brine shrimp to feed small larval fish (Patil *et al*. 2005). The most common microalgae species used as feed are *Chlorella, Tetraselmis, Isochrysis, Pavlova, Phaeodactylum, Chaetoceros, Nannochloropsis, Skeletonema* and *Thalassiosira* (Spolaore *et al*. 2006; Hemaiswarya *et al*. 2010). *Isochrysis galbana* is one of the microalgae species commonly used as natural feed in fish farming, namely rotifer feed, marine organism larvae such as molluscs, crabs, and fish. *Isochrysis galbana* is easily digested and contains many nutrients (Iwamony *et al*., 2024). Isochrysis galbana is one of the species of the Isochrysidales order, which is included in the haptophyta division. Unlike other microalgae belonging to Isochrysidales such as Emiliana huxleyi, *I. galbana* does not have a dimorphic life cycle and does not form calcareous cocoliths (Bendif *et al*., 2013). The nutritional composition of Isochrysis galbana is 12% to 14% lipid, 50% to 56% protein, and 10% to 17% carbohydrate (Milledge, 2011). *Isochrysis galbana* is one of the most promising carbohydrate-rich microalgae in the aquaculture industry. *Isochrysis galbana* is one of the most suitable nutrient sources for rapid growth. Microalgae have a short growth phase, according to Safitri (2023) states that the growth phase of microalgae consists of 4 phases, namely: a) Lag or Adaptation Phase, b) Logarithmic or Exponential Phase, c) Stationary Phase and d) Death Phase. The growth phase in microalgae occurs for 7 days of maintenance. So that microalgae are suitable for larvae, but one of the causes of low growth and survival in the early development of larvae that still rely on food from natural feed is the availability of feed. The purpose of this study was to analyze the percentage increase in the density of the amount of natural feed cultured with different volumes of culture media.

METHODS

Sterilization of Equipment

In laboratory-scale natural feed culture activities, the equipment used is first sterilized by washing the equipment using detergent and boiling the equipment. Equipment that is sterilized by boiling is usually made of plastic such as aeration hoses, 5-10 liter jars, toles lids, and aeration stones. As for glass culture equipment, sterilization is carried out using an autoclave at a temperature of 121◦C for approximately 4 hours. This is in line with the statement of Bangun *et al*. (2015), which states that sterilization is a process to completely inactivate living microbes. Sterilization of research equipment is washed and dried. After equipment such as aeration hoses is completely dry, steam them in boiling water for 15 minutes, then cool and dry them before reusing them. Equipment such as Erlenmeyer flasks are cleaned like other equipment and dried until completely dry. After that, the mouth of the Erlenmeyer flask is

closed with sterile cotton and gauze, and finally covered with aluminum foil and sterilized in an autoclave. On a laboratory scale, the media water after going through the filtration process is collected in a 300 liter fiber tank, sterilized with halamide at a dose of 10 ppm and left for approximately 24 hours. This is done to even out the balance of chlorine content in the water. The chlorinated water is placed in a container-shaped storage tank and prepared as medium water.

Preparation of Culture Media

I. *galbana* culture activities in the Laboratory use KW21 and silicate fertilizers for microalgae cultures using jars with a capacity of 5-10 liters with a dose of 15-20 ppm/liter of water. The ingredients of KW21 fertilizer are 49 g/L nitrogen (N), 4 g/L phosphoric acid (P), boron, manganese, iron, zinc, cobalt, EDTA, complex amino acids, a mixture of vitamins (B1, B12, biotin, and others). This is in line with the statement of Mukhlis (2018), which states that commercial fertilizer KW21 49 g/L nitrogen (N), 4 g/L phosphoric acid (P), boron, manganese, iron, zinc, cobalt, EDTA, complex amino acids, vitamin mixtures (B1, B12, biotin, etc.). KW21 fertilizer is very effective in improving the quality and quantity of natural feed, which is very important in fish farming to ensure optimal nutrient availability for the organisms being farmed. Before the P. lutheri seed spreading process is carried out, make sure all equipment and media are sterile. The culture process is carried out by preparing a water medium with a volume of 250 ml to 1 liter that has been sterilized using an autoclave, then aeration is given and then KW21 fertilizer is added as much as 1 ml/L of water and silicate as much as 0.5 ml/L of water and left for approximately 30 minutes. The next stage is the spreading of pure seeds as much as 1:1 between the volume of water and the volume of seeds or adjusting the condition of the seeds. After that, it is covered using aluminum foil to avoid contamination from other microorganisms. According to Setyawati *et al*. (2017), laboratory-scale cultivation is the development of plankton in a controlled and maintained space with the aim of preserving and producing plankton. Furthermore, according to Sopian *et al*. (2019), the culture process begins with sterilization of equipment and culture materials to prevent or even eliminate pathogens.

