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Abstract. One of the limiting problem in fish larvae rearing is the availability of live feed such as zooplankton and phytoplankton. *Spirulina platensis* is a phytoplankton with important uses in fish larvae culture either as a live feed or an ingredient supplementation in the feed. However, its cultivation has high economic costs associated with the growth medium. One of the alternative medium that potentially reduce costs and environmentally friendly is tofu wastewater. The purpose of this research is to evaluate the combination of tofu wastewater with urea for the cultivation of *S. platensis*. We investigated the growth of *S. platensis* in tofu wastewater containing various concentrations of urea: 0 g (control), 10 g, 20 g, 30 g, or 40 g urea added to 3 L diluted (2:1) tofu wastewater. The *S. platensis* productivity parameters were measured are population density, specific growth rate, and doubling time. The population density growth was significantly different, while the specific growth rate and doubling time were not significantly different for the various urea concentrations. The optimum growth was observed with 10 g urea per 3 L medium. This combination of tofu wastewater with urea could be a cost-effective medium for the cultivation of *S. platensis*.

1. Introduction

Feed is a limiting factor in aquaculture and represents about 60% of aquaculture production costs [1]. For all species of fish, the availability of natural food is a crucial factor in rearing larvae. One of the most important natural feeds for larvae rearing is microalgae [2](Lu et al 2004). Microalgae are used as a main source of supplement feed nutrition and to improve water quality [3].

Various microalgae are used as natural feed, including species in the genera *Chlorella*, *Tetraselmis*, *Isochrysis*, *Pavlova*, *Phaeodactylum*, *Chaetoceros*, *Nannochloropsis*, *Skeletonema*, and *Thalassiosira* [3]. Another potentially useful microalga for larvae rearing is *Spirulina platensis*, a filamentous cyanobacterium with nutritional significance as a protein source and immunomodulator for fish [4].

Cultivation of *S. platensis* can utilize wastewater from industry effluents as a growth medium [5] [6]. This is an ecofriendly solution to the problem of industrial waste. Home industry effluents contain organic matter in carbohydrates, protein, amino acids, lipids, inorganic nutrients sodium, and potassium phosphate, and ammonium salts. The economic cost of nutrients in microalgae production is considerable. One wastewater effluent useful for *S. platensis* cultivation is tofu production [6]. Tofu wastewater contains abundant nutrients for *S. platensis* growth [5] and is neither toxic nor hazardous

[7]. Another important, cost-effective nutrient for *S. platensis* cultivation is urea [8]. Media used for microalgae cultivation need to include both nitrogen and phosphate [9].

Tofu wastewater has been used to cultivate *C. vulgaris* [5] and *Chlorella pyrenoidosa* [6][7]. Thus, it is an alternative, environmentally friendly medium for microalgae cultivation, and its use would decrease the environmental impact of discarded tofu effluent. Therefore the purpose of this research evaluated the combination of tofu wastewater with urea for the cultivation of *S. platensis*. We investigated the growth of *S. platensis* in a medium of tofu wastewater supplemented with urea to determine the optimum combination of urea and tofu wastewater for *S. platensis* cultivation.

2. Materials and Methods

2.1. Sample and experiment medium

S. platensis was obtained from a local farmer in Magelang regency, Central Java, Indonesia. The strain originated in Balai Besar Perikanan Budidaya Air Payau, Jepara, Central Java. The tofu wastewater was acquired from a local tofu factory in Purwokerto Utara sub-district, Banyumas, Central Java.

2.2. Medium preparation

The medium for cultivation consisted of tap water, tofu wastewater, and urea (Pupuk Sriwijaya). Before the tofu wastewater was used, its pH was adjusted to 9 by adding NaOH [6](Wang *et al*., 2018). Then the medium was sterilized at 121°C. Tap water was homogenized with the tofu wastewater at a ratio of 1:2, with a total volume of 3 L. Urea (0 g, 10 g, 20g, 30 g, and 40 g) was added to the media. All media were aerated using an aerator.

2.3. Water quality measurement

The water quality parameters measured were temperature and pH. The temperature was measured each day in the morning and evening using a thermometer. The pH was measured once daily using universal pH paper (Merck). Throughout the research, the water quality was within the normal range for *S. platensis* cultivation, the pH was 9, and the temperature was maintained between 27°C and 29°C.

