Original Research Article

EFFECTIVENESS OF UREA FERTILIZER WITH DIFFERENTCONCENTRATIONS ON *Tetraselmischuii*CULTURE ON LABORATORY SCALEAT TECHNICAL IMPLEMENTATION UNIT OF SOUTH BRACKISHANDMARINE CULTURE (UPTD PAPLWS)

Abstact

Tetraselmischuii is a species of phytoplankton which has a high nutritional content, namely 70.287% protein, 0.795% lipid and 2.180% carbohydrate. The aim of this research was to determine the best urea fertilizer concentration for *Tetraselmischuii* cell density on a laboratory scale. The research method used was a Completely Randomized Design (CRD) experimental method, with 4 treatments and 4 replications. The treatments in this study consisted of treatment A, namely 1 ml/L KW21 fertilizer (control), treatments B, C and D using 30 mg/L, 45 mg/L, 60 mg/L Urea fertilizer with a ZA concentration of 40 mg/L and TSP 5 mg/L. Observations were carried out for 24 hours. The parameters observed included *Tetraselmischuii* population density, growth rate, generation time and air quality. The results showed that treatment D produced the best results with a population density of 232.75 x 104 cells/ml, a growth rate of 0.31 cells/ml/day and the fastest generation time of 15.41 hours.

Keywords :*Tetraselmischuii*, Urea Fertilizer, Population Density, Growth Rate, Generation Time

INTRODUCTION

Natural feed has many advantages, especially in cultivation activities. Some of the advantages are affordable prices(Raharjo *et al.* 2016),fits the fish's mouth opening and has complex nutrition so that the organism's nutritional needs are met (Rihi 2019). Natural food is very diverse in nature, including plankton, namely zooplankton and phytoplankton which play an important role in meeting nutritional needs during larval life (Arif 2014).

Phytoplankton or vegetable plankton are microscopic organisms in the form of plants because they can photosynthesize to produce organic matter or autotrophs, their life floats and floats along with the movement of water (Nontji 2008). Microalgae that have high nutritional content and are commonly found in Indonesia are *Chlorella vulgaris*, *Chaetoceroscalcitrans*, Nannochloropsisoculata and *Tetraselmischuii*(Suminto dan Susilowati 2017).

*Tetraselmischuii*is a species of phytoplankton which has a high nutritional content, namely 70.287% protein, 0.795% lipid and 2.180% carbohydrate(Hermawan *et al.* 2017).

Tetraselmischuii has thin cell walls and autolysis walls so that it is easily digested by larvae(Costa *et al.* 2004), and has a relatively small body size so that it fits the mouth opening of fish and shrimp larvae (Harun *et al.* 2010).

*Tetraselmischuii*requires nutrition for its growth. These nutrients usually come from inorganic fertilizers, namely all types of fertilizers made from inorganic chemicals and are usually made by factories, for example urea, ZA, TSP, and pro-analyst fertilizers such as KW21 fertilizer. (Amini dan Syamsidi 2006).

According to Mukhlis *et al.*(2017)KW21 fertilizer is a pro-analyst fertilizer currently used in laboratory scale *Tetraselmischuii* culture. This fertilizer has liquid form so the nutrients in it will be easily absorbed. The method of administering fertilizer is directly, thus ensuring safety and convenience for users. Furthermore, KW21 makes the growth of *Tetraselmischuii* stable because it has complete content with 49 g/L nitrogen, 4 g/L phosphoric acid, boron, manganese, iron, zinc, cobalt, EDTA, complex amino acids and a mixture of vitamins (B1, B12, biotin and others) which are quite reliable on a laboratory scale. In addition, KW21 is expected to maintain peak density for longer after *Tetraselmischuii* develops. However, KW21 has a high price and it is relatively difficult to get it because it has to be imported from Japan, so its use is highly considered. Therefore, research is needed on alternative fertilizers whose effects are the same or close to the density level of KW21.

The compound most needed in fertilizing phytoplankton is nitrogen. One of the nitrogen supplies comes from urea fertilizer, which is usually used by farmers for plant growth and development in agricultural activities(Ramadhani *et al.* 2016). Urea fertilizer is an artificial fertilizer to stimulate the growth of phytoplankton, forms chlorophyll which causes cells to be greener in color and is a building block for protein and fat(Fery *et al.* 2020). This fertilizer is cheaper and easily available on the market.The aim of this research was to determine the best urea fertilizer concentration for *Tetraselmischuii* cell density on a laboratory scale

MATERIAL AND METHODS

The maintenance period for *Tetraselmischuii* lasts for 6 days. Research activities were carried out in two places, namely at the PSDKU UnpadPangandaran Fisheries Laboratory to carry out the sterilization process and Plankton Laboratory of Technical Implementation Unit of South Brackishand Marine Culture (UPTD PAPLWS) to carry out the culture and observation process.