Research Preparation

This research was conducted in a laboratory with a Completely Randomized design with 3 treatments and 5 replications. The first treatment used a culture media volume of 500 ml, the second treatment used a culture media volume of 1000 ml and the third treatment used a culture media volume of 5000 ml

RESULT

The results of plankton density in 500 ml Erlenmeyer culture on a laboratory scale can be seen in Figure 1. Based on the graph of the results of the calculation of the density of *I. galbana* phytoplankton in 500 ml Erlenmeyer culture media, the results of the density calculation on the first day were 1,140,000 cells/ml, and experienced a significant increase on days 2 to 4, namely 2,540,000 - 14,000,000 cells/ml. On the first day, phytoplankton entered the lag phase or the phytoplankton adaptation phase with the media and nutrients in the new culture media. Meanwhile, on days 2 to 4, phytoplankton entered the exponential phase, where in this phase phytoplankton began to utilize the nutrient content in the culture media and light sources for the photosynthesis process and phytoplankton cell division. In addition, on days 2 to 4, phytoplankton growth was influenced by the place where phytoplankton grew, such as pH, temperature, and air humidity. On the 5th day, the growth of phytoplankton in 500 ml Erlenmeyer culture media was 24,800,000 million cells/ml. On the 5th day, the growth of phytoplankton entered the stationary phase, where in that phase the growth of phytoplankton

was relatively more balanced between growth and cell death. In this phase, the cell size becomes smaller because each cell continues to divide even though the nutrients in the culture media are running out. On the 6th to 7th day, the growth of phytoplankton began to decline again, namely 22,300,000-19,140,000 cells/ml, because on that day the phytoplankton began to enter the death phase.