2.4. Experimental design

Several aquaria (20 × 25 × 20 cm) were used for cultivation. The aquaria were placed on a shelf in three rows of five aquaria each. Each was illuminated with a PL lamp (30 watts, Philips). The experimental protocol was a completely randomized design (RCD). The research was conducted for 8 days in an indoor laboratory at the Faculty of Fisheries and Marine Science, Jenderal Soedirman University, Purwokerto.

2.5. Collection of data

The data collected included the population density of *S. platensis*, its specific growth rate, and the doubling time. The density (d) of *S. platensis* was measured each morning between 8:00 and 9:00 a.m. using a hemocytometer. The sample was taken from the middle of the cultivation medium. The density was calculated according to:

$$d = \frac{\Sigma K}{5} \times 25 \times 10^4 \qquad \text{(Eq.1)}$$

where ΣK is the total population of *S. platensis* observed in five squares of the hemocytometer, 25 is the number of squares in the hemocytometer, and 10^4 is a constant hemocytometer.

The specific growth rate (μ) was calculated from the initial and final densities during the cultivation period, according to the method of Njouondo[10]:

$$\mu = \frac{Ln(X2) - Ln(X1)}{t2 - t1} \quad \text{(Eq.2)}$$

Where X_2 and X_1 are the final and initial densities, respectively, t_2 is the final time of cultivation, and t_1 is the initial time of cultivation. The specific growth rate was noted as cells/day.
The doubling time was calculated from the specific growth rate, according to the equation [11]:

$$g = \frac{\text{Ln } 2}{\mu} = \frac{0.633}{\mu} \quad \text{(Eq.3)}$$

2.6. Data analyses
The data are shown as means ± SE. The increase in population density, the specific growth rate, and the doubling time were analyzed using one way analysis of variance (ANOVA). The analyses were performed using the software SPSS Statistics 23 (IBM). The significance of each treatment was determined using the Tukey test.

3. Results

3.1. Growth of population density of S. platensis
The population density of *S. platensis* was measured each day during 8 days of cultivation **Figure 1**.