Tools and materials used in the research include 500 ml glass bottles, blowers, TL lamps, dropper pipettes, microscopes, hand counters, litmus paper, thermometers, digital scales, refractometers, lux meters, label paper, stationery, cameras, aluminum foil, pipettes measuring, petri dish, 15 L jar, Haemacytometer, cover glass, aeration stone, aeration hose, funnel, cotton, magnetic stirrer, 500 ml Erlenmeyer flask, autoclave, 24 well plate *Tetraselmischuii* inoculum, urea fertilizer, ZA, TSP, KW21, alcohol 70%, sea water 25-32 ppt, chlorine, potassium permanganate, sodium thiosulfate and chlorine tester.

This research consisted of 4 treatments with 4 repetitions. The total number of containers used in the research was 16 containers. The treatments tested were the use of 1 ml KW21 fertilizer (treatment A) as a control, 30 mg.L-1 urea fertilizer (treatment B), 45 mg.L-1 (treatment C), 60 mg.L-1 (treatment D) and each treatment that uses urea fertilizer is given ZA fertilizer with a concentration of 40 mg.L-1 and TSP fertilizer of 5 mg.L-1. The fertilizer is crushed using a pestle and mortar.

Data analysis was carried out to determine the effect of treatment on the response of test parameters. Data on population density, growth rate and generation time were analyzed using variance or F test with a 95% confidence level. If there is a significant difference, then the test is continued with Duncan's multiple distance test, while the water quality is analyzed descriptively.

Preparation of culture medium

Seawater culture media was collected in a 15 L container, given 60 ppm chlorine and left for 24 hours accompanied by strong aeration to make it homogeneous. If for 24 hours it still contains chlorine (known from the smell of chlorine and the results of the chlorine test), sea water is given 30 ppm sodium thiosulfate for 24 hours. Then put them into culture bottles of 500 ml each and sterilized using an autoclave together with a solution of urea, ZA and TSP fertilizer media for 20 minutes at a temperature of 121^{o}C at a pressure of 1 atm.

***Tetraselmischuii* culture**

Initial inoculum density of *Tetraselmischuii* used was 5x 10^5 cells/mL. The inoculum is placed in a culture bottle containing sterile seawater and fertilizer according to the treatment. Cell density calculations using a hemocytometer under a microscope were carried out every day for 6 days. The initial density calculation will use the formula (Ruliaty *et al.* 2019) :

$$V_1 \times N_1 = V_2 \times N_2$$

Where :

V_1 = Volume of *Tertraselmis*needed (ml)

N_1 = Inoculum population density (cell/ml)

V_2 =Volume of cultured water (ml/L)

N_2 = Desired initial density (cell/ml)

Cell density

Calculation of population density is calculated using the following formula according to (Isnansetyo & Kurniastuty 1995):

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

Where :

P = Cell density

N = Average number of cells

Growth rate

The growth rate of phytoplankton is calculated using the formula according to (Fogg 1987) in (Hermawan *et al.* 2017):

$$k = \frac{lnWt - lnWo}{T}$$

Where :

k = Growth rate (cell/ml/day)

Wt = Number of cells after time t (cell/ml)

Wo = Initial cell number (cell/ml)

T = Culture time from Wo to Wt(day)

Generation time

Phytoplankton generation time is calculated using the formula according to (Isnansetyo & Kurniastuty 1995):

$$G = \frac{T}{3{,}3(logWt - logWo)}$$

Keterangan :

G = Generation time (hour)

Wt = Number of cells after time t (cell/ml)

Wo = Initial cell number (cell/ml)

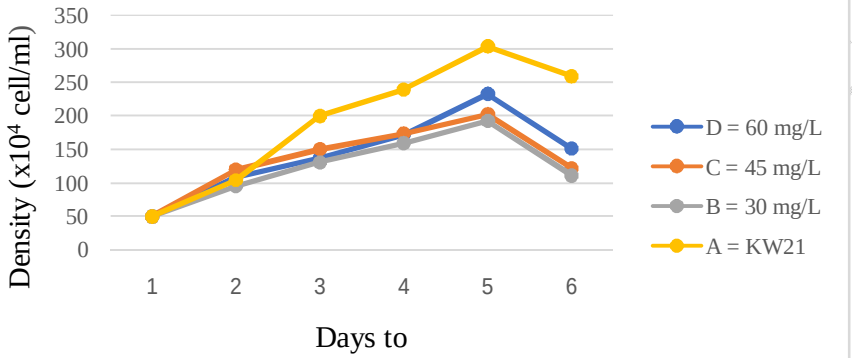
T = Culture time from Wo to Wt(hour)

RESULT AND DISCUSSION

Cell density of *Tetraselmischuii*

The adaptation phase of all treatments in this research occurred within 1 day. This is influenced by the environment of the previous *Tetraselmischuii* seedlings with the culture process such as age, species, media and culture location still remaining the same so they have adapted. This is in accordance with Pujiono (2013) explanation, namely that several things influence the timing of the adaptation phase, including age and cell type. The adaptation time will take longer if the culture medium has less nutrients. because cells must produce enzymes that match the type of nutrients present.

