Global Scientific JOURNALS

GSJ: Volume 8, Issue 6, June 2020, Online: ISSN 2320-9186
www.globalscientificjournal.com

# The impact of fermented rabbit urine usage on the growth of *Spirulina fusiformis*

**Ibnu Dwi Buwono [1]*, Annisa Nurjannah[2], Ujang Subhan[3], Zahidah[4]**

*[1,3,4] Lecturer Staff at Faculty of Fisheries and Marine Science Padjadjaran University, Jl. Raya Bandung-Sumedang KM.21, Jatinangor, Sumedang District, West Java 45363 Indonesia*

*[2]Alumni at Faculty of Fisheries and Marine Science Padjadjaran University, Jl. Raya Bandung-Sumedang KM.21, Jatinangor, Sumedang District, West Java 45363 Indonesia*

** Corresponding author e-mail: 0812ibnu@gmail.com*

***Abstract***

The purpose of this research is to identify the optimum concentration of fermented rabbit urine as the alternative for the Pro Analysis fertilizer to increase the growth of *S. fusiformis* population. Four treatments of rabbit urine concentration used were 4ml/L (treatment A), 6 ml/L (treatment B), 8 ml/L (treatment C) and Zarrouk medium (treatment D, as the control). The design of this research used complete randomized design (CRD) with four treatments and three replications. The highest density average of *S. fusiformis* cell at its peak of growth is treatment D (190.723 x $10^6$ cell/ml), followed by treatment B (172.120 x $10^6$ cell/ml), treatment A (151.637 x $10^6$ cell/ml), and treatment C (140.800 x $10^6$ cell/ml). Treatment B (6 ml/L) was not significantly different with treatment D (control) based on the Duncan test ($p > 0.05$). The treatment B (6 ml/L) cell density average at the peak of population are 94,27 % from treatment D (control/Pro Analysis fertilizer). The growth phases (lag phase, exponential phase, stationary phase, and declination phase) of each treatments happened equally and at the same time interval. Rabbit urine serves as an alternative for the Pro Analysis fertilizer for cultivating *S. fusiformis* needed by herbivorous fish larvae.

***Keywords***: *blue green algae, fermented rabbit urine, fertilizer, growth*

## 1. Introduction

*S. fusiformis* is a type of microalgae that is used as a natural food source for fish and crustaceans larvae hatchery with its high and complete nutritional content (55.22% protein, 21.32% carbohydrate, 8.67% fat, 9.57% ash, 4.68% phycocyanin and 0.43% chlorophyll, it also contains 17 amino acids, 9 of which are essential amino acids [1].

The commonly chosen type of fertilizer in *S.fusiformis* culture is the standardized PA (Pro Analysis) fertilizers such as Walne, Guillard, and Zarrouk. The expensive price of PA fertilizers leads to the search for alternative fertilizers that could produce high amount of nutrition and cell density, with a lower price and easy to obtain [2].

Biourine is a type of urine that is taken from livestock, especially ruminants, and is fermented first before use. Biourine is obtained from anaerobic fermentation of urine with additional nutrition using nitrogen-fixing microbes and other decomposers. Because of this, the nitrogen in the biourine will be higher than the average urine [3].

Rabbit urine contains higher nitrogen and phosphor compared to other livestock. Rabbits urine contains a higher value of N and P which are 2.72% and 1.1%, respectively, compared to other livestock such as cows (N (0.5%), P (0.2%)) and sheep (N (1.5%), P (0.33%) [4].

Nitrogen and phosphor are chemicals needed by the phytoplankton in a large amount to live and for the growth of *S. fusiformis*. The fulfillment of nutrients for *S. fusiformis* depends greatly on its availability in the culture media. The complete nutrient composition and the precise nutrient concentration will determine the production of biomass and the nutrition content of microalgae [2]. Nitrogen is the most important element for *S. fusiformis* cell growth in the cell metabolism activity such as catabolism or assimilation, especially protein biosynthesis and also an important component in the chemical make up of amino acid, amide, nucleotide, and nucleoprotein, it is also plays a pivotal role in cell division [5]. Therefore, when there is an optimum nitrogen concentration in the culture media, the cell metabolism activity will also go well, including chlorophyll synthesis, because high chlorophyll will led to well photosynthesis process and the growth of *S. fusiformis* will be optimum.