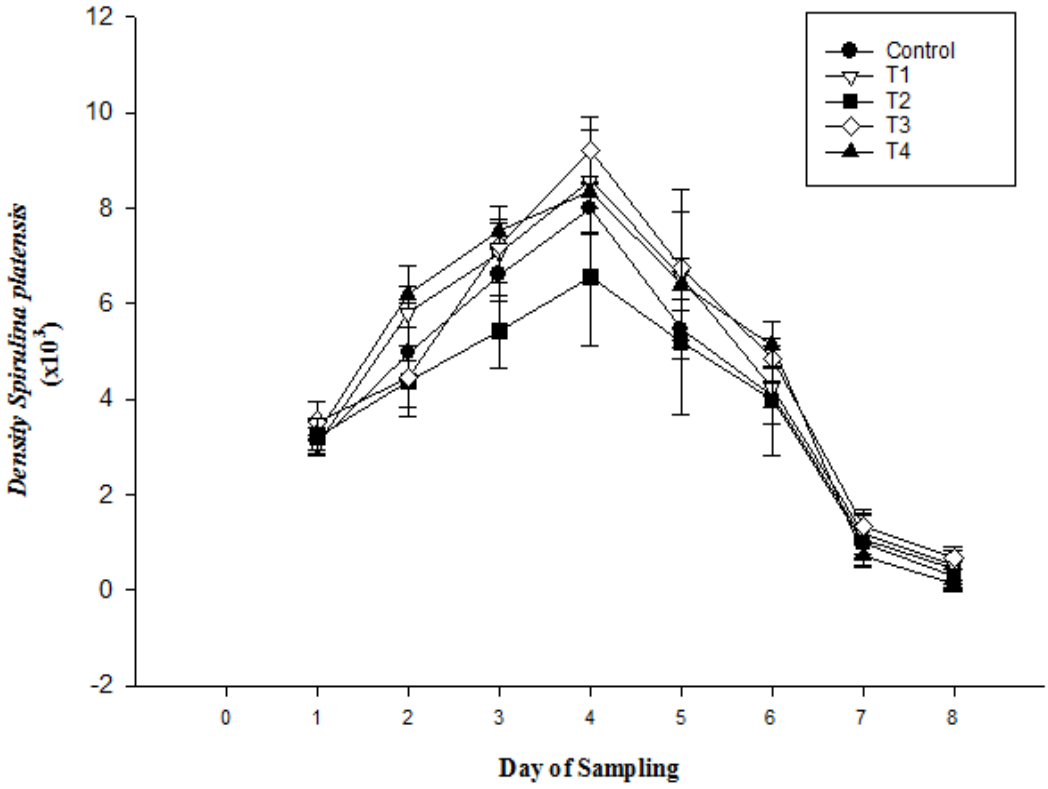

Figure 1. Population density of S. platensis durng 8 days of cultivation. The growth media consisted of 3 L diluted (2:1) tofu wastewater at pH 9 and 27°C to 29°C, containing various amounts of urea: 0 g (control), 10 g (T1), 20 g (T2), 30 g (T3), or 40 g (T4) (n = 3 replicates ; data shown mean ± SD)

Although each growth medium produced a different population density, in all of them, the peak density occurred on day 4. After 4 days, all media showed a decreasing population density. A dramatic decrease occurred on day 7, at which the S. *platensis* began to die.

The population densities on the initial day of cultivation ranged from 3016.67 to 3575 cells/mL. During days 2 to 4, the density of *S. platensis* increased. The density was significantly different ($P < 0.05$) for each concentration of urea in the cultivation medium. On days 2 and 3, the highest density was exhibited by T4 (40 g urea), and the lowest density was exhibited by T2 (20 g urea). On day 4, the highest density was exhibited by T3 (30 g urea), T2 exhibited the lowest. From day 5 onward, all populations decreased in density, with no significant differences ($P < 0.05$) between them. The final densities were between 7400 and 116.67 cells/mL. T2 exhibited the lowest peak density, including that of the control.

Table 1. The total population of S. platensis on each day during cultivation. The growth media consisted of 3 L diluted (2:1) tofu wastewater at pH 9 and 27°C to 29°C, containing various amounts of urea: 0 g (control), 10 g (T1), 20 g (T2), 30 g (T3), or 40 g (T4).

Days	***S. platensis* density (cells/mL)**				
	Control	**T1**	**T2**	**T3**	**T4**
Day 1	3100.00^{a}	3016.67^{a}	3183.33^{a}	3575.00^{a}	3250.00^{a}
Day 2	4950.00^{ab}	5783.33^{ab}	4316.67^{a}	4425.00^{ab}	6183.33^{b}
Day 3	6600.00^{ab}	7050.00^{ab}	5416.67^{a}	7200.00^{b}	7516.67^{b}
Day 4	8000.00^{ab}	8566.67^{ab}	6550.00^{a}	9300.00^{b}	8333.33^{ab}
Day 5	5466.67^{a}	6566.67^{a}	5200.00^{a}	7400.00^{a}	6383.33^{a}
Day 6	4066.67^{a}	4233.33^{a}	3950.00^{a}	4800.00^{a}	5100.00^{a}
Day 7	1050.00^{a}	1183.33^{a}	1000.00^{a}	1333.33^{a}	716.67^{a}
Day 8	450.00^{a}	516.67^{a}	283.33^{a}	683.33^{a}	116.67^{a}

Data shown mean±SD
Superscript letters indicate significant differences

3.2. Specific Growth Rate

The specific growth rate was determined from the population density in each growth medium at the beginning and day 4 (day of peak density) of the cultivation period. Each growth medium gave rise to a different specific growth rate. Higher concentrations of urea did not necessarily promote increased growth. The specific growth rates are shown in **Figure 2**.

The specific growth rate of *S. platensis* was most significant in the T1 medium (10 g urea), at 0.26 ± 0.02 cells/day. It was lowest in the T2 medium (20 g urea), at 0.11 ± 0.05 cells/day. For control, T3 (30 g urea) and T4 (40 g urea) shown at 0.24±0.04; 0.24±0.01 dan 0.24±0.03 cell/days, respectively The differences between treatments were not significantly different ($P > 0.05$), suggesting no significant effects of the various urea concentrations on growth.

