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Phycoremediation of anaerobic digested dairy manure wastewater using Chlorella vulgaris

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Abstract. Microalgae have been used in a process of pollutants removal from wastewater, a process known as phycoremediation. In this study we used Chlorella vulgaris to remediate anaerobically digested dairy manure wastewater (ADDMW) which contained a high concentration of nitrogen and phosphor. The light intensity of 50, 100 µmol/m²s and sunlight were applied to the microalgal culture. Percentage of reduction in ammonium and orthophosphate content in the media were measured. Protein content in microalgal biomass was measured using hot-TCA method. At the light intensity of 100 µmol/m²s, microalgal productivity in biomass reached its high level i.e. 0.149 ± 0.03 gL⁻¹d⁻¹ with its protein content $10.74 \pm 0.63\%$. However, the highest specific growth rate $(0.343 \pm 0.034 d^{-1})$ with the shortest doubling time $(2.028 \pm 0.141 \text{ d})$ were reached under sunlight conditions. The reduction rate of ammonium and orthophosphate content in the medium were 36-48 mgL⁻¹h⁻¹ and 5-6.62 mgL⁻¹h⁻¹ respectively in a culture which was inoculated with C. vulgaris. C. vulgaris can be used as phycoremediator for ammonium and orthophosphate, and ADDMW medium can supply C. vulgaris with appropriate nutrients. Moreover, light intensities of 50 and 100 µmol/m²s can be used in the indoor culture of microalgae.

1. Introduction

Indonesian Directorate General of Environmental and Forestry Pollution and Damage Control reported that in 2015 nearly 68 percent of river water quality in 33 provinces of Indonesia was heavily polluted, the other 24 percent were in moderately polluted status, 6 percent were mildly polluted and only around 2 percent meets the water quality standards. Pollutants in river water can be contributed by domestic or household wastes, farm waste, industrial and agricultural wastes [1]. Phycoremediation is the use of algae to reduce or metabolized pollutants from waste, which includes nutrients and xenobiotics [2].

Cattle farming in Indonesia recently reached a population of beef cattle of 16,092,561 and dairy cattle 533,860 in 2016 [3]. Liquid waste generated from the livestock industry is usually disposed to the river. One type of waste is farm wastewater which has passed anaerobic digester to produce biogas or commonly referred to as anaerobically digested dairy manure wastewater (ADDMW). This waste is characterized by high BOD (Biological Oxygen Demand) and COD (Chemical Oxygen Demand) value, and high nitrogen ammonium content and requires relatively expensive chemical processes in a series of waste treatment processes [4, 5]. To overcome this, remediation of farm wastewater can be a costeffective alternative.

Microalgae are known to absorb inorganic nitrogen and phosphorus from wastewater. Another advantage of using microalgae in bioremediation (phycoremediation) is that microalgae can absorb carbon dioxide (CO₂) from the air during photosynthesis, providing an oxygenation process of treated

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wastewater, and microalgae can absorb organic micropollutants from waste. Therefore, the algae biomass produced can be used in the production process of products that can be categorized as human and animal foods, cosmetics, and biofuels [6]. Most studies have been carried out on unicellular Chlorophyceae, especially members of the *Chlorella* and *Scenedesmus* families. Species from this family are often used because of their dominance in the freshwater environment [7, 8]. *Chlorella vulgaris* is a freshwater microalgae that has spherical cells with a diameter ranging from 2 to 10 micrometers. Rapid growth and flexible cultivation techniques of this type of microalgae can be used in the food industry and wastewater treatment [9]. Microalgae *C. vulgaris* has high protein content which reaches 51-58% of its dry weight [10], which can be a potential source of food and feed.

Whitton *et al.* [6] state that there are several parameters that influence the growth of microalgae, namely temperature, light, cultivation method, and treatment time or hydraulic retention time (HRT). Because microalgae are photosynthetic organisms, metabolic processes associated with nutrient absorption and growth are driven by light, therefore light is the key parameters for microalgae reactors. Light intensity is an important factor that can affect growth, lipid accumulation, and fatty acid composition of microalgae [11].

Seeing the importance of light intensity for the continuity of microalgae metabolism and metabolic processes that influence the absorption of nutrients from waste, this study will focus at the effect of light intensity on the growth of unicellular autotrophic microalgae *Chlorella vulgaris* and the reduction of ammonium and orthophosphate levels from anaerobic digested dairy manure wastewater (ADDMW).

