BIOTEKNOLOGI
Volume 15, Number 2, November 2018
Pages: 51-54

ISSN: 0216-6887
E-ISSN: 2301-8658
DOI: 10.13057/biotek/c150201

Cattle urine as a low-cost medium for accelerate growth, biomass productivity, and lipid production of *Botryococcus braunii*: Future energy

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Manuscript received: 10 September 2018. Revision accepted: 9 October 2018.

Abstract. *Hidayati Z, Arofah AN, Fakhira J. 2018. Cattle urine as a low-cost medium for accelerate growth, biomass productivity, and lipid production of* Botryococcus braunii*: future energy. Bioteknologi 15: 51-54.* The increasing necessities of global energy can trigger an energy crisis. Microalgae is a unicellular microorganism which has potency as bioenergy, such as biofuel, and efficient alternative to substitute fossil resources. *Botryococcus braunii* is a microalgae that has a high lipid concentration of around 25-80%, and it is this characteristic which shows its potency to produce biofuel. The research purpose was to utilize cattle urine as an alternative medium for growth of *B. braunii*. Some of the components in cattle urine are 95% water, urea, 2.5% mineral salt, hormone, enzyme, 69% nitrogen, sulfur, and magnesium. The components of cattle urine can substitute macronutrient and micronutrient in others synthesis medium. This research was designed by using Random Design Complete (RAL) with seven variables: control culture *B. braunii* (medium macronutrient+ micronutrient Johnson); cultures *B. braunii* with 5%, 7.5%, 10%, 12.5%, 15%, and 17.5% concentration of cattle urine+micronutrient Johnson. The methods of this research were divided into three steps: preparation, testing, and analysis of biomass also lipid extraction. The data from the observations of biomass were analyzed with linear regression equation. All of data was analyzed in Microsoft Excel. The results showed in culture *B. braunii* with a 12.5% of concentration cattle urine, that it can accelerate the growth of cell *B. braunii* being maximal which production 1.425 mg/L biomass in days 12. Additionally, *B. braunii* culture with 12.5% of concentration cattle urine can accelerate biomass productivity of *B. brauni* which production 9.018 g/L biomass. Whereas with just added cattle urine as medium is not given significantly to lipid production.

Keywords: Biofuel, *Botryococcus braunii*, cattle urine

INTRODUCTION

Energy is one of the most important sectors for life. The increasing necessities of global energy can trigger an energy crisis. In Indonesia, the necessities of global energy will increase from 144 billion Tones Oil Equivalent (TOE) to 1.049 billion TOE in 2050, with growth quickly 5.7% per year (Nurzaman et al. 2015). Microalgae is a unicellular photosynthetic organism that has great potential in the biotechnology industry, especially as a source of renewable energy (Ermavitalini et al. 2017). A renewable energy resource is energy that unlimitedly available in nature (Festus and Ogoegbunam 2015). The provider of using microalgae as an alternative is because microalgae have a short life cycle, capability to synthesized high lipid, and can survive in extreme conditions (Carvalho et al. 2011 and Gumbira 2016). Although production of biodiesel from microalgae still lacks industrial applicability because it requires a higher cost than using fossil (Ermavitalini et al. 2017).

Botryococcus braunii is one species of microalgae that has potency to produce biofuel. *B. braunii* is a unicellular and green microalga that exist in lakes, river, salty, and marine water. The cell size of *B. braunii* ± 15-20µm, and they live in colonies, and are non-motile (Tasic 2016). The chlorophyll of *B. braunii* consists of chlorophyll a, b, and c that amount ±1.5-2.8% (Saputro 2015). The characteristic of this species is lipid, which amounts to 15-80% of the dry weight biomass (Asma et al. 2015). The high lipid productivity can be influenced by factors like light intensity, media, temperature, harvesting technique, culture technique, extraction technique, etc. Medium culture of *B. braunii* is like BG11, BBM-3N, Jaworski's medium, Johnson, etc.

Media composition can have a significant effect on the growth rate and the final concentration of microalgae. Microalgae are known to grow more abundantly in nutrient-rich mediums(Blair et al. 2013). The utilized medium synthetic in large scale can burden the cost production by 60-70% (Patmawati et al. 2014). So, a solution is needed to modify the medium, like by using cattle urine. Cattle urine is one of the alternative medium cultures for growing *B. braunii*. The contents of cattle urine can fulfill macronutrient and micronutrient medium synthetic. Cattle urine contains about 69% nitrogen and other micronutrients (Sharma and Rai 2015). Some of the components in cattle urine are 95% water, urea, 2.5% mineral salt, hormone, enzyme, 69% nitrogen, sulfur, and magnesium (Manalu 2010). The benefits of using cattle urine can be because it is low cost to substitute micronutrient of synthetic Johnson, and can decrease pollution, especially in surface water and on land.