3.3. Doubling time of S. platensis

The doubling time represents the average amount of time needed for an individual microalga to replicate itself. The doubling times were calculated from the specific growth rates and are shown in **Figure 3**. All of the media containing urea enabled a shorter doubling time than T4 2.98±0.40 days. The doubling time for T3 and control were 2.87±0.15 days and 2.97±0.5 days, respectively, except T2. The shortest doubling time occurred in the T1 medium (10 g urea) at 2.67±0.17 days, while the longest was in the T2 medium (20 g urea) at 4.23±1.31 days. The results were not significantly different ($P > 0.05$) for the various media.

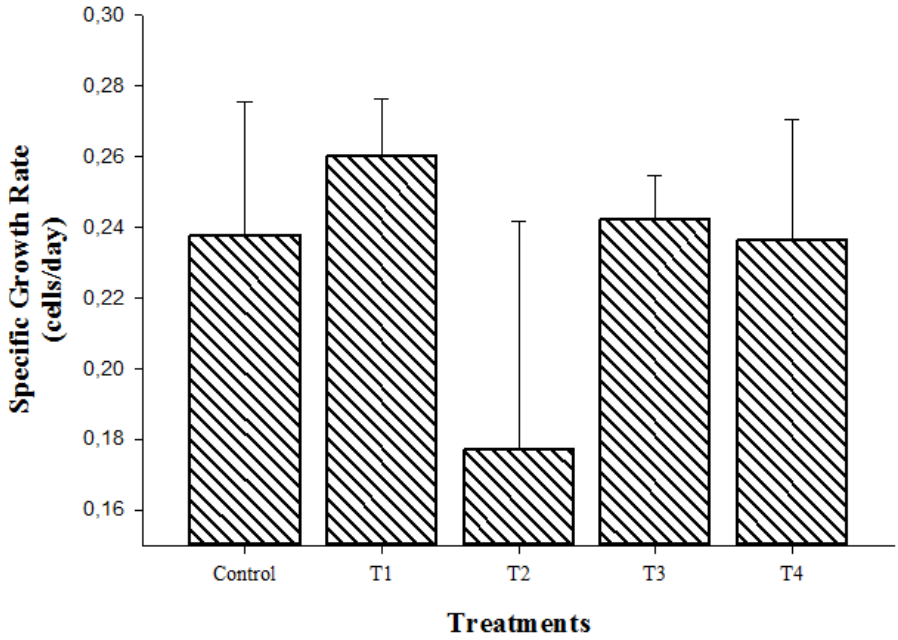

Figure 2. Specific growth rate S. platensis during the first 4 days of cultivation. The growth media consisted of 3 L diluted (2:1) tofu wastewater at pH 9 and 27°C to 29°C, containing various amounts of urea: 0 g (control), 10 g (T1), 20 g (T2), 30 g (T3), or 40 g (T4). (n = 3 replicates ; data shown mean ± SD)

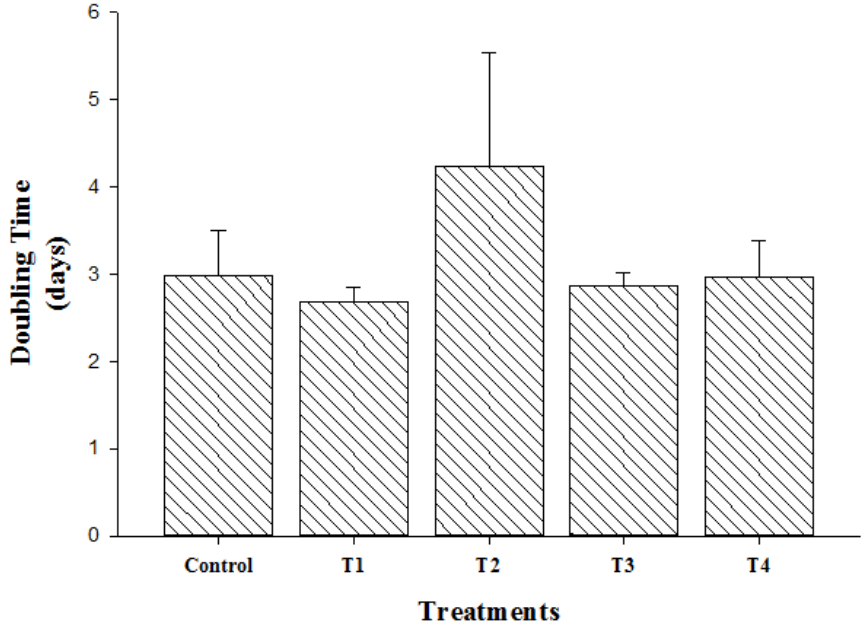

Figure 3. Doubling times of S. platensis during cultivation in various media. The growth media consisted of 3 L diluted (2:1) tofu wastewater at pH 9 and 27°C to 29°C, containing various amounts of urea: 0 g (control), 10 g (T1), 20 g (T2), 30 g (T3), or 40 g (T4).

3.4. Discussion

The population density of *S. platensis* grew well for 4 days in all growth media, after which it dropped precipitously. The growth of *S. platensis* is influenced by the availability of nutrients in the medium [12]. The major nutrient is nitrogen ammonia, nitrite, and nitrate [13]. Urea should influence *S. platensis* growth under cultivation; when it is supplemented at optimal levels, the population density of *S. platensis* should increase [8][14]. Urea consists of about 46% nitrogen in a single molecule ($CO(NH_2)_2$) [15](Sukumaran *et al*., 2018), which may facilitate the absorption of the nutrient for its growth. Meanwhile, tofu wastewater consists of other nutrients that stimulate microalgae growth, including carbon, phosphorus, and nitrogen. According to Faisal *et al*. [16], tofu effluent consists of 1.24% nitrogen, 5.54 ppm phosphate, and 7.72% protein. Therefore, tofu wastewater contains nitrate and phosphate that microalgae can use for growth [5]. Nitrate is a considerable nutrient for *S. platensis* [8]. It is a general factor in microalgae growth, and phosphate is often a limiting growth factor [17]. The above considerations were the rationale for using tofu wastewater with supplemental urea to facilitate microalgae cultivation in this study.

The population density of *S. platensis* in this study did not exhibit a lag phase (Figure 1) in any of the media but exhibit exponential growth immediately. Madkour *et al*.[8] reported that *S. platensis* does not go through a lag phase when the inoculant is harvested in the exponential phase. The density growth pattern observed in our study indicates that *S. platensis* retained its exponential phase of growth through the first 4 days of cultivation, reaching peak density at day 4. On day 5, the population density of *S. platensis* began to decline, presumably because of insufficient nutrients, and by day 8 the population density was dramatically reduced. Insufficient nutrients in the medium negatively impacts microalgae growth [10][18]Although the *S. platensis* was dying by the end of the cultivation period, the initial rapid growth until day 4 demonstrates that urea in tofu wastewater is a viable, inexpensive cultivation medium that can replace standard Zarouk medium [19].

The specific growth rate is inversely related to doubling time, as we observed. The highest specific growth rate and lowest doubling time was observed in the T3 medium (30 g urea), while the lowest specific growth rate and most significant doubling time was observed in the T2 medium (20 g urea). The doubling time is required for a microalgae population to double its density [20]. Thus, a higher doubling time indicates a lower specific growth rate, presumably due to limited medium nutrients. Sukumaran *et al*. [15 noted that inconsistent levels of nutrients in a medium could negatively impact growth and microalgae productivity. Specific growth rate and doubling time have previously been used to characterize the productivity of various media for cultivation of *S. platensis* and other species [11][21]. In the current study, higher urea concentrations did not necessarily translate into greater productivity. The T4 medium (40 g urea) was less productive than the T3 medium (30 g urea), perhaps because excessive urea was detrimental. According to Utomo *et al*. [12], too high a nutrient concentration is toxic for microalgae under cultivation.

4. Conclusion

The combination of urea with tofu wastewater promoted growth of *S. platensis*. The optimum we observed was 10 g urea, with a peak population density occurring at day 4. Thus, 4 days of cultivation before harvesting is optimal. Simultaneously, it also revealed good growth performance in specific growth rate and doubling time. Further research should aim to increase the scale of cultivation and expand the research to other microalgae species.

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