2. Materials and Methods

2.1. Materials

2.1.1. Microalgae Chlorella vulgaris. Chlorella vulgaris was obtained from the Balai Besar Perikanan Budidaya Air Payau (Center for Brackish Water Aquaculture) Jepara, Central Java, Indonesia. Microalgae C. vulgaris was propagated in Bold's Basal Medium. Acclimatization was carried out in the ADDMW medium which had been diluted 2.5 times.

2.1.2. Anaerobic digested dairy manure wastewater (ADDMW). Wastewater was taken from cattle farms owned by the Faculty of Animal Husbandry, Padjadjaran University, Jatinangor, West Java, Indonesia. Once taken, the wastewater passes through two filtering stages, the first stage is by using fat-free cotton and then using filter paper. After filtered, ADDMW is stored on a plastic container at room temperature. Before cultivation, the wastewater was diluted 2.5 times (40% wastewater content) with distilled water. Dilution of the wastewater medium was conducted to reduce the turbidity of the medium [12], this wastewater dilution method was previously also carried out by Wang et al. [13] and Miao et al. [14].

2.2. Methods

2.2.1. Cultivation of Chlorella vulgaris. Microalgae cultivation was conducted using a 1 L glass bottle.. The experiment was carried out using four replications. In general, the treatment given was a variation in light intensity i.e., 50 and 100 μ mol/m²s, and sunlight. The cultures were inoculated with C. vulgaris except in the control. Research design is shown in Table 1.

Table T Research design.			
Madium	Culture	Light Intensities	Sample Treatment
Mediulli	conditions	(µmol/m ² s)	Code
ADDMW diluted 2.5 times	Not inoculated with <i>C. vulgaris</i>	50	WN 50
		100	WN 100
		Sunlight	WN S
	Inoculated with – <i>C. vulgaris</i> –	50	W 50
		100	W 100
		Sunlight	W S
BBM half-strength	Inoculated with <i>C. vulgaris</i>	Sunlight	BBM S

Table 1 Research design

*W = wastewater; WN = Wastewater not inoculated; BBM = Bold Basal Medium; S = sunlight

The light source that used was a white fluorescent lamp (Phillips Cool daylight TL-D) with intensity settings done by adjusting the distance between culture and lights source. Intensity measurement was carried out using lux meter (Milwaukee MW 700) in lux units, conversion to μ mol/m²s for fluorescent lamps was carried out according to Ahn et al. [15] in equation (1):

$$PPFD\left(\frac{\mu mol}{m^2 s}\right) = \frac{Intensity\left(lux\right)}{i} \tag{1}$$

i is a conversion factor, with a value of 74 for fluorescent lamp and 54 for sunlight.

The microalgae used to inoculate was uninformed at ~10⁷ cell/mL. The Bold's Basal Medium chemical medium was made in half-strength for cultivation. The pH of the culture was set at 7 ± 0.3 at the start of cultivation and rearrangement was carried out on the 5th day of cultivation. The absorbance of culture was measured every day at a wavelength of 680 nm. For cultures which were not inoculated with *C. vulgaris* was also given aeration and the same cultivation conditions as the culture which inoculated. Aeration was given through a hose using an aerator with an air flow rate set at 1 L/minute. Kinetic analysis was done by calculating the specific growth rate (μ), doubling time (dt), and biomass productivity (r). The calculation was done with equations (2), (3), and (4) according to Barsanti & Gualtieri [16] and Blair *et al.* [17]:

$$\mu(d^{-1}) = \frac{\ln(N_2/N_1)}{t_2 - t_1} \tag{2}$$

$$dt(d) = \frac{\ln\ln 2}{\pi} \tag{3}$$

$$r\left(\frac{g}{Ld}\right) = \frac{N_2 - N_1}{t_2 - t_1} \tag{4}$$

 N_1 and N_2 are biomass concentration (g/L) when t_1 and t_2 .

2.2.2. C. vulgaris biomass harvesting. Biomass harvesting was carried out by sampling culture with a volume of approximately 400 mL from each treatment. Then the culture was centrifuged at 5000 RPM for 10 minutes to separate the medium with microalgae biomass. The centrifuge was carried out repeatedly until all biomass in each treatment was obtained. Then the sample was centrifuged again in the microcentrifuge with a speed of 15000 RPM for 5 minutes at room temperature for better separation. Furthermore, the biomass obtained was freeze-dried for further analysis.

2.2.3. Ammonium (NH_4^+) levels measurement. The first step was harvesting the culture with approximately 15 mL in a centrifuge tube. Then the culture was centrifuged (Heraeus Labofuge 200) at 5000 RPM for 10 minutes, to separate the microalgae biomass with the waste medium. Measurement was done by Nessler method.