This research aims to use cattle urine as an alternative medium to accelerate growth *B. braunii*, biomass productivity, and production of Lipid cell *B. braunii*.

MATERIALS AND METHODS

This research was conducted between April and July 2018 at the Laboratory of Microalgae, Biology Department Faculty of Mathematics and Natural Sciences Padjadjaran University and Central Laboratories Padjadjaran University, Sumecang, Indonesia. The optimization of concentration medium cattle urine in this research was utilized Random Design Complete (RAL) toward Johnson medium (Agustini 2012). The methods of this research are divided into:

Preparation

Tools and materials preparation

The tools that were used in this research were: reaction tube, analytic digital scales, plastic bottles 1L, Erlenmeyer, aerator, spectrophotometer UV-VIS 680nm, dry oven, centrifuge, and Soxhlet extraction. Whereas the materials that were used in this research were: culture of *Botryococcus braunii* from Algae Laboratories Padjadjaran University, Cattle urine was taken from Ciparanje Faculty of Animal Science Padjadjaran University, 70% alcohol, NaOH, EDTA, n-hexane solvent, and Johnson medium synthesis.

Preparation of starter culture Botryococcus braunii

Microalgae *Botryococcus braunii* was cultivated in plastic bottle amount 4L by used Johnson synthesis. The cultures gave 2500 lux light intensity, radiation period 24 L, at temperature 25-27^{O}C, and aeration condition 3 days.

Testing of medium cattle urine

Cattle urine was taken from Ciparanje Faculty of Animal Science Padjadjaran University. First, neutralization was done from alkali condition. The culture of *B. braunii* was then made. 21 plastic bottles were divided to be: 1 bottle as control and 6 bottles as treatment with 3 replications. The concentration of cattle urine was made 5%, 7.5%, 10%, 12.5%, 15%, and 17.5%. Cattle urine with the respective concentration was made in every bottle treatment, with also 1mL EDTA added. Culture *B. braunii* in aeration condition for 15 days, 2500 lux light intensity, at temperature 25-27^{O}C, and radiation period 24L.

Biomass analysis

The curve diagram of growth *B. braunii* was analyzed by absorbance measurement (Optical Density) every 24 hours with using scanning spectrophotometer UV-VIS 680 nm. 1 mL culture of *B. braunii* was taken by syringe, and measurement of biomass *B. braunii* was analyzed by centrifuge. 10 mL culture of *B. braunii* was taken by syringe in tube centrifugation. The process of centrifugation was for 15 minutes 3000 rpm, and then separated between supernatant and pellet, put the tube centrifugation in oven and dried for 24 hours at 45^{O}C. Formula that used for dry weight biomass (Susanto et al. 2012):

$$\text{Biomass: } \frac{\text{Dry weight biomass (g)}}{\text{Culture volume (L)}}$$

Extraction lipid

The method of lipid extraction was by using Soxhlet extraction. After 15 days, the culture of *B. braunii* which has harvesting, had 1 mL NaOH as flocculant added and kept in 24 hours. Biomass of *B. braunii* was filtered by fabric filter < 20 um, dried using oven during 18 hours in 45^{O}C, then dry biomass of *B. braunii* was extracted with Soxhlet technique and using n-hexane as a solvent (Al-Hothaly et al. 2015). The formula for measurement content lipid (Hamedi et al. 2016):

$$\text{Lipid content: } \frac{\text{Weight of extracted lipid}}{\text{Weight of dried biomass}} \times 100$$

Data analysis

Data from the observations of biomass were analyzed with linear regression equation that used a control culture of *B. braunii*. The results are related between absorbance (Optical Density) and gram/liter biomass *B. braunii*. All of data was analyzed by Microsoft Excel.