Based on previous statements, rabbit urine has a good potential as an alternative for Pro Analysis fertilizer because of its lower price could optimize *S. fusiformis* production better and as an alternative for handling livestock waste that if not utilized could pollute the environment. The purpose of this research is to identify the optimum concentration of fermented rabbit urine that is used as fertilizer in the culture media to increase the growth of *S. fusiformis.*

## 2. Research method

### 2.1. Time and place

This research is conducted on April 15, 2018 – June 11, 2018 in the Invertebrate Laboratory of Faculty of Fisheries and Marine Science, *Universitas Padjadjaran*. The inoculum of *S. fusiformis* was obtained from the Natural Feed Laboratory of Faculty of Fisheries and Marine Science, *Universitas Padjadjaran* while the rabbit urine was obtained from a rabbit farm in Banjarnegara.

### 2.2. Instruments and materials

The instruments that was used include jars, aerators, measuring cups, scales, pipettes, hand counter, microscope, haemocytometer, pH meter, DO meter and TL Lamps. As for the materials, it includes *S. fusiformis* inoculum, fermented rabbit urine, zarrouk medium, and also $FeCl_3$, $NaHCO_3$, and NaCl as the mixing ingredients for the fermented rabbit urine treatments.

### 2.3. Cell multiplication

The research procedures consist of the stage of preparation of tools and materials, rabbit urine fermented, environmental preparation and *S. fusiformis* culture media, spreading of *S. fusiformis* inoculum, and observations covering *S. fusiformis* population growth and water quality of culture media.

#### 2.3.1. Spreading of *S. fusiformis* inoculum

The *S. fusiformis* inoculum is inserted into the media with a density of 100,000 cells / ml. Calculation of the number of *S. fusiformis* inoculums for culture using the following formula which explain below [6].

$$V1 = \frac{N2 \text{ X } V2}{N1}$$

V1 = Volume of inoculum for initial stocking(ml)
N1 = Density of the planktonic inoculum(sel/ml)
V2= Volume of desired culture media(ml)
N2 = The desired density of the planktonic inoculum(sel/ml)

**2.3.2. Measurement of *S. fusiformis* cell density**

Observation of water quality as *S. fusiformis* culture medium includes parameters of temperature, pH, DO, $CO_2$, nitrate and phosphate. Temperature and DO parameters were measured daily (morning, afternoon and night), pH parameters were measured daily, while nitrate and phosphate were measured at the beginning and end of the culture process. Calculation of cell density in *S. fusiformis* culture is done by taking samples every day using a pipette, then put it in the chamber of haemocytometer.

Formula the density of the number of cells according to explain below [7] :

$$D = \frac{N1+N2+N3+N4}{X} \text{ x } 16 \text{ x } 10^4$$

D = Microalgae density(sel/ml)
N1=Number of microalgae in the top right box
N2=Number of microalgae in the lower right box
N3 =Number of microalgae in the top left box
N4 =Number of microalgae in the lower left box
X =The number of sample boxes calculated

**2.3. Methods and data analysis**

The research method used is the experimental method with complete randomized design (CRD) that consists of four treatments and three replications. The treatments in the research are as follow:

A: 4 ml/L of fermented rabbit urine
B: 6 ml/L of fermented rabbit urine
C: 8 ml/L of fermented rabbit urine
D: Pro Analysis Fertilizer (Zarrouk medium)

The data analysis of *S. fusiformis* cell desnity is done using Analysis of Variance statistics and difference between treatments was done using the Duncan test. The water quality data (temperature,pH, DO, $CO_2$, $NO_3^-$, dan $PO_4^-$) is analyzed using the comparative descriptive analysis method.

**3. Results and discussion**

**3.1. *S. fusiformis* cell density and growth pattern**

The control treatment (Zarrouk medium) has the highest maximum population growth is obtained in, while on the treatments using fermented rabbit urine, the cell density of *S. fusiformis* increased on the 4 ml/L to 6 ml/L concentration. The fermented rabbit urine concentration of 8 ml/L has the lowest average cell density, which is the treatment with the highest fermented rabbit urine concentration (Figure 1).

