

THE POTENCY OF MICROALGAE (*CHLORELLA VULGARIS*) IN REDUCING THE LEVEL OF AMMONIUM AND ORTHOPHOSPHATE IN BIOSLURRY MEDIA

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ABSTRACT

In biogas production using dairy manure, an anaerobic digestion process also produced bioslurry as a byproduct, also known as Anaerobically Digested Dairy Manure Wastewater (ADDMW), which is rich in nutrient. The availability of some organic compounds such as ammonium, phosphate, and other nutrients make ADDMW potentially to be used as growth media to grow autotrophic organism, including microalgae. Furthermore microalgae potentially could reduce organic content in ADDMW, thus play a role as phycoremediator to organic waste water. In this study we used ADDMW as a medium for the growth of microalgae, determining its level of reduction in ammonium and orthophosphate content of ADDMW. The result showed that microalgae *C. vulgaris* can be grown in ADDMW media and can reduce its organic content i.e. ammonium (95%) and orthophosphate (65-78%), therefore potential to be used as phycoremediator.

Key words: *bioslurry, ADDMW, microalgae, Chlorella vulgaris, phycoremediation*

I INTRODUCTION

Livestock farming is a type of activity that produces large amount of waste. The waste comes from the metabolic process of cows (faeces and urine) and remaining feeds. Farmers who do not have sufficient waste treatment technologies and a lack of knowledge of the environment choose to dispose the waste directly to open stream. These activities will undoubtedly have a negative impact on the environment, such as the occurrence of eutrophication due to the uncontrolled supply of nutrients (ammonium and phosphate), increased biological oxygen demand (BOD), changes in aroma and reduced the clarity of water (Taufikurahman and Ismi, 2019¹). Increasingly the need for beef and cow's milk from the human community certainly requires a good waste treatment system to maintain environmental conditions.

The use of cattle dairy waste as a substrate in biogas production is an option that can be chosen by farmers. From the anaerobic process will be produced other products in addition to biogas, ie the bioslurry. Bioslurry is a by product from anaerobically digestion process and still contains ammonium and total phosphate (TP) with concentrations reaching respectively 136 and 34 mg / L (Sooknah and Wilkie, 2004)². For companies that process livestock breeding waste in raw materials for the production of large quantities of biogas, bioslurry is also another problem because it is produced continuously in sufficient quantities. The nutrients contained in the bioslurry, such as ammonium and phosphate, can be used as a source of nourishment in the microalgae growing medium. Nowadays, the cultivation of microalgae generally uses a synthetic medium that certainly requires costs in its

supply, which will therefore influence the selling price of products processed by microalgae. The cultivation of microalgae using a waste medium will provide two advantages at the same time, reduced cultivation costs and environmentally friendly waste treatment.

Chlorella vulgaris plays an important role in the food chain of the aquatic system as a source of nourishment for other organisms (Richmond, 2004)³. The high protein content in microalgae biomass makes it feasible to use it as an alternative mixture of feed production, considering that the intake of protein in animal feed currently adheres to soy flour and fish meal that compete directly in its uses as ingredients for human consumption. Furthermore, Ursu et al. (2014)⁴ states that there are 18 amino acids present in microalgae proteins. Of these 18 amino acids, 9 of them are essential amino acids for humans.

II MATERAILAS AND METHODS

2.1.Determination of Photoperiodism and Dillution Factor

The experimental design for determining photoperiodization and the mean dilution factor ADDMW can be seen in Table 1.

Tabel 1. Photoperiodism and Dillution Factor Variation

Photoperiodism	Dilltion Factor	
16:8	2,5	5
12:12		
8:16		

The bioslurry filtrate produced by filtration using cotton was diluted using 2.5 and 5 times. The dilution was carried out to reduce the ammonium concentration in the soil so that it is not toxic to microalgae. A dilution is also performed to reduce the turbidity of the medium so that light penetration is not blocked in the culture bottle. A total of 720 ml of diluted medium was mixed with 80 ml of inoculum concentrating 10^7 cells / ml. The cultivation was carried out on the photobioreactor with lighting derived from a 100 μ mol photon m⁻²s⁻¹ fluorescent lamp (Philips Cool White, TLD 36 Watt). The culture is kept at a temperature of 27-30°C and pH 7 -8. Aeration is given at a rate of 1 L / minute. The cultivation was carried out for 10 days.