RESULTS AND DISCUSSION

The effect of cattle urine media on the growth and biomass productivity of *Botryococcus braunii*

The results from this research showed in every treatment of concentrations of cattle urine gave preference to increase growth cell *B. braunii* for 15 days. The produce of biomass in day 12 (exponential phase) culture that was not given treatment (control) only production 0.156 mg/L biomass. Cattle urine concentration of 5% can produce 0.632 mg/L biomass. Cattle urine concentration of 7.5% can produce 0.763 mg/L biomass. Cattle urine concentration of 10% can produce 0.829 mg/L biomass. Cattle urine concentration of 12.5% can produce 1.425 mg/L biomass. Cattle urine concentration of 15% can produce 1.248 mg/L biomass. With finally, cattle urine concentration of 17.5% can produce 1.309 mg/L biomass. The average in every treatment was presented in Table 1 and the curve of growth cell *B. braunii* was presented in Figure 1.

These results are similar to biomass productivity (absorbance value) in Figure 1. The culture of *B. braunii* was not given a treatment (control) only produced 1.554 g/L biomass. Cattle urine concentration of 5% can produce 3.850 g/L biomass. Cattle urine concentration of 7.5% can produce 4.572 g/L biomass. Cattle urine concentration of 10% can produce 4.841 g/L. Cattle urine concentration of 12.5% can produce 9.018 g/L biomass. Cattle urine concentration of 15% can produce 8.883 g/L. Finally, cattle urine concentration of 17.5% can produce 8.907 g/L biomass. These results are presented in Figure 2.

Table 1. The average of biomass (absorbance value) cell *Botryococcus braunii* during15 days culture.

Day	Average of biomass						
	Control	5%	7.50%	10%	12.50%	15%	17.50%
0	0.018	0.028	0.035	0.033	0.034	0.056	0.039
1	0.038	0.016	0.026	0.031	0.068	0.071	0.079
2	0.059	0.075	0.088	0.121	0.132	0.165	0.174
3	0.063	0.115	0.123	0.154	0.204	0.273	0.293
4	0.101	0.143	0.161	0.182	0.308	0.336	0.333
5	0.062	0.176	0.178	0.239	0.47	0.504	0.489
6	0.133	0.258	0.322	0.382	0.63	0.647	0.642
7	0.154	0.29	0.376	0.435	0.743	0.767	0.732
8	0.144	0.34	0.488	0.551	0.978	0.9	0.902
9	0.165	0.448	0.638	0.662	0.967	1.044	1.1017
10	0.153	0.488	0.642	0.719	1.215	1.107	1.13
11	0.155	0.576	0.73	0.8	0.8	1.177	1.213
12	0.156	0.632	0.763	0.829	1.425	1.248	1.309
13	0.163	0.543	0.53	0.51	1.249	1.231	1.238
14	0.149	0.506	0.407	0.423	1.118	1.185	1.18
15	0.141	0.398	0.455	0.294	1.084	1.107	1.08

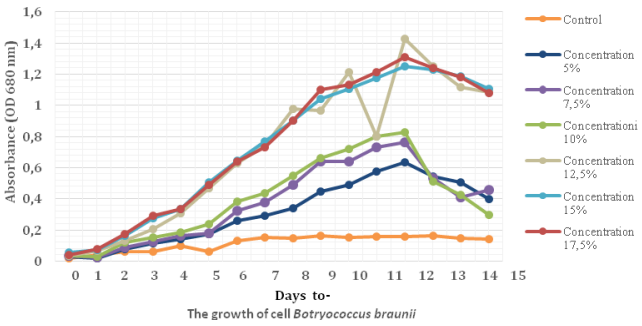

Figure.1 The growth of cell *Botryococcus braunii* using cattle urine as medium for 15 days.

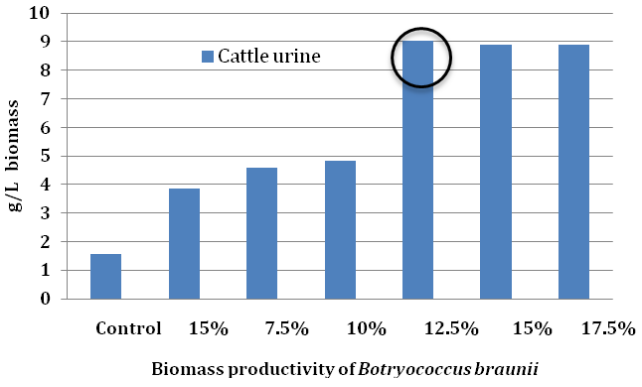

Figure 2. Biomass productivity of *Botryococcus braunii* with using cattle urine as medium.