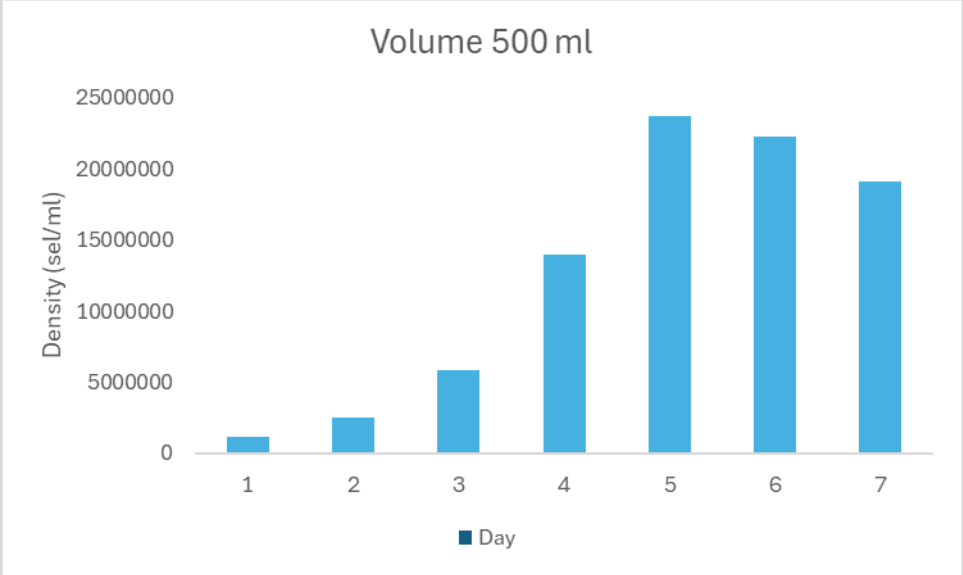

Figure 1. Density of *I. galbana* at a volume of 500 ml

In Figure 2, the results of the calculation of the density of I. galbana phytoplankton in 1,000 ml Erlenmeyer culture media obtained the results of the density calculation on the first day as many as 1,140,000 cells/ml, and experienced a significant increase on days 2 to 4, namely 5,340,000 - 11,800,000 cells/ml. On the first day, phytoplankton entered the lag phase or the phytoplankton adaptation phase with the media and nutrients in the new culture media. Meanwhile, on days 2 to 4, phytoplankton entered the exponential phase, where in this phase phytoplankton began to utilize the nutrient content in the culture media and light sources for the photosynthesis process and phytoplankton cell division. In addition, on days 2 to 4, phytoplankton growth was influenced by the place where phytoplankton grew such as pH, temperature, and air humidity. On the 5th day, the growth of phytoplankton in 1,000 ml Erlenmeyer culture media was 18,500,000 cells/ml. On the 5th day, the growth of phytoplankton entered the stationary phase, where in that phase the growth of phytoplankton was relatively more balanced between growth and cell death. In this phase, the cell size becomes smaller because each cell continues to divide even though the nutrients in the culture media are running out. On the 6th to 7th day, the growth of phytoplankton began to decline again, namely 16,340,000-11,040,000 cells/ml, because on that day the phytoplankton began to enter the death phase.

In Figure 3, the graph of the results of the calculation of the density of *I. galbana* phytoplankton in 5,000 ml Erlenmeyer culture media shows that the density calculation results on the first day were 1,140,000 cells/ml, and experienced a significant increase on days 2 to 4, namely 2,900,000 - 8,600,000 million cells/ml. On the first day, phytoplankton entered the lag phase or the phytoplankton adaptation phase with the media and nutrients in the new culture media. Meanwhile, on days 2 to 4, phytoplankton entered the exponential phase, where in this phase phytoplankton began to utilize the nutrient content in the culture media and light sources for the photosynthesis process and phytoplankton cell division. In addition, on days 2 to 4,

phytoplankton growth was influenced by the place where phytoplankton grew, such as pH, temperature, and air humidity.

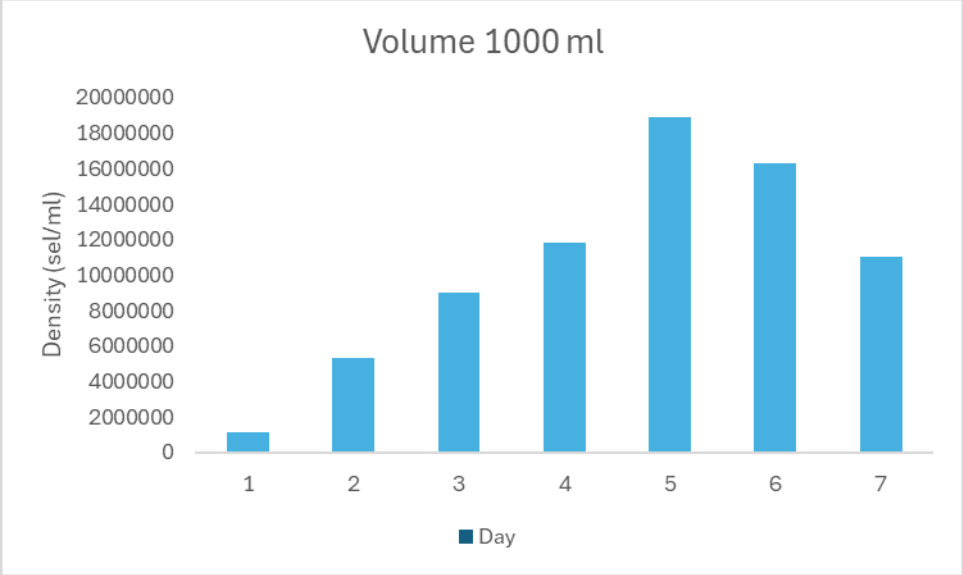

Figure 2. Density of *I. galbana* at a volume of 1000 ml

On the 5th day, the growth of phytoplankton in 5,000 ml Erlenmeyer culture media was 10,150,000 cells/ml. On the 5th day, the growth of phytoplankton entered the stationary phase, where in that phase the growth of phytoplankton was relatively more balanced between growth and cell death. In this phase, the cell size becomes smaller because each cell continues to divide even though the nutrients in the culture media are running out. On the 6th to 7th day, the growth of phytoplankton began to decline again, namely 8,900,000-6,600,000 cells/ml, because on that day the phytoplankton began to enter the death phase.