2.2.Growth Media And Inoculum Adding Frequency

The experiment was using ADDMW which is diluted 2,5 times, and Bold Basal Media (BBM) as standard growth media. The addition of inoculum in ADDMW media was varied in its adding frequency i.e. once, first 3 days and everyday, while for control the innoculum was added at once. For control using ADDMW no innoculum was added (Table 2).

Table 2. Inoculum Adding Frequency Variation

Medium	Inoculum Adding Frequency
ADDMW 2,5x	Once (80 mL)
	First 3 Days (26,7 mL/day)
	Everyday (8mL/day)
BBM ½ strength (biomass control)	Once (80 mL)
ADDMW 2,5x (waste control)	- (Without Inoculum)

2.3. Ammonium Content

The ammonium test is performed using the Nessler method. The sample will pass through the centrifugation process to obtain the supernatant. Centrifugation was performed at a rate of 4500 g for 5 minutes. A total of 10 mL of supernatant was added with 0.2 mL of nessler reagent then stirred using vortex. After 10 minutes, the sample absorbance was measured using a spectrophotometer with a wavelength of 420 nm. The absorbance value obtained is converted into ammonia concentration using the equation of the standard curve that has been carried out.

2.4. Orthophosphate Content

The reagents used for the test are ammonium molybdate and SnCl₂. The sample was passed the centrifugation process at a rate of 4500 g for 5 minutes to obtain the supernatant. A total of 25 ml of the test sample is put in erlenmeyer and then added with 1 mL of ammonium molybdate reagent while stirred slowly. After 20 minutes, 2 drops of SnCl₂ solution are added to the sample. The absorbance of the samples was tested using a spectrophotometer with a wavelength of 650 nm. The absorbance value is then converted into orthophosphate concentration using the equations obtained from the standard orthophosphate curve.

III Result and Discussion

3.1. Ammonium Content

The ammonium content in the medium will be directly related to the accumulation of metabolites in microorganisms. The accumulation of biomass occurs higher when the soil has a sufficient nitrogen and phosphate content or in other words does not show nitrogen and phosphate deficiency (Amaro et al., 2011)⁵. The ammonium content was analyzed in ADDMW medium with changes in the frequency of inoculum addition. The profile of changes in the ammonium content in the ADDMW medium is shown in Table 5 below:

Table 5. Amonium Reduction Percentage

Inoculum Adding Frequency	Initial Concentration (mg/L)	Final Concentration (mg/L)	Reduction Percentage (%)
Once	123,992	5,349	95,69 ± 0,767
First 3 Days	141,799	5,915	95,82 ± 0,124
Everyday	141,798	4,117	97,10 ± 0,135
Without Inoculum (BBM)	147,835	6,204	95,80 ± 1,462

Microalgae used ammonium for the supply of nitrogen which will then be used to carry out some metabolism process, such as protein formation and energy formation. Statistical tests have shown that the frequency of addition of inoculum did not significantly influence the reduction of ammonium levels in the medium. In each treatment a reduction of ammonium levels of more than 95% was found. In the previous research by Wang et al. (2010)⁶, a reduction of 93.6% ammonium levels in wastewater manure that went through the stages of anaerobic bacteria digesting using remediator *C. vulgaris* agent.