This method uses Rochelle salt solution made by mixing 50 g of Na-K-Tartrate solids with 100 mL of distilled water. Then a total of 12.5 mL of sample was added with 1 drop of Rochelle salt solution which had been made beforehand, then added 0.25 mL of Nessler reagent and the sample was incubated for 10 minutes right after the reagent was added. Then the sample absorbance was measured at a wavelength of 420 nm. Ammonium concentration was based on the NH₄Cl standard curve equation. Ammonium reduction rate (R_i) and efficiency calculations were carried out with equations (5) and (6) according to Delgadillo-Mirquez et al. [18] and da Fontoura et al. [19]:

$$R_i = \frac{S_0 - S_f}{t_f} \tag{5}$$

Reduction eff (%) =
$$\frac{S_0 - S_f}{S_0} \times 100\%$$
 (6)

 S_0 is concentration when t_0 and S_f is concentration when t_f .

2.2.4. Orthophosphate (PO_4^{3-}) levels measurement. Orthophosphate measurement preparation was carried out in the same way as ammonium measurement. Phosphate level measurement was carried out by the Stannous Chloride (SnCl₂) spectrophotometric method. This method used two types of reagents namely SnCl₂ reagent and ammonium molybdate solution. The sample absorbance was measured at a wavelength of 660 nm. The concentration of orthophosphate ions in wastewater was based on the calculation of the KH₂PO₄ standard curve. The rate calculation and reduction efficiency were carried out with equations (5) and (6).

2.2.5. Nitrate (NO_3^{-}) levels measurement. Calculation of nitrate levels from wastewater follows Cataldo et al. [20] using salicylic-sulfuric acid (H₂SO₄) reagent. The absorbance of the solution was measured at a wavelength of 410 nm. Nitrate concentration can be known by the equation of the standard curve of Potassium Nitrate (KNO₃⁻).

2.2.6. Protein extraction and quantification. The process of protein extraction from C. vulgaris biomass was based on the hot-TCA method based on Slocombe et al. [21] with some modification. The extraction process started with 3 mg of freeze-dried biomass of C. vulgaris was added with 250 μ L 24% (w/v) trichloroacetic acid solution in a microcentrifuge tube. This mixture was then vortexed several times until it was homogeneous and incubated at 950C at a water bath for 15 minutes. Subsequently, this mixture was centrifuged with microcentrifuge (Eppendorf 5430 R) at 15,000g at 40C for 20 minutes. The supernatant produced by the centrifuge was separated and the pellet was added with 0.5 mL 0.1 N NaOH. This mixture was vortexed several times until homogeneous. Then the mixture was pre-incubated at 55°C on a water bath for 3 hours. The sample was then cooled to room temperature and centrifuged back at 15,000g at room temperature for 20 minutes and the supernatant was further tested.

2.2.7. Protein quantification was carried out by the Bradford method based on Kruger [22]. 0.1 ml supernatant was taken and added with 5 ml Bradford reagent. Bradford's reagent was made by dissolving 100 mg of Comassie Brilliant Blue in 50 mL of 95% ethanol. The solution was then mixed with 100 mL 85% H₃PO₄ and added with distilled water up to the volume of 1 L. The reagent then was stored in a dark bottle and filtered with Whatman no. 1 filter paper before use. Protein extracts that have been added to the reagent are then incubated for 20 minutes and the absorbance was measured at 595 nm. Protein concentration was known by the equation of the BSA (Bovine Serum Albumin) protein standard curve.

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2.2.8. Data representation and analysis. Statistical analysis performed such as the calculation of averages, standard deviations, and Analysis of Variance (ANOVA). Representation and overall data analysis were done with Microsoft Excel 2016.

3. Results and Discussions

3.1. Kinetics of C. vulgaris growth

The highest specific growth rate and biomass productivity of *C. vulgaris* is shown by the treatment light intensity of 100 μ mol/m²s with ADDMW medium (W 100), while for the parameters of specific growth rate (μ) and doubling time (dt) the highest value is indicated by the culture cultivated in sunlight (W S) (Table 2).