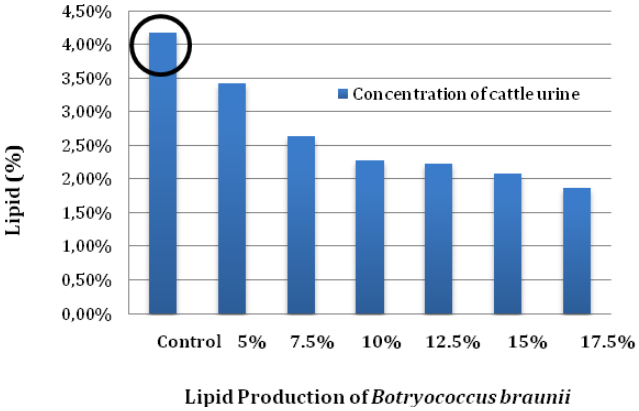

Figure 3. Lipid production of *Botryococcus braunii* with using cattle urine as medium.

The effect of media cattle urine on lipid production of *Botryococcus braunii*

This research showed that cattle urine can affect lipid production of cell *B. braunii*. Although, with some concentrations cattle urine not significantly affecting lipid production. The results from lipid extraction showed that culture of control *B. braunii* can more produce 4.17% lipid than using cattle urine (treatment). Cattle urine concentration of 5% can produce 3.43% lipid. Cattle urine concentration of 7.5% can produce 2.63% lipid. Cattle urine concentration of 10% can produce 2.28% lipid. Cattle urine concentration of 12.5% can produce 2.23% lipid. With cattle urine concentration of 17.5% producing 1.67% lipid. The results are presented in Figure 3.

Discussion

Algae growth was evaluated daily by optical density (OD) measurements at 680nm. The growth cell of *B. braunii* begins with lag phase; in lag phase cell *B. braunii* still adapts toward culture medium. In this research, the lag phase occurs in 0-3rd day for all treatment; the long duration of lag phase is because of the length culture as inoculum which was too long (Fog and Thake 1987). The growth of cell *B. braunii* which was showed by absorbance value, which increases between the low distance and it can show metabolism of cells *B. braunii*. The lag phase also showed that the cleavage of the cell was very low and it can make the cell in 4-9th day the culture *B. braunii* had a log/exponential phase. In exponential phase, the cell *B. braunii* was cleaved very quickly, so the cell can grow so fast. In 10-11th day it had a decreasing phase, this was because the cell *B. braunii* began to cleave slowly. While the next day it was still growing in increase and 13-15thday had a stationary phase, followed by decrease in growth. In this research, the culture with treatment (give cattle urine) can give effect to growth and biomass productivity of cell *B. braunii*. Algae growth was described to follow in five different phases: divided to lag or acclimatization phase, log growth phase, declining growth phase, stationary phase, and death (lysis) phase (Moazami et al. 2012).

The results showed that the culture with cattle urine concentration of 12.5% can accelerate growth cell *B.*

braunii by 1.425 mg/L in 12th day and can accelerate biomass productivity which produces 9.018 g/L biomass. Concentration of 12.5% of cattle urine has more nutrients than other concentrations. The cell of *B. braunii* still has tolerated with concentration of 12.5% cattle urine (Sharma and Rai 2015). If nutrients are added too much it can be toxic and result in the death of the cell of *B. braunii* (Gumbira 2016). But it was different with the result of lipid extraction that showed in culture non-treatment (control) that can produce high lipid amounts of 4.17% rather than culture that was given the treatment (used cattle urine). The culture that used cattle urine did not give significant effect to lipid production of cell *B. braunii*. The culture with low concentration of cattle urine can produce higher lipids, than culture that used a high concentration of cattle urine. The availability 69% of N in cattle urine can increase to form protein than lipid (Manalu 2010), so the culture that added cattle urine only produced a few lipids, because the cell *B. braunii* had formed protein more than formed lipid. Biomass concentration increased, with an increasing concentration of nitrogen in medium culture (Amin et al. 2013). Besides, this research was influenced by some factors. This research suggests to continue observing the effects of using cattle urine in other species of microalgae and considering the factors are like nutrient, pH, light intensity, temperature, salinity, harvesting technique, extraction technique, and etc. (Juneja et al. 2013). To prove that cattle urine can give effect for accelerate growth, biomass productivity, and can increase lipid production, future research must try in other species of microalgae.

ACKNOWLEDGEMENTS

Thank you for Indonesian Ministry of Riset, Technologie and High Education, Department of Biology, Padjadjaran University, and Dr. Joko Kusmoro, as supervisor of this research.

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