Nitrification bacteria present in the medium to convert the ammonium content into nitrate (Wang et al., 2015)⁷. nitrifying bacteria that live in heterotrophic aerobics and can grow well in the growing system for growing conditions are very suitable for supporting the growth of bacteria (aeration and ammonium availability as a nitrifying substrate). This is evidenced by the presence of high nitrate levels due to the nitrification process in the ADDMW medium as originally almost no increase of about 5 ppm at the end of cultivation (data obtained from the control treatment test, in the form of ADDMW medium by aeration and exposure light without the addition of inoculum). Furthermore, the ammonium in the medium is also used by microalgae to grow. The presence of nitrifying bacteria has an impact on the reduction of the ADDMW ammonium content. Therefore, a reduction of the ammonium profile levels over a whole variety of relatively different treatment, which means that the reduction of ammonium in the soil remains the case if it is caused by consumption of microalgae or bacterial nitrification.

3.2.Orthophosphate Content

Phosphate is one of the macronutrient elements necessary for plants to grow. Phosphate will be used by microalgae for the synthesis of phospholipids, nucleic acids and cell division (Martinez et al., 1999⁸, Richmond, 2004³). The orthophosphate content in the ADDMW medium is shown in Table 6 below.

Table 6. Orthophosphate Reduction Percentage

Inoculum Adding Frequency	Initial Concentration (mg/L)	Final Concentration (mg/L)	Reduction Percentage (%)
Once	7,451	1,598	78,55 ± 1,788
First 3 Days	6,151	1,824	70,35 ± 2,800
Everyday	5,768	2,001	65,30 ± 2,373
Without Inoculum (BBM)	7,451	1,598	78,55 ± 1,788

Based on statistical tests, the frequency of inoculum addition significantly influenced the decrease in orthophosphate levels in the ADDMW medium. The greater amount of biomass in the crop system with variations in the addition of the "Once" inoculum is the main factor causing the highest decrease in orthophosphate levels in the medium.

IV CONCLUSION

Reduction percentage of the overall ammonia levels at all of inoculum adding frequency variation is relatively the same (from 95.69 ± 0.77 to 97.10 ± 0.135). Reduction percentage in orthophosphate levels makes a different result by all variation, i.e 78.55 ± 1.787 (once), 70.35 ± 2.800 (first 3 days), and 65.30 ± 2.373 (daily).

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REFERENCES

- [1] Taufikurahman, T. and Istiqomah, I.N. (2019). Phycoremediation of anaerobic digested dairy manure wastewater using *Chlorella vulgaris*. IOP Conference Series Earth and Environmental Science 299:012013. DOI: 10.1088/1755-1315/299/1/012013
- [2] Sooknah, R. D. dan Wilkie, A. C. (2004). Nutrient Removal by floating aquatic macrophytes cultured in anaerobically digested flushed dairy manure wastewater. Ecological Engineering. 22: 27-42.

- [3] Richmond, A. (2004). Biological principles of mass cultivation. In: Richmond A (ed) Handbook of microalgal mass culture. Biotechnology and Applied Phycology. Blackwell, Oxford.566.
- [4] Ursu, A., Marcati, A., Sayd, T., Sante-Lhoutellier, V., Djelveh, G., dan Michaud, P. (2014). Extraction, Fractination, and Functional Properties of Proteins from the Microalgae *Chlorella vulgaris*. Bioresource Technology. 157: 134-139.
- [5] Amaro HM, Guedes A, Malcata FX. (2011). Advances and perspectives in using microalgae to produce biodiesel. Anton Leeuw. 88(10):3402–10.
- [6] Wang, L., Wang, Y., Chen, P., dan Ruan, R. (2010). Semi-continous cultivation of *Chlorella vulgaris* for treating undigested and digested dairy manures. Appl Biochem Biotechnol. 162:2324-2332.
- [7] Wang, M., Yang, H., Ergas, S. J., dan van der Steen, P. (2015). A novel shortcut nitrogen removal process using an algal-bacterial consortium in a photo-sequencing batch reactor (PSBR). Water Resource. 15(87):38-48.
- [8] Martinez, M., Jiménez, J. M. and El Yousfi, F. (1999). Photoautotrophic consumption of phosphorus by *Scenedesmus obliquus* in a continuous culture. Influence of light intensity. Process Biochemistry. 34(8): 811-818.