Modification of Bean Sprout and Urea Media to Spirulina platensis Culture

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Abstract— The development of microalgae culture *Spirulina platensis* is interesting until now, this was caused *S. platensis* has multi-function in several sector. High price of commercial media for the culture needs prompting to search alternative medium which is not expensive, such as sprouts extract (ET) and Urea. The Data of research from the experiment on commercial media and alternative using *Standard Error of the Mean* (SEM) are : The threatment K (Kontrol) with commercial media "walne", the growth of *S. Platensis* cell is 22105 cell/ml and protein levels is 22.17%. Treatment P4 with alternative media (6% ET+120 ppm urea) showed that result of growth of *S. Platensis* cell is not too different to the threatment K, the result is 21136 cell/mL and protein level is 18.55%. Both experiment showed that the difference of growth cell between both experiment is not significant. Another result of treatment which used alternative media are: Treatment P2 (6% ET+100 ppm) growth cell is 16.383 cell/mL, protein level is 17.07%; Treatment P1 (4% ET+ 100ppm) growth cell is 10.7669 cell/mL, protein level 10.10%. The lowest of growth cell is treatment P3 (4% MT+120 ppm), growth cell is 10.769 cell/mL and protein level 13.81%. The conclusion is combination of ET and urea able to replace medium of microalga culture, it is like commercial medium "walne" as the culture medium for the growth of *S. platensis*.

Keywords—Spirulina platensis, Bean Sprout, Urea.

INTRODUCTION

Spirulina platensis or cyano bacterium is blue green algae shaped filaments and resembles a spiral (helix). Besides the unique form of *S. platensis,* protein contentained in *S. spirulina* also usefull as raw material for the food industry, health, and pharmacy. Ciferri, 1983 mentioning that protein *Spirulina platensis* is 60-70%, about 85-95% of the protein can be digested properly, while the fat was low enough that was 1.5-12%. The contain of *S.Platensis* is not only that, another contains are various vitamins such as vitamin B1, B3, B6, B12, pro vitamin A and vitamin E [17]. The good content in the *S. platensis* make the green algae demanded in some industries, therefore the cultivation of *S. platensis* has remarkable progress which are provided to each of field.

The various ways of culture has been done to multiply the seed through the culture medium, either the technical culture media which is expensive up to culture media alternative which is not. Culture cell of *S. platensis* in semi massive scale in laboratory needs good attention, mainly related to the provision of nutrient for the growth. Nutrient elements or nutrient in media culture is very important to keep the quantity, quality and stability of production cell *S. platensis*. The cells production of *S. platensis* is nfluenced by eight big component of media factor, such is the intensity of light, temperature, size of inoculation, charge dissolved solids, salinity, the availability of macro and micronutrients (C, N, P, K, S, Mg, Na, Cl, Ca and Fe, Zn, Cu, Ni, Co and W) [15], whereas the technical culture media that the availability of macro and micronutrients needs is fulfilled, such as Walne media and Guillard.

The expensive price of media culture types of PA (Pro analysis) like medium walne, became the basis to search alternative culture media that is able to produce nutrients and high cell density with an economical price and easy to get, one of them use of extracts of bean sprouts and urea (Setyoningrum *et al.,* 2014; Utomo *et al.,* 2000; Amanatin *et al.,* 1995). The use of extracts of bean sprouts in microalgae culture indicating cell growth has been done on some of research, which are types of *Chlorella* spp. [11] and *Scenedesmus* sp. [12]. Utilization of bean sprouts for the growth microalgae are believed because the bean sprouts contain high amounts of phosphate, vitamins such as thiamine, riboflavin, pridoksin, tryptophan, pantothenic acid, vitamin K and vitamin C.

But the availability of nitrogen in bean sprouts does not exist, therefore required the addition of an economical type of commercial fertilizer as a source of nitrogen, namely fertilizer urea. Urea solute will form ammonium (NH_4^+) which will be assimilated by the mikroalga and converted into glutamate as one of the constituent amino acids [8]. The use of urea fertilizer in microalga culture indicating cell growth has been done on several research, *Porphyridium* sp. [3], *Chlorella vulgaris* and *Scenedesmus* sp. [7]. Through the urea fertilizer and bean sprouts extract modified each extra bean sprouts both concentration and urea fertilizer, are expected to provide a solution in the form of an alternative culture medium which can produce phase growth of *Spirulina platensis* which is expected produce the highest protein levels.