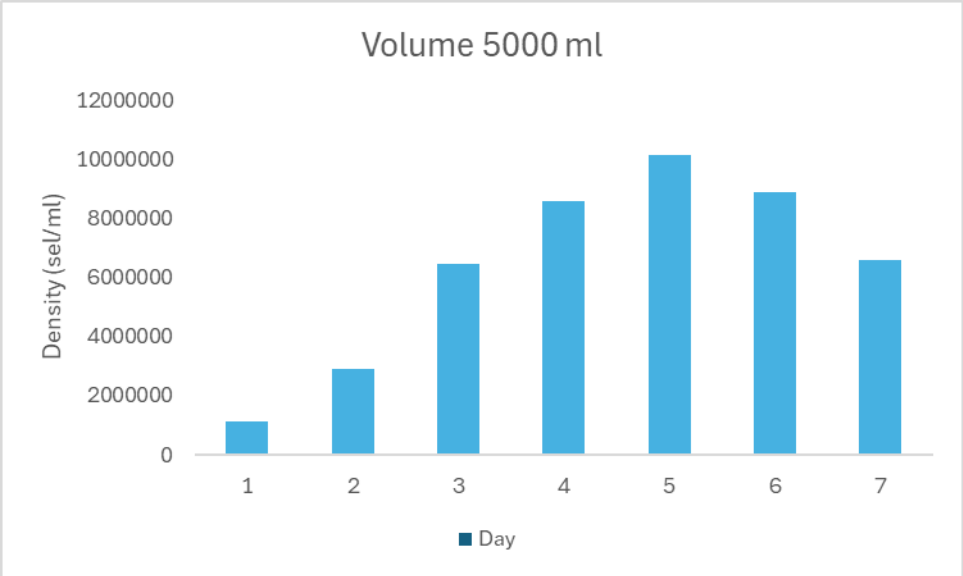

Figure 3. Density of *I. galbana* at a volume of 5000 ml

DISCUSSION

At this stage, the nutrients in the media are depleted and the microalgae can no longer survive. This is in line with Safitri's statement (2023) which states that in the delayed or adaptation phase, microalgae cells begin to adapt to physiological environmental conditions. Then after the algae are added to the culture medium, physiologically the organism undergoes metabolism, but cell division has not occurred so that cell density has not increased. Logarithmic or exponential phase. This phase begins with cell division with a constant growth rate. Under optimal cultivation conditions, the growth rate reaches its optimal point in the logarithmic phase. In the exponential phase, the growth rate is influenced by the growth location, including pH, nutrient content, temperature, and humidity. At this stage, cells require more energy than other stages, and cells are most sensitive to environmental conditions. Stationary phase: At this stage the reproduction rate is the same as the death rate, so that the addition and reduction of the number of cells are relatively the same or balanced, and cell density remains constant. During this stage, the cell size decreases because each cell continues to divide even though the nutrients have run out. Death phase: This phase is characterized by little cell division and large cell death. At this stage, the microalgae are no longer able to survive on steady state (without receiving nutrients), so their population returns to the starting point and the graph shows a sharp decline.

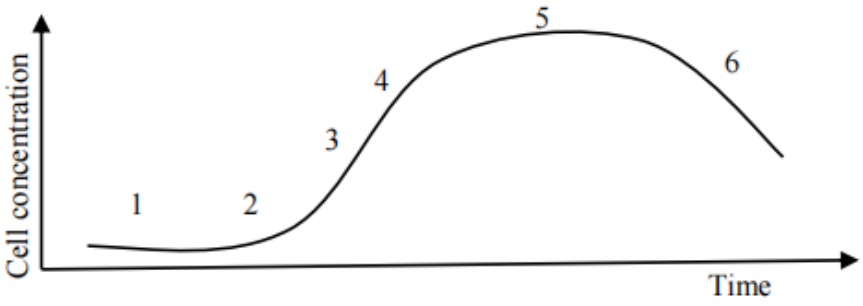

Figure 4. Growth curve of microalgae culture in batch with phases; (1) lag, (2) acceleration, (3) growth, (4) deceleration, (5) stationary, and (6) death
(Source: Azmi, 2018)

Table 1. Density of Natural Feed *I. galbana* at volumes of 500 ml, 1000 m, 5000 ml

N0	Density (sel/ml)		
	Volume 500 ml	Volume 1000 ml	Volume 5000 ml
1	1.140.000	1.140.000	1.140.000
2	2.540.000	5.340.000	2.900.000
3	5.840.000	9.040.000	6.450.000
4	14.000.000	11.800.000	8.600.000
5	23.700.000	18.900.000	9.100.000
6	22.300.000	16.340.000	8.900.000
7	19.140.000	11.040.000	6.600.000

Based on Table 1, the highest density results are found in the 500 ml Erlenmeyer culture medium with a density of 23.7 million cells/ml. Factors that can affect phytoplankton density are light intensity, water quality, and the amount of nutrients contained. In addition, the volume of the container is one of the supporting factors that can affect phytoplankton density. This is in accordance with the statement of Padang et al. (2015) who said that one of the environmental

conditions that affects phytoplankton growth is light. Light intensity is very important in the process of photosynthesis because it is related to the amount of energy received by phytoplankton for photosynthesis. In addition, the size of the container can also affect phytoplankton density.