Fig 1. Cell Density of *T. chuii*



The average cell density results of *Tetraselmischuii* in treatment A reached peak density on the fifth day with a cell number of 303,75 x 10^4 cells/ml, for treatment B it reached peak density with a cell number of 192,5 x 10^4 cells/ml. The growth in the number of *Tetraselmischuii* cells in treatments C and D reached peak density with cell numbers of 202,25 x 10^4 cells/ml and 232.75 x 10^4 cells/ml. Next, the population density results were tested using Analysis of Variance (ANOVA). Treatment A had the best growth compared to other treatments. The results were not significantly different from treatment D and significantly different from treatments B and C. So the best use of urea fertilizer is to use treatment D.

Treatment A produces the highest cell density because it uses KW21 fertilizer which contains more complete nutrients, resulting in the highest population density. This is in accordance with the statement of Regista *et al.*(2017)that the growth of microalgae is not only influenced by the availability of adequate macro nutrients such as carbon, nitrogen, phosphorus, but is also influenced by the availability of micro nutrients such as iron, manganese, zinc, cobalt, copper which are sufficient and according to needs.

Micronutrients are actually only needed in small amounts, but they play an important role in the growth of *Tetraselmischuii.* As statedIsnansetyo & Kurniastuty (1995) that

microalgae require various types of inorganic compounds, both macro nutrients (N, P, K, S, Na, Si and Ca) and micro nutrients (Cu, Mg, Fe, Mn, Zn, Mo, Co , B and others). Every nutrient contained in the fertilizer media is actually said to be complete because in the statement Leksono *et al.*(2017) that Urea and ZA fertilizers contain nitrogen while TSP fertilizer contains phosphate. This fertilizer is good for phytoplankton culture because it is easily soluble in water. This is in accordance with the statement of Wirabumi *et al.*(2017)stated that nutrients dissolved in water bodies are directly utilized by phytoplankton for their growth so that their population and abundance increases. After experiencing the exponential phase, on the sixth day all treatments experienced a stationary phase. According to Kawaroe *et al.* (2010)the stationary phase can be identified from the amount that does not increase or is the same as before. However, in this research this phase is thought to occur in less than 24 hours so the number of *Tetraselmischuii*produced cannot be observed.

The next phase is the death phase which occurs on the sixth day with each treatment having a decreasing population density. Reduced nutrient availability and decreased water quality parameters cause cells not to grow and develop optimally so that cell density will decrease(Prihastama *et al.* 2016).

Table 1. Effect of Fertilizer Concentration on *Tetraselmischuii* Density ($x10^4$ cells/ml) at Peak Population Time.

Treatment	Time to Reach Peak Population (day)	Maximum Population Density ($x10^4$cells/ml)
A	5	303,75 ± 17,21 [c]
B	5	192,50 ± 11,73 [a]
C	5	202,25 ± 21,42 [ab]
D	5	232,75± 42,30 [bc]

Note: The average value followed by the same letter is not significantly different at the 5% level.

Growth rate of *Tetraselmischuii*

Treatment D is an agricultural fertilizer medium that produces the highest daily growth rate, namely 0.31 cells/ml/day, which is not significantly different from treatment A. This is thought to be because the fertilizer content in treatment D is in accordance with the needs of *Tetraselmischuii* so that it can accelerate the growth rate and population size. Apart from that, this research also shows that the higher the concentration of urea fertilizer, the greater the growth of *Tetraselmischuii*.

The lowest growth rate was in treatment B, namely 0.27 cells/ml/day, thus showing significantly different results from treatment D (Table 2). This is caused by less nitrogen, even though it is needed for the formation of chlorophyll, where chlorophyll is needed in the photosynthesis process. According to Amanatin & Nurhidayati (2013) that when the concentration of the nitrogen element is reduced, the formation of chlorophyll will be hampered so that the photosynthesis process is also hampered. The inhibition of the photosynthesis process resulted in the growth of *Tetraselmischuii* being hampered.

Table2. Effect of Fertilizer Concentration on Daily Growth Rate of *Tetraselmischuii* (cells/ml/day) During Peak Population.

Treatment	Daily Growth Rate (cells/ml/day)
A	$0,36 \pm 0,011^{c}$
B	$0,27 \pm 0,012^{a}$
C	$0,28 \pm 0,020^{ab}$
D	$0,31 \pm 0,034^{bc}$

Note :The average value followed by the same letter is not significantly different at the 50% level.