METHOD

Research done in the laboratory of Fisheries product technology, Faculty of Agriculture, University of PGRI Banyuwangi, test levels of proteins has been done in Quality control and Testing UPT Results fisheries, Marine and Fisheries Agency. Isolates of *S. platensis* which was gotten from great hall of brackish water aquaculture development (BBPBAP) of Jepara. Basic culture medium used a sterile filter sea water from coastal waters Boom Banyuwangi, while the object of research which has done in the modification of the culture of *S. platensis* Extracts are bean sprouts (ET), urea fertilizer, and control as media culture pro analysis is walne.

Walne media that has been used includes several solution among others, nutrient solution: 20 g of $NaH_2PO.2H_2O$, 100 g of $NaNO_3$, 5 g of Na_2EDTA, 40 g of $NaSiO_3$, 0.36 g of $MnCl_2H_2O$, 1.3 g of $FeCl_3$, 10 g of H_2BO_3, 1L of akuades, and aqueous trace metal solution among others: 21 g of $ZnCl_2$, 2 g of $CoCl_2.6H_2O$, 0.9 g of $(NH4)_6.Mo_7O_{24}.4H_2O$, 20 g of $CuSO_4.7H_2O$, 3.15 gr of $FeCl_3.6H_2O$, 100 ml of akuades , as well as vitamin solution: 0.1 g of vitamin B12, 20 gr of thiamin, 0.1 g of biotin.

Media extracts of bean sprouts (ET) was made from 500 gr of green beans which has been boiled for 1.5 hours with a 2.5 L akuades, then filtered with the kassa are coated cotton. Concentration of ET that has been done for ET's treatment is 4% and 6% form the stock solution (v/v). Media ET which has been made then modified with the addition of urea fertilizer commercial shaped pollen with each dose of 100 ppm and 120 ppm.

The methods which has been used were experimental methods using the RAL (Complete Random Design) consisting five treatments, and each of observation was done by three times.

Tabel 1. Test Treatment in Research

Treatment	Description
P1	K : Pupuk Walne
P2	4%ET+100ppm Urea
P3	6%ET+100ppm Urea
P4	4%ET+120ppm Urea
P5	6%ET+120ppm Urea

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Seeds *Spirulina platensis* which was used was starter *S. platensis*, it was grown in sterile seawater filter media with support from walne media culture to reach a phase of exponential growth. Each treatment test was inoculated as many 10% volume of media culture. Observation on growth of *S. platensis* was done every 24 hours during the exponential phase cultures and the stationary phase.

The protein level has been tested at the end of harvesting of *S. platensis*. Before protein was tested, the culture of *S. platenisis* was separated between supernatant and pellet (rough fibers) using a centrifuge with a speed 6000 rpm for 20 minutes, then platenisis s. pellets dried to a powder using a dryer. Then a 15 grams of *S. platensis* powder was conducted a test protein by the titration method. Other supporting parameters are water quality including temperature, salinity and pH that has been done every 24 hours. The Data of research about growth of *S. platenisis* then analyzed with the standard Error of the Mean (SEM) using Microsoft excel.

RESULT AND DISCUSSION

The culture results of growth of *S. platensis* cell was done during 10 days and result of every treatment has growth pattern almost the same. The lag phase takes place in H2 at each treatment unless treatment P1, entered a phase of exponential progress in H4 every treatment. At the treatment K, P4 and P2 are having a stationary phase of growth or peaks in the H8, and almost in every test treatment but P3 entered a phase of death in H10, each treatment result can be seen in Figure 1.