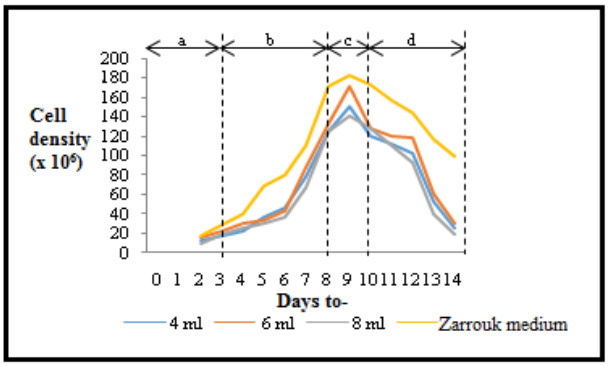


**Fig. 1:** Density of *S. fusiformis* cells cultured for 14 days. a = lag phase, b = exponential phase, c = stationary phase, d = declination phase.

The Zarrouk medium has a standardized macro and micro nutrients ratio [8]. The micro nutrients are Fe, Mn, Mg, and Cl. The Zarrouk medium has been commonly used for *S. fusiformis* cultivation in the laboratory settings, so *S. fusiformis* has adapted to grow in said media. Therefore, the Zarrouk medium can be utilized faster by *S. fusiformis* cells for its growth. Treatment B (6 ml/L) shows a higher density average of *S. fusiformis* cell than treatment A (4 ml/L). The suspected cause is that up to 6 ml/L fermented rabbit urine concentration, *S. fusiformis* cells could still utilize the macro nutrients increase in urine to optimize its growth.

The nutrient in the artificial fertilizer is one of the crucial factors in supporting the success of microalgae cultivation. Based on the preceding statement, a nitrogen and phosphor contents analysis is conducted in this research to identify the percentage of both elements in rabbit urine. Based on the test result of the soil chemistry and plant nutrition laboratory of *Universitas Padjadjaran*, the nitrogen and phosphor composition in rabbit urine are shown in Table 1.

**Table 1:** The Nitrogen and Phospor Contents in Rabbit Urine Analysis

| No | Test Sample | Paramenters | Result |
|---|---|---|---|
| 1 | Rabbit urine | N | 2,09 % |
| | | $P_2O_5$ | 0,69 % |
| 2 | Fermented rabbit urine | N | 2,20 % |
| | | $P_2O_5$ | 0,93 % |

Based on Table 1, there is a difference between the result of nitrogen and phosphor parameter analysis on the rabbit urine before and after it is fermented. The value of nitrogen content after the fermentation process increased by 0.11%, while the phosphor content increased by 0.24%. Fermentation is a process of organic compounds breakdown into simple compounds that involves microorganisms. Biourine is obtained from the anaerobic fermentation of urine with the additional nutrition using a nitrogen-fixing microbes and other decomposers, therefore the nitrogen content in biourine will be higher than the average urine [3]. The average density result of *S. fusiformis* cells shows that the fermented rabbit urine has an impact on the growth of *S. fusiformis* cells. Based on the preceding statement, an analysis of variance is done to identify the degree of` influence of the treatment towards the average value of *S. fusiformis* growth at the peak of population. Based on result of Table 2, it can be concluded that giving fermented rabbit urine in different concentrations and using Zarrouk medium as control causes a tangible impact on the growth of *S. fusiformis* population (F hit > F tab 0.05). Therefore, the Duncan distance test is done with 95% confidence interval to identify the difference between each treatments.

**Table 2:** Density of *S. fusiformis* cells (peak population)

| Treatments | Average density (cell/ml) | Peak day population |
|---|---|---|
| 4 ml (A) | 151,637 x $10^6$ $^{bc}$ | 9 |
| 6 ml (B) | 172,120 x $10^6$ $^{ab}$ | 9 |
| 8 ml (C) | 140,800 x $10^6$ $^{cd}$ | 9 |
| Media zarrouk (D) | 182,580 x $10^6$ $^{a}$ | 9 |

The value followed by different letters indicated significant difference ($p < 0.05$).

The treatment of 6 ml/L fermented rabbit urine was not different with the control treatment based on the Duncan test results at peak population. Based on the average cell density at peak population, treatment B produces a cell density of 94.27% from the control density at peak population (harvest time). The result of the research using the fermented rabbit urine shows that the population growth of *S. fusiformis* experiences four growth phases, which are adaptation, exponential, stationary, and declining growth phase. The observed growth pattern in this research in all of the treatments is shown on Figure 1. The Lag phase is marked by a slow growth due to the little to no energy that was used for growth. The phase happened because of the energy possessed by cells is aimed for self-adaptation towards the culture condition, and also to maintain metabolism stability, which causes the little energy used for the growth [9].