Generation Time

Generation time is the speed of time required for phytoplankton to divide their cells twice. After a cell divides into 2, each becomes a daughter cell which then divides again into 2 and so on. According to Boleng (2015)generation time is influenced by several growth factors such as nutrient availability, light intensity, pH, oxygen, genetics and so on. If these factors are available in optimum quantities for phytoplankton to divide their cells, then within a certain period of time the phytoplankton population will increase to greater numbers.

Based on the calculation results (Table 3), treatment D has the fastest generation time, namely 15.41 hours, which is not significantly different from treatment A, namely 14.65

hours. This can happen because the nutritional content in the media can meet growth needs and accelerate cell division of *Tetraselmischuii*(Pujiono 2013). Meanwhile, treatment B experienced a slower time, namely with a generation time of 15.92 hours.

Table3. Effect of Fertilizer Concentration on Generation Time (Hours) of *Tetraselmischuii* at Peak Population Times

Treatment	Generation time (hour)
A	14,65 ± 0,14[a]
B	15,92 ±0,19 [b]
C	15,79 ±0,29 [b]
D	15,41 ±0,47 [a]

Note :The average value followed by the same letter is not significantly different at the 50% level.

Water Quality

Water quality values during the research were relatively stable because the culture process was carried out intensively so that environmental conditions were relatively homogeneous and easy to control. The water quality parameters observed during the research were temperature, salinity and pH. Each parameter was observed every day while the light intensity was carried out at the beginning of the study with a value of 5530 lux. The temperature of the culture media in the four treatments ranged from 26.9^{o}C–28^{o}C, salinity ranged from 29–34 ppt, pH value 7.

Nutrient composition and concentration influence phytoplankton growth, but apart from that salinity and light intensity can also influence cell density and growth rate (Hotos & Avramidou 2021). The results of this research produced an average salinity value ranging from 29-34 ppt. This value is in accordance with research by Rostini (2007)that *Tetraselmischuii* can live in a fairly wide range of salinity values, namely 15-36 ppt with optimal conditions of 25-35 ppt. The research results of Noriko *et al.*(2015) explained that salinity affects the density and growth rate of microalgae, where the level of microalgae population density will vary according to the level of salinity in the environment. According to Wardani *et al.*(2022) that decreases or increases in salinity values during culture can still be tolerated. The salinity value can naturally increase due to respiration from organisms in the water which can increase the mineralization process which causes salt levels to increase in the culture process.

The growth of organisms is related to pH because it can affect enzyme activity. Apart from that, pH can also affect the metabolism and growth of microalgae and change the availability of nutrients which can affect the physiology of *Tetraselmischuii* cells (Afriza et al. 2015). The research results show that the average pH value for each treatment is 7 which can be seen in Table 4. This value is in accordance with the explanation of Pratiwi et al. (2015) that the pH suitable for plankton growth is 7-8.5.

According to Indrastuti *et al.*(2014)light is one of the main factors that influence the cultivation of microalgae, because light is an important part of photosynthetic pigments which provide energy for microalgae life. Lack of light can cause the photosynthesis process to not take place optimally, which can affect the growth of microalgae. However, there is also another opinion that providing light intensity can be said to be optimal depending on the cultivation objectives themselves (Gonzalez *et al.* 2021).

This research used 65 watt white light with a light intensity of 5530 lux. This value is in accordance with the opinion of Isnansetyo & Kurniastuty (1995) that the optimal value for the growth of *Tetraselmischuii* is between 5,000-1,0000 lux. According to Kusdarwati *et al.*(2011) Light intensity plays a very important role, the need is very large depending on the depth and density of the cultivation itself, and the light can come from nature or from lamps. White light has the most complete and good light components for this process because it is a combination of various rays and has the highest intensity so it contains the most energy among all other colors of light and the resulting wavelength is 2500 lux. Light intensity and the photosynthesis process have a very close relationship, although increasing light intensity is not always followed by an increase in the photosynthesis process. Each color of light has a different wavelength and has different penetration and absorption capabilities. The growth of microalgae can be assisted by light energy to carry out the photosynthesis process. But this energy depends on light intensity, photoperiod and light quality (Muyassaroh *et al.* 2018).

CONCLUSION

The conclusion from the research that has been carried out is that treatment D (Urea 60 mg/L, ZA 40 mg/L and TSP 5 mg/L) produces the highest density of *Tetraselmischuii*, namely 232.75 x 104 cells/ml, growth rate 0.31 cells/ml ml/day and the fastest generation time is 15.41 hours. These results have provided a high population density level approaching the results of KW21 fertilizer which has a density value of 303.75 x 104 cells/ml, a growth rate of 0.36 cells/ml/day and a generation time of 14.65 hours. Then from an economic perspective it can be recommended because it can cut production costs.

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