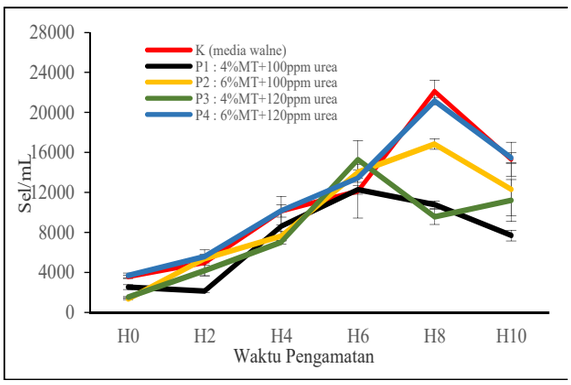

Figure 1. Spirulina platensis On Growth Curve of Each Treatment

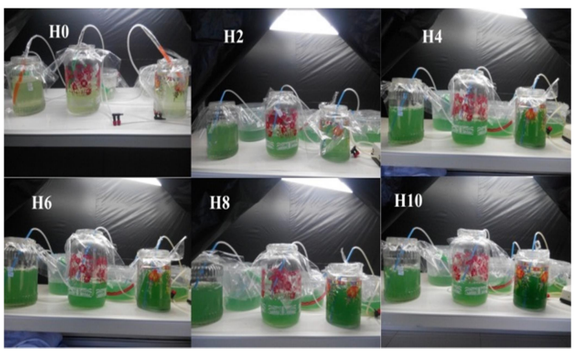

Figure 2. Changes of Pigment on Spirulina platensis During Ongoing Culture

Can be seen in Figure 1, that there is a difference of peak growth of *S. platensis* in every treatment. The stationary phase at the treatment K, P4 and P2 takes place at H8, while treatment of the P1 and P3 are having a stationary phase in H6, the phase patterns indicated that treatment K, P4, P2 have longer growth cell compared to P1 and P3. From the entire treatment results that obtained, the treatment K (a medium walne fertilizer) is good performance compared to other treatments, because stationary phase cells on growth reached 22105 cells/mL.

The best treatment with media culture alternative is treatment P4 (6% + 120ppm MT of urea) total cell growth reached 21136 cells/mL, in this case treatment P4 superior compared to other alternative culture media treatment. Entering a phase lag of death in H10, the difference of growth of *S. platensis* in treatment K and P4 is significant, it can be seen the result of *standard error mean*, each growth are 15315 cells/mL and 15448 cells/mL, while the lowest growth occurred in treatment P1 (4% + 100ppm MT of urea) with a value of 7690 cells/mL.

The highly growth of cells on the treatment K was caused walne fertilizer as medium that has the micro as well as macro nutrients needed for growth mikroalga. Compound in fertilizer as nutrient solution, walne trace metal solution, and vitamin solution is a source of nitrogen like the *S. platensis* one compound $(NH4)_6.Mo_7O_{24}.4H_2O$ (Borowitzka 1988), besides fertilizer walne is often used in the making of the previous starter, so the s. platensis has adapt well with fertilizer walne that supports growth.

On the other hand the use of fertilizer on alternative treatment P4 also provides good results even closer total growth at the treatment of K, that is because the composition of the bean sprouts and urea granting greater than other alternative fertilizer treatment, namely 6% ET + 120ppm urea. Nitrogen derived from urea (CH_4N_2O) as well as phosphate and some kinds of vitamins contained in bean sprouts is a necessary nutrient elements in the formation of chlorophyll, where is the chlorophyll is also very necessary for the process of photosynthesis. The lower the concentration of the element nitrogen that is given then the chlorophyll formation becomes obstructed, causing the slowness of growth of *S. platensis* (Riyono 2007). The Growth of *S. platensis* during a culture also supported by visual, there is a difference in color on the culture of *S. platensis* in the early research (H0) until the end of the study (H10). At the end of research, culture of *S. platensis* has a green colour which is more concentrated than the early research with pale green color (Figure 2), it does a proven that there is increase of growth cell of *S. platensis* and the influence of fertilizer in every treatment of pigment color *S. platensis*.

a. The Test Of Protein Levels

From the result of protein test that has been conducted at the time of harvesting of *S. platensis* in H10, there are the differences of protein levels in every threatment, as in table 2.