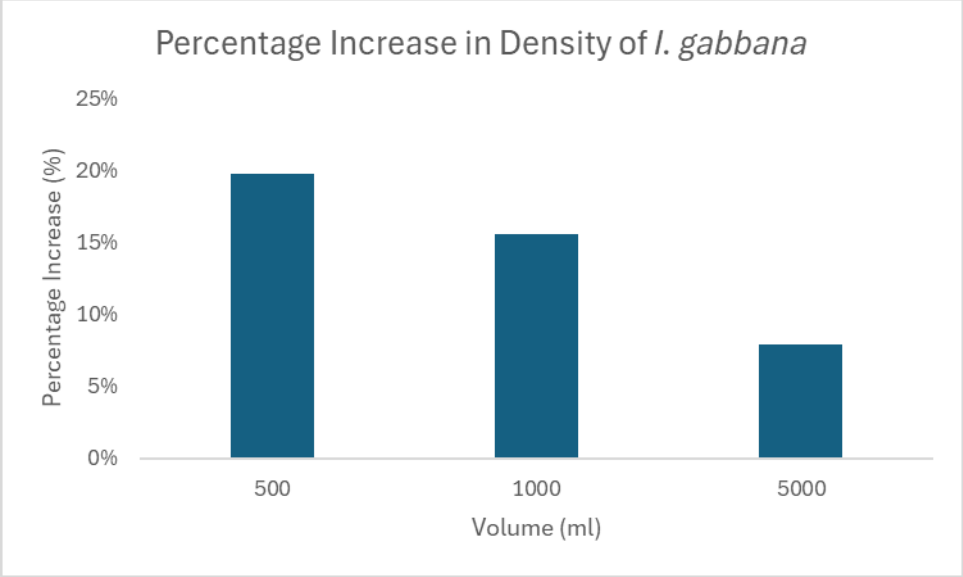

Figure 5. Percentage of Density Increase of *I. galbana* at different volumes

Based on Table 1, it can be concluded that the growth of I. galbana phytoplankton in culture media with volumes of 500 ml, 1,000 ml, and 5,000 ml reached its peak on the 5th day, with a density of 24,800,000 cells/ml for culture media with volume of 500 ml, for culture media with volume of 1,000 ml reached a peak phase of 18,900,000 cells/ml on the 5th day, and for culture media with volume of 5,000 ml reached a density of 9,100,000 cells/ml. In this case, what affects the height of plankton growth is because plankton are able to utilize the nutrients in the water which is known as the exponential phase which is characterized by a significant increase in the number of cells. This is in accordance with the opinion of Sumsanto and Muahiddah (2023) who stated that in the exponential phase, inoculated cells utilize nutrients in the medium so that cells can grow and produce more. In the logarithmic phase, the inoculated cells experience maximum cell division or double the previous number. On the 6th day, the cells enter the stationary phase, and the number of cells decreases towards the declination phase (death phase). On the 7th day, the plankton density decreases in the declination phase (death phase). This is influenced by the availability of nutrients, light, temperature and age of the plankton. Figure 5 shows that the highest increase in the percentage of phytoplankton density is in the 500 ml culture media volume of 20%, then in the 1000 ml culture media volume there is an increase in the percentage of phytoplankton density of 16% and in the 5000 ml culture media volume of 8%. This study is in accordance with the opinion of Sumsanto and Muahiddah (2023) who stated that the best phytoplankton culture is to use a culture with a culture media volume between 250 and 500 ml. The resulting cell growth produces up to more than 700x 104 cells/ml (Sumsanto and Muahiddah, 2023).

CONCLUSION

Based on the research that has been done, it can be concluded that the Erlenmeyer method culture carried out with the treatment of different volumes of culture media produced for a 500 ml volume culture media produced a density increase of 20%, then for a 1000 ml volume culture media produced a density increase of 16% and for a 5000 ml volume culture

media produced an increase in density of 8%. So that with a 500 ml volume culture media can effectively have an increase in the density of *I. galbana*.

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