Table 2 Specific growth rate (μ), doubling time (<i>dt</i>), and biomass productivity values				
	Treatment	μ (d ⁻¹) ± SD	dt (d) \pm SD	Biomass productivity $(gLd^{-1}) \pm SD$
	W 50	0.320 ± 0.058	2.231 ± 0.405	0.097 ± 0.015
	W 100	0.333 ± 0.017	2.089 ± 0.103	0.149 ± 0.031
	W S	0.343 ± 0.024	2.028 ± 0.141	0.131 ± 0.006
	BBM S	0.296 ± 0.049	2.418 ± 0.488	0.093 ± 0.036
	TTT TTTTTTTTTTTTT	XX7	1.10.0111.00	

*W = Wastewater, WN = Wastewater not inoculated, S = Sunlight, BBM = Bold Basal Medium



*W = Wastewater, WN = Wastewater not inoculated, S = Sunlight, BBM = Bold Basal Medium

Figure 1 Growth curve of C. vulgaris exposed to different light intensities in ADDMW medium

W 100 culture experienced the shortest lag phase of 2 days, while for W 50 was 4 days, W S was 5 days, and BBM S was 7 days (Figure 1). In the lag phase, culture is still adapting to the new environment with a growth rate with values close to zero that indicates the rate of reproduction and cell death are almost equal [16]. At high light intensity, the time to reach the cell division checkpoint (in this case is called the commitment point-the point when the cell is ready to divide) becomes shorter, this is because the time to reach the commitment point is influenced by the growth rate that is affected by the rate photosynthesis [23]. The intensity of light affects the rate of photosynthesis by providing sufficient light

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energy for the electron transport process in chloroplasts, with the time to reach a commitment point is shorter, the more daughter cells can be formed (in the same time span), this is what is likely to make the W 100 culture experience the shortest lag phase, followed by W 50, then W S. The intensity of sunlight in the study period shows relatively low values when compared with fluorescent lamps (in the range of 24-94 μ mol/m²s). The culture grown on half-strength BBM medium (BBM S) has the longest lag time. It is most likely due to different Nitrogen sources in the BBM medium, namely nitrate (NO₃⁻) because of the re-adaptation process carried out because the inoculum of *C. vulgaris* comes from the ADDMW medium.

After experiencing the lag phase, all the treatment that cultivated in ADDMW has a specific growth rate that is not significantly different (p>0,05), which indicates that the possibility after adapting to the cultivation conditions, culture can experience growth with a speed that is not much different regardless of the treatment given.

3.2. Reduction of Ammonium (NH_4^+) Levels

The fastest reduction of ammonium ions occurs in the treatment of light intensity of 50 and 100 μ mol/m²s grown which inoculated with *C. vulgaris* (W 50 and W 100) (Figure 2). Both had almost the same reduction rate on the first 3 days of cultivation, i.e. 48.70 ± 0.098 for W 50 and 48.55 ± 0.251 for W 100 with an efficiency of 92.8% and 93.67%, respectively (Table 3). The large standard deviation at the beginning is caused by a non-homogeneous medium (which can be caused by imperfect stirring) and the deviation that happened later can be caused by the different activities of microalgae and maybe the other microorganisms too (this have a high probability to happen as the medium was not sterilized). In general, the reduction efficiency did not differ significantly between culture which inoculated with *C. vulgaris* and which was not (p> 0.05) which gave a value of 92-95%, this result is consistent with the results obtained by Wang *et al.* [24], namely microalgae *C. vulgaris* can almost reduce all NH₄⁺ from waste regardless of its initial concentration. For the same light intensity, cultures that were inoculated with *C. vulgaris* resulted in a higher ammonium reduction rate than cultures that were not inoculated with significant differences (p<0.05). This shows that *C. vulgaris* can be a good candidate for ammonium phycoremediation agent.



*W = Wastewater, WN = Wastewater not inoculated, S = Sunlight

Figure 2 Ammonium reduction in ADDMW medium

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In the first 3 days, all cultures (W 50, W 100, W S) were still experiencing a lag phase (Figure 1). The cultures were still adjusting to the cultivation conditions, which showed that the culture had not grown to its full potential. However, it is seen that the ammonium level has decreased quite sharply from the first day (Figure 2), compared to when the culture has begun to enter the exponential phase (for W 50 from day 4, W 100 from day 3, and W S from day 5). This result shows that there are other reduction mechanisms besides ammonium absorption by microalgae namely indirect remediation mechanism. The process of microalgae cultivation in waste makes pH rise due to several things, namely the formation of hydroxyl radicals (OH⁻) due to absorption of inorganic carbon such as bicarbonate, absorption of protons (H⁺) from the dissociation process of water (H₂O) for the process of cotransport from NO₃⁻ and PO₄⁻ through the cell membrane and the change in pH facilitates indirect remediation [6]. At pH of more than 7, equilibrium between ammonium (NH₄⁺) and ammonia (NH₃) shifts towards the formation of NH