Table 2. Protein Test Results

Treatment	Levels of Protein
K (Pupuk walne)	22.17%
P1 (4%ET+100ppm urea)	10.10%
P2 (6%ET+100ppm urea)	17.06%
P3 (4%ET+120ppm urea)	13.81%
P4 (6%ET+120ppm urea)	18.55%

The results of testing protein rough s. platensis in table 2 can be known that K treatment, showed the highest protein of 22.17% compared to other treatments. While the fertilizer alternative that has the highest protein levels was P4 of treatment 18.55%, and the lowest is the treatment of P1 10.10%. This as a prove that the high growth of s. platensis in treatment K, as well as an alternative manure treatment on P4 (Figure 1) also have an good impact to the levels of a protein that was produced, as well as the opposite that was happen in treatment P1 that result in the lowest levels of the protein

due to a combination of urea fertilizer ET and granting the bit.

The K indicates that treatment elements nitrogen ($(NH_4)_6.Mo_7O_{24}.4H_2O$) which is in fertilizer walne went well resulting in a total cell protein levels and rough *S. platensis*. Micro nutrient levels as well as the complete macro in the fertilizer walne serves as a component of life that is used for the synthesis of proteins in the cells of *S. platensis*. The same results of occurred on treatment P4 which generate the levels of protein and total cell concentration high because urea fertilizer ET and the treatment more than P1, P2, and P3. Nitrogen will affect the growth of *S. platensis* in cell metabolism activities such as biosynthesis or catabolism in particular assimilation of proteins [4]. The statement supported [6] that the nitrogen is also an important ingredient of constituent amino acids, amides, nucleotide, and nukleo proteins, and cell division for life. Hence the concentration of nitrogen in the culture media is optimal then the activities of cell metabolism is also running as well, including the synthesis of chlorophyll a maximum effect on growth.

b. The Test Of Water Quality

In addition to media culture (fertilizer) required for the growth of *S. platensis* nutrients, water quality such as temperature, salinity and pH is also required as a factor supporting growth. Following are the results of water quality parameters produced during culture *S. platensis* in progress:

Table 3. Water Quality Test Results

Treatment	Environmental conditions		
	Suhu (°C)	Salinitas (ppt)	pH
K	30-35	30-40	7-8
P1	32-34	30-37	7-9
P2	31-34	29-40	7-9
P3	30-32	30-40	7-8
P4	30-32	30-40	7-8

The results of the measurement of water quality parameters in the culture of *S. platensis* include temperature, salinity, pH in every treatment showed an almost uniform results. Temperature measurement results showed media ranged from 23°C-35°C, Ciferri (1983) stated that the optimal temperature for the *S. platensis* is 23°C-35°C, based on it can be noted that the resulting of temperature parameters supports growth culture of *S. platensis*.

The results of the measurements of salinity showed media ranged between 30ppt-40ppt, during ongoing culture showed a gradual increase in salinity from the culture begins to harvest culture. Richmond (2004) stated that, the optimal salinity for *Spirulina* sp. was 30ppt-60ppt, so that the resulting salinity levels of the *S. platensis* supports growth. The increasing value of salinity until reach to 40ppt was caused the increasing of concentration of salt which is associated with a decrease in the rate of photosynthesis that impact the total growth of *S. platensis*, when the rate of photosynthesis increase will have an impact on increasing the growth of *S. platensis* until entering a phase lag of death which is also an increase in salt concentration or salinity [10].

The results of measurements of the pH of the media shows ranging between 7-9, the pH value is still within the limits of optimal growth of *S. platensis* ranging between 7-9 [13]. Similar things with a salinity that the value of pH during culture showed gradual increases of culture begins to harvest culture of *S. platensis*, it is caused due to the biological processes that occur. pH influence to solubility and availability of mineral ions, then affecting the solubility and availability of mineral ions by cells. pH also influence to the performance of the enzyme that could inhibiting the process of photosynthesis and growth of microalga, by the inhibition of photosynthesis would have an impact on the decline in the growth phase or lag that occurs and results in death in oil pH [9].

CONCLUSION

The conclusions of this research are media extracts of bean sprouts and fertilizer urea can be used as alternative fertilizer replacement media kultur pro analyst (walne fertilizer) in culture *S. platensis*. ET and modified urea fertilizer, with the concentration of ET 6% urea 120ppm and get the best results compared to other alternative manure treatment concentration, namely on phase lag of death total cells of *S. platensis* as much 15448 cells/mL and protein levels of 19%.

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