The exponential phase is marked by the rapid increase of the cell amount. This increase is because the microalgae actively dividing themselves and creating proteins and cell plasma component makeup that is needed in the growth process). The stationary growth process is marked by the balance between the growth and the mortality rate, because of the increase of population density is balanced with the mortality rate, there is no population growth [9].

The increase of cell concentration in cultivation will increase the self-shading that will lead to the decline of growth rate. The shade formation of spirulina cells are in tandem with the increasing cell density. The higher the cell density, the fewer light will penetrate the media because of the blockage [9].

### 3.2. The water quality of the culture media

The growth of *S. fusiformis* in the culture media is influenced by the nutrition contents and the environment of the preservation media. The results of water quality in the culture media during this research are shown in Table 3.

**Table 3:** Culture Media Water Quality

| Parameters | Time | Unit | Range | Optimum Standard |
|---|---|---|---|---|
| Temperature | Morning | °C | 25.50-26.90 | 25-35 [10] |
| | Afternoon | | 26.40-27.90 | |
| | Night | | 24.90-25.90 | |
| DO | Morning | mg/L | 4.70-5.90 | 4.65-6.27 [11] |
| | Afternoon | | 4.60-5.60 | |
| | Night | | 4.90-6.10 | |
| pH | Initial | - | 9.02-9.05 | 7.20-9.50 [12] |
| | Last | | 9.06-9.09 | |
| $CO_2$ | Initial | mg/L | 2.67-3.64 | < 12 [13] |
| | Last | | 2.85-4.49 | |
| Nitrate | Initial | mg/L | 0.97-1.40 | 0.90-3.50 [14] |
| | Last | | 0.50-0.94 | |
| Phosphate | Initial | mg/L | 0.84-0.94 | 0.05-20 [14] |
| | Last | | 0.51-0.91 | |

In laboratory settings, the change on water temperature is affected by room temperature and light intensity . The higher temperature in the morning and afternoon is caused by the high room temperature and light intensity, while the lower temperature at night is caused by the low room temperature and low light

intensity at night [12]. The increasing temperature to a certain limit can accelerate the metabolism process and increase *S. fusiformis* growth. The treatment using 8 ml of fermented rabbit urine has the highest temperature average, but this treatment also has a lower density of *S. fusiformis* cells than other treatments. This is caused by the impact of other water quality parameters or nutrient availability in the culture media which is affecting the growth of *S. fusiformis* cells [10].

The pH value is an important factor for the growth of *S. fusiformis*. Spirulina is usually able to live well in neutral pH and more tolerant to base than acid because *S. fusiformis* uses $CO_2$ efficiently even though it is available in a very low concentration [15].

The oxygen availability in the culture media is an important factor for the phytoplankton because it is directly used to form organic molecules through photosynthesis. The differences on DO average is affected by the environmental difference in the morning, afternoon, and night.

$CO_2$ in the culture media of *S.fusiformis* is still in its optimum limit. The range value of $CO_2$ at the end of the cultivation process is higher than the initial cultivation. The higher the $CO_2$ concentration, the biomass production will also increase. $CO_2$ is absorbed by microalgaes and used for the biofixation process for producing biomass [15].

The nitrate and phosphate contents at the end of this research seems to decrease compared to the beginning of this research. This shows that nitrate and phosphate is used by spirulina to fulfill the needs of nutrients [16]. But on the treatment using 4 ml of fermented rabbit urine, the phosphate content instead increased. Furthermore stated that the phosphate source besides from the media also came from the decomposition of dead algae cells, which is also affected by the cells of *S. fusiformis* absorbing phosphate[16].

## 4. Conclusion

The highest *S. fusiformis* cell density average at peak growth is on treatment D (Zarrouk medium) by 190.723 x $10^6$ sel/ml, followed by treatment B (6 ml/L of fermented rabbit urine) by 172.120 x $10^6$ sel/ml, treatment A (4 ml/L of fermented rabbit urine) by 151.637 x $10^6$ sel/ml, and the lowest is treatment C (8 ml/L of fermented rabbit urine) by 140.800 x $10^6$ sel/ml. The usage of fermented rabbit urine is impacting the growth of *S. fusiformis* cells and the optimum concentration of fermented rabbit urine usage on the culture media is 6 ml/L with the cell density of 94.27% from the cell density using Zarrouk medium at peak population.

## Acknowledgement

The author would like to thank the fisheries and marine sciences faculty, University of Padjadjaran for facilitating the place of this research.

## References

[1] Setyaningsih,I., A.T.Saputra, Uju, "Chemical Composition and Content of Spirulina fusiformis Pigments at Different Harvesting Age in Fertilizer Media", *Indonesian Fisheries Product Processing Journal*, Vol (1), No.(14), (2011), pp: 63-69.

[2] Amanatin, D.R., T. Nurhidayati, "The Effect of Combination of Tauge (MET) Extract Media Concentration with Urea Fertilizer on Spirulina sp", *Journal of Science and Art Pomits*, Vol (2), No. (2), (2013), pp 2337-3520.

[3] Mutryarny E., Endruani dan U. L. Sri, "Utilization of Lamb Urine to Increase Growth and Production of Mustard (Brassica Juncea L) Varieties of Tosakan", *Agricultural Scientific Journal.*Vol (3), No (2), (2014), pp: 11 – 17.

[4] Badan Penelitian Ternak. 2005. *Annual Report 2005*. Bogor.

[5] Rafiqul, L.M., K.C.A. Jalal, M.Z, Alam, "Enfiromental Factors for Optimizations for Spirulina biomass in Laboratory Culture", *Biotechnology Journal*, Vol (4)No. (1), (2005), pp: 19-22.

[6] Edhy, W. A, J. Pribadi dan Kurniawan, *Plankton in PT. Centralpertiwi Bahari. A Biological Approach and Plankton Management in Shrimp Cultivation*,Mitra Bahari, (2003).

[7] Fauziah, M.Hatta, "The effect of giving vermicompost (former worm) with different doses in Skeletonema costatum culture", *Acta Aquatica Journal*, Vol (2), No (1), (2015), pp: 11-17.

[8] Dianursanti, "Industrial Tofu Wastewater as a Cultivation Medium of Micoalgae Chorella vulgaris", *Energy Procedia* Vol (47), (2014), pp: 56-61.

[9] Vonshak, A., *Spirulina: growth, physiology and biochemistry. In Vonshak, A. (Ed.), Spirulina platensis(Arthrospira): physiology, cell-biology and biotechnology*. Taylor and Francis Ltd. Bristol, (1997), pp: 46-

47.
[10]Suminto, "Use of Types of Technical Culture Media for Production and Spirulina Plantesis Cell Nutrition Content*", Fisheries Scientific Journal*, Vol(4), No (2), (2007), pp: 53-61
[11]Astiani, F., I. Dewiyanti, S.Mellisa, "The Effect of Different Culture Media on Growth Rate and Biomass Spirulina sp.", *Scientific Journal of Marine and Fisheries Students Unsyiah*, Vol (1), No. (3), (2016), pp:441-447.
[12]Bangun, H.H., S.Hutabarat., C.Ain, "Comparison of Spirulina Platensis Growth Rates at Different Temperatures in Laboratory Scale", *Diponegoro Journal Of Maquares*, Vol (4), No (1), (2015), pp:74-81.
[13]Susanti,M., N.Kanada, T.A.Pribadi, "Plankton Abundance and Distribution in Kedungombo Reservoir Waters", *UNS Student Scientific Journal*, Vol (1), No (4), (2010), pp: 1-8.
[14]Anderson, R. A., *Algal Culturing Technique*, Elsevier Academic Press UK, 2005, pp: 344 – 351.
[15] Setiawan, S., M. Sari, M., Yuliusman, "Mechanism of $CO_2$ Absorption by Using Phytoplankton", *Scientific Journal of Biotechnology*, Vol (1) No (19), (2008), pp:115-119 hlm.
[16]Budiardi, T., N. P. Utomo., A. Santosa, "Nutritional Growth and Nutrient Content Spirulina sp. on Different Photoperiods", *Journal of Indonesian Aquaculture*, Vol (9), No (2), (2010), pp: 146–156.