The optimal n/p ratio of shrimp culture waste liquid fertilizer on growth of *Chlorella vulgaris*

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Abstract. Environmental quality protection is done by processing shrimp waste into materials of high use value, including liquid fertilizer. The value of N/P ratio in shrimp culture waste fertilizer is still relatively low so it needs to add urea as a source of nitrogen to increase the N/P ratio in fertilizer. The purpose of this research was to determine the optimal N/P ratio in shrimp culture waste fertilizer to the growth of *Chlorella vulgaris*. The research method used in this research was an experimental method, A completely randomized design with 5 treatments and 4 replications. The treatments used are giving different N/P ratios from shrimp culture waste fertilizer enriched by urea, with N/P ratio 10:1, 15:1 and 20:1, whereas Walne fertilizer with N/P ratio 17:1 and shrimp culture waste fertilizer with N/P ratio 5.5:1 as a control. Data analyze was processed use ANAVA and followed by DMRT. The results of the research showed that the using of different N/P ratios in shrimp culture waste fertilizer enriched by urea gave a significantly different effect on the population and growth rate with the highest population average of 1030x10⁴ cell/ml and an average growth rate of 203x10⁴ cell/ml/day during the exponential phase.

1. Introduction

Increased production targets led to changes in the pattern of shrimp culture previously used extensively pattern changed into a pattern intensive shrimp culture activities with a density of 500 fish/m² produce nitrogen waste load of 406.57 kg and 100.33 kg phosphorus retention 30.47% N and P 16.59% [1]. This culture system resulted in degradation of the environment due to the production process, followed by sewage containing organic matter and nutrients, is both particles or dissolved. Shrimp culture wastes are includes ammonia, organic matter and solids [2]. The efforts to improve sewage treatment is to try utilization of shrimp culture for cultivation of natural food, one of which is *Chlorella vulgaris* with the content perfectly adequate nutritions are proteins, amino acids, unsaturated fatty acids, vitamin C, K, B1, B6, B12, and beta carotene [3]. The successful cultivation of *Chlorella vulgaris* is very dependent on the availability of nutrients, especially nitorgen and phosphorus.

Shrimp culture waste can be used as fertilizer to stimulate the growth of *Chlorella vulgaris* but the organic material must be converted first into inorganic materials to be used by microalgae [2]. The content of inorganic which can be used by microalgae for growth is ammonium (NH₄⁺), nitrate (NO₃⁻) and phosphate (PO₄³⁻) [4]. Processing of organic matter into inorganic material in the waste can be added Effective microorganisms to shrimp culture waste in the manufacture of fertilizer, Rachman [5] reported that shrimp culture waste was fermented by EM has a total N content of 0.46% and P₂O₅ of 6.25%. Value ratio of N/P in the fermented waste is still low and insufficient *Chlorella vulgaris* that
needs to be enriched with nitrogen to increase the ratio of N/P on shrimp culture waste and value engineering done on the element phosphorus as a limiting factor in the growth of Chlorella vulgaris.

Excellence fermented shrimp culture waste by Effective Microorganism (EM) and added nitrogen as a provider of high nutrient in promoting the growth of Chlorella vulgaris. So that further research is needed to determine the growth of Chlorella vulgaris in the culture medium using a liquid fertilizer of shrimp culture wastes fermented with different of N/P ratio. The purpose of this study was to determine the relationship of N/P ratio on shrimp culture waste fertilizers on growth of Chlorella vulgaris and to know the optimal of N/P ratio in waste shrimp culture fertilizer on the growth of Chlorella vulgaris.

2. Materials and methods

2.1 Research design

The method used in this research is experimental methods. The experimental design used is completely randomized design with 5 treatments and 4 replications. The independent variable in this research is the different of N/P ratio in the effluent of shrimp culture waste added urea in the culture medium of Chlorella vulgaris, the dependent variable is the number of population and the rate of growth of Chlorella vulgaris, and variable control that is the origin of a pure culture of Chlorella vulgaris, waste shrimp culture, urea, effective microorganism and culture environmental conditions or water quality include temperature, salinity, pH and light intensity.

The treatment in this research is the addition of nitrogen (N) in the shrimp culture waste fertilizer with different of N/P ratio. Determination of the variation of N/P ratio in this research using 5 treatments.

Table 1. Treatment Used in Research

| Treatment | Addition of N Elements (Urea / 80ml) | The value of N (mg/L) | P values (mg / L) | The ratio of N/P | Giving volume (ml / L) |
|-----------|------------------------------------|----------------------|------------------|-----------------|----------------------|
| P1        | Walne                              | x                    | 1510             | 87.24           | 17:1                 | 1 ml                 |
| P2        | SFW                                | x                    | 1510             | 270             | 5.5:1                | 7 ml                 |
| P3        | SFW + Urea                         | 29 mg                | 1510             | 151             | 10:1                 | 4 ml                 |
| P4        | SFW + Urea                         | 61.9 mg              | 1510             | 100             | 15:1                 | 3 ml                 |
| P5        | SFW + Urea                         | 94.75 mg             | 1510             | 75              | 20:1                 | 2 ml                 |

2.2 Shrimp culture waste fertilizer enriched urea

Shrimp culture waste obtained from intensive shrimp culture in ponds IBAP Lamongan. waste in the form of solids are dried for 7 days under the sun, after the shrimp culture waste have dried so fermentation is carried out with the first step of activation effective microorganism containing the bacteria Lactobacillus casei and Saccharomyces cerevesiae with each minimum density of 2.0x10⁸ cells/mL and 3.5x10⁶ cells/mL, the bacteria used are as starter in the fermentation to decompose organic matter into inorganic material that will more easily absorbed by microalgae [6].

The ratio used in EM activation is 1:1:20 namely EM: Molasses: Aquades [3] 500 mL, and cultivated for 12 hours [7]. later to be used as a liquid fertilizer that dilution of waste shrimp culture in the ratio 1:2 [8] or 1 kg of shrimp culture waste was added to 500 mL of EM and Aquades 1500 mL, then mixed well and fermented for 14 days in anaerobic, and then filtered and sterilized along nitrogen and fosfor content are tested. Values of N and P test results used as a basis in the addition of urea.

2.3 Sterilization equipment and materials

Shrimp culture waste liquid fertilizer was added to flask and covered with cotton and wrapped tightly with plastic wrap, then sterilized by autoclave at 121 °C and 1 atm pressure for 15 minutes [9]. The sea water is sterilized by chlorine 60 ppm for 24 hours and then added Natrium thiosulfate 40 ppm and aerated for 24 hours to neutralize chlorine [10]. Sterilization of the medium and tools aimed at preventing any unwanted bacteria in cultivation [11].
2.4 Environment and culture media
Media using 500 ml of sea water with salinity 30 ppt, then fertilized with the provision of specified volumes (7.3 ml/L; 4 ml/L; 2.7 ml/L and 2 ml/L) and fertilizer walne (1 ml/L). Environmental conditions and the media must be in optimal condition.

2.5 Spreading inoculants
Chlorella vulgaris pure inoculum obtained from the Institute for Brackish Water Aquaculture Situbondo with a density of 9.1x10^6 cells/mL. Initial density of Chlorella vulgaris used is 100,000 cells/mL [12]. Calculation of volume administration with the desired cell density can be calculated by the formula:
\[ V1 \times V2 \times N1 = N2 \]

Note:
\( V1 = \) Volume of seed for initial stocking (mL)
\( N1 = \) Density seed / stock Phytoplankton (cells/mL)
\( V2 = \) volume of the desired culture medium (L)
\( N2 = \) Density of Phytoplankton desired seed (cells/mL)

2.6 Cultivation of Chlorella vulgaris
Cultivation on chlorella vulgaris in this research conducted for 7 days to determine the growth of Chlorella vulgaris begining from adaptation phase to a decline phase. Water quality is an important factor in supporting the growth of phytoplankton culture, so the stability should be maintained in culture. some parameters that need to be taken care of is the salinity, temperature, light intensity, supply oxygenated and pH.

2.7 Population and Growth Rate Calculation of Chlorella vulgaris
Chlorella vulgaris cell density calculation can be performed using Haemocytometer [13] were observed under a microscope with a magnification of 100 x, with formula [14]:
\[
\text{Density cells/mL} = \frac{na + nb + nc + nd + ne}{5 \times 4} \times 10^6
\]

\( na, nb, nc, nd \) and \( ne \) = Total of phytoplankton cells in the box a, b, c, d and e
\( 4 \times 10^6 \) = Area of a small box of a, b, c, d and e

The growth rate calculation with formula [15]:
\[
\text{The rate of growth} (\text{cell/ml/day}) = \frac{Nt - (Nt-1)}{\Delta t}
\]

\( Nt \) = Total of cells at the specified time
\( Nt-1 \) = Total of cells 24 hours before the time specified
\( \Delta t \) = Difference in cell count time (days)

2.8 Data analysis
Data were analyzed using One Way Analysis of Varian (ANOVA) RAL and tested further by Duncan's Multiple Comparison Test (DMRT).

3. Results and Discussion
3.1 Result
The use of shrimp culture waste fertilizer enriched by urea with different of N/P ratio provide the significantly different effect \( p < 0.05 \) to the population and growth rate of Chlorella vulgaris. Further test results used Duncan's Multiple Range Test showed that there were significant differences among the treatments. On the first day and the second P3 with a ratio of N/P 10:1 is the highest population with average value and significantly different with other treatments, while P5 with a ratio of N/P 20:1 is the average value of the lowest population of all treatments. The third day and the fourth highest
population of *Chlorella vulgaris* on P3 ratio of N/P 10:1 were not significantly different to P4 ratio of N/P 15:1 and the lowest population at P5 ratio of N/P 20:1.

The highest population peak on the fifth day that the P3 ratio of N/P 10:1 with an average density 1030x10^4 cells/mL and was significantly different from other treatments. P3 ratio of N/P 15:1 with an average density of 881x10^4 cells/mL is not significantly different from the P1 ratio of N/P 17:1 with an average density of 826x10^4 cells/mL, while the peak of the lowest population that is at P5 ratio of N/P 20:1 with an average density of 511x10^4 cells/mL were not significantly different from the P2 ratio of N/P 5.5: 1 with an average density of 720x10^4 cells/mL. The sixth and seventh day average *Chlorella vulgaris* population highest in the ratio of N / P 10: 1 which is significantly different from the other treatments, while the average lowest population that is in the ratio of N/P 20:1. Cultivation for seven days, P3 ratio of N/P 10:1 was significantly different from all treatments, P4 ratio of N/P 15:1 is not significantly different from the P1 ratio of N/P 17:1, while P2 ratio of N/P 5.5:1 is not significantly different from the P5 ratio of N/P 20:1.

### Table 2. The average population of *Chlorella vulgaris* with a ratio of N/P Different

| Treatment | P1 | P2 | P3 | P4 | P5 |
|-----------|----|----|----|----|----|
| The ratio of N/P | 17:1 | 5:5:1 | 10:1 | 15:1 | 20:1 |
| Day 0 | 10 | 10 | 10 | 10 | 10 |
| Day 1 | 90 ±10,8^b | 73 ±6,2^c | 120 ±7,3^a | 95 ±11,3^b | 43,7 ±7,8^d |
| Day 2 | 286 ±19,8^b | 193 ±19,8^b | 400 ±53,6^a | 315 ±28,3^b | 135 ±28^d |
| Day 3 | 588 ±66,7^b | 418 ±15,3^b | 701 ±103,8^a | 619 ±57,2^b | 295 ±45,4^d |
| Day 4 | 711 ±65,8^b | 625 ±83,2^b | 823 ±71,2^a | 746 ±43,4^b | 511 ±37,5^d |
| Day 5 | 826 ±35,9^b | 720 ±68^d | 1030 ±102^a | 881 ±67,9^b | 630 ±65,2^d |
| Day 6 | 697 ±24,5^b | 539 ±74,9^b | 817 ±54,5^a | 743 ±41,8^b | 520 ±33,9^d |
| Day 7 | 513 ±66,2^b | 441 ±48,7^d | 677 ±43,9^a | 524 ±46,8^d | 368 ±38,7^d |

The average value of the highest growth rate in the first and second day is P3 with a ratio of N/P 10:1 and significantly different with other treatments, while P5 with a ratio of N/P 20:1 is the average value of lowest growth rate, which not significantly different from the P2 ratio of N/P 5.5:1 on the second day. Highest growth rate in the third day is on P4 ratio of N/P 15:1 at a growth rate average of 304x10^4 cells/mL/day were not significantly different from P1, P2 and P3, but significantly different from the P5 ratio of N/P 20:1 with average density 160x10^4 cells/mL/day.

On fourth day the highest growth rate average of *Chlorella vulgaris* is in ratio of N/P 20:1 (P5) were not significantly different from the P2 ratio of N/P 5:1, but significantly different from the P1, P3 and P4. The fifth day the highest average growth rate of *Chlorella vulgaris* is in ratio of N/P 10:1 were not significantly different from P4 ratio of N/P 15:1, but significantly different from P1, P2 and P5. The transformation data result of *Chlorella vulgaris* average density is shown in Table 3.

### Table 3. The average of *Chlorella vulgaris* Growth Rate with Different of N/P Ratio

| Treatment | P1 | P2 | P3 | P4 | P5 |
|-----------|----|----|----|----|----|
| Day 1 | 80 ±10,8^b | 63 ±6,2^c | 110 ±7,3^a | 85 ±11,3^b | 33 ±7,7^d |
| Day 2 | 196 ±26^b | 120 ±20,2^c | 280 ±51,2^a | 220 ±18,6^b | 91 ±29^c |
| Day 3 | 301 ±61,9^a | 225 ±6^b | 300 ±61,4^a | 304 ±76,8^b | 160 ±57,5^b |
| Day 4 | 123 ±36,3^b | 206 ±77,3^a | 122 ±33,9^b | 127 ±25,2^b | 215 ±29,3^a |
| Day 5 | 115 ±48,8^b | 95 ±19^b | 206 ±80,4^a | 134 ±43,9^b | 118 ±29,7^d |

### 3.2 Discussion

The different of N/P ratio in shrimp culture waste fertilizer provide effect on the population and the growth rate of *Chlorella vulgaris*. Reproduction of *Chlorella vulgaris* occurs vegetatively, wherein the structuring proteins such as peptides, enzymes, chlorophyll and genetic material in the cell of microalgae are affected by nitrogen [16], while the process of the formation of ATP [17], acid DNA
and RNA nucleic affected by phosphorus [18]. So that the ratio of N/P greatly affect the growth of the individual into adult cells and stem cells produce tillers, or early phase of cell growth, cell maturation phase of the child until the hatching phase [19]. Nitrogen and phosphorus are also an important element that is used in the process of photosynthesis, where the results of photosynthesis is used for cell growth of *Chlorella vulgaris*. The total of nitrogen molecules also affects the formation of pigment chlorophyll a. The growth is characterized by increasing the size and total of cells [20], with a balanced nutrient needs of the cell can grow well.

The growth of phytoplankton consists of five phases which adaptation, exponential phase, the decline of rate phase, stationary phase and death phase [21]. The growth of *Chlorella vulgaris* using shrimp culture waste fertilizer enriched by urea with different N/P ratio have four growth phase that is a phase of adaptation that occurs after the stocking of inoculant into culture medium, exponential phase occurred on the first day of the culture until the fifth day that the peak population, decline phase rate occurs on the fourth to the fifth day and the deaths phase occurred on the sixth day until the seventh day.

Liquid fertilizers of shrimp culture waste is containing nitrogen 207.20 mg/L and phosphorus 37.11 mg/L with a ratio of N/P 5.5:1. Setting the ratio of N/P is done by adding urea to increase the nitrogen content of the shrimp culture waste fertilizer to obtain the different of N/P ratio, according to the ratio N/P is desirable in this research that the ratio of N/P 10:1, 15:1 and 20:1. Shrimp culture waste fertilizer with a ratio of N/P 10:1 produces the highest population which occurred on the fifth day with an average density of 1030x10^4 cells/ml, which is in contrast that needs optimal of N/P ratio for *Chlorella vulgaris* growth is 10:1 [22], the result is in contrast which states that *Chlorella vulgaris* grows optimum in ratio of N/P 15:1 [23] and 16:1 [24].

Peak population of *Chlorella vulgaris* cultivated using shrimp culture waste fertilizer with a ratio of N/P 10:1 result in an average density of 1030x10^4 cells/ml higher than walne fertilizer with a ratio of N/P 17:1, which produces an average density of 826x10^4 cells/ml. The growth of *Chlorella vulgaris* influenced by several factors such as the availability of macro and micro nutrients in fertilizer, seed quality *Chlorella vulgaris*, water quality and environmental cultivation media, a source of nitrogen, the concentration of nitrogen and phosphorus [24,25] and the ratio of N/P in media culture [26].

*Chlorella vulgaris* cultured using shrimp culture waste fertilizer with ratio of N/P 10:1 produces a peak density of 1030x10^4 cells/mL, which was higher than the population of *Chlorella vulgaris* cultivated using nodule peanuts fertilizers with a ratio of N/P 8:1 produces a peak density 147,35x10^4 cells/mL [27], and the use of *Azolla pinata* fertilizers with a ratio of N/P 7:1 produces a peak density of 120x10^4 cells/mL [28]. In the other research showed that the ratio of N/P 10:1 to produce maximum biomass is reached 2.97 g/ L/day [29].

Shrimp culture waste fertilizer with ratio of N/P 10:1 in addition to producing the highest population of *Chlorella vulgaris*, also resulted in the highest growth rate. The growth rate of *Chlorella vulgaris* influenced by individual growth into adulthood and speed of reproduction stem cells produce tillers, and the growth rate will be comparable with the concentration of nutrients [30], so that the composition of the nutrients contained in the culture medium is a crucial fast or slow to the growth of microalgae cultivated, Nutrition with a different quality and quantity may affect the population, production of tillers, biomass and cell metabolism in organisms [31].

*Chlorella vulgaris* growth rate would increase if adequate nutrient needs for growth, and the growth rate decreased because of increased cell density, accompanied by a limited total of nutrients in the culture medium [32]. In addition the growth rate cell of *Chlorella vulgaris* is also affected by the water quality in the cultivation media [33] including CO₂ which an important role in photosynthesis and metabolic activity of *Chlorella vulgaris* [34]. Especially the cultivation environment light intensity [35] also an important role in cell growth rate.

The use of shrimp culture waste fertilizer with a different ratio of N/P affects on the growth rate of *Chlorella vulgaris*. The fastest growth rate in *Chlorella vulgaris* cultivated happened on the third day in a row that the ratio of N/P 15:1, 17:1, 10:1, 5.5:1 and 20:1, in this phase of the cell has a productivity high because each cell capable of developing into 10,000 cells/ml within 24 hours with sufficient nutrients [36]. While on the fourth to fifth day the growth rate was declined, it is because of the competition between *Chlorella vulgaris* cells to compete for nutrients and space is limited to the cultivation medium [37].
Using of shrimp culture waste fertilizer enriched by urea with a ratio of N/P 10:1 provides the highest growth rate during the exponential phase with the average of growth rate is $203 \times 10^4$ cells/ml/day, while the lowest growth rate is the ratio of N/P 20:1 with an average growth rate is $123,4 \times 10^4$ cells/ml/day. The amount of constant rate of growth indicates that the division process *Chlorella vulgaris* becomes faster so that accretion of cells per unit time will be greater than the increase of time itself [38].

*Chlorella vulgaris* cultivated using shrimp culture waste fertilizer with the same of nitrogen concentration and different of phosphorus concentrations, produces a different growth rate. Use of shrimp culture waste fertilizer enriched nitrogen with a ratio of N/P higher and lower than the ratio of N/P 10:1 resulted in a population and the average *Chlorella vulgaris* growth rate was lower, this is because the phosphorus becomes a limiting factor in the growth algae [39]. Shortage of phosphorus concentration in the cultivation medium can disrupt the process of ATP which causes cell growth to be limited, while the excess phosphorus concentrations can inhibit the assimilation of phosphorus compounds in microalgae cells [40].

Population and growth rate of *Chlorella vulgaris* influenced by environmental factors of cultivation include salinity, temperature, pH and light intensity. The culture medium used for the cultivation of *Chlorella vulgaris* using shrimp culture waste fertilizer with a different ratio of N/P range of 30-35 ppt salinity, pH 7-9, 27.7-29.8 °C temperature and light intensity 2275 lux. Water quality in the cultivation media in accordance with the optimum needs of *Chlorella vulgaris* is 25-34 ppt salinity [41], pH 4.5-9.3 [42], 25-34 °C temperature and light intensity 2000-4000 lux [43].

4. Conclusion

The use of shrimp culture waste fertilizer enriched by urea with a different of N/P ratio provide significantly different effect on the population and growth rate of *Chlorella vulgaris*. The use of shrimp culture waste fertilizer enriched by urea with a ratio of N/P 10:1 resulted in a population and optimal growth rate, with the highest population of $1030 \times 10^4$ produce cells/mL, and the growth rate average is $203 \times 10^4$ cells/mL/day during the exponential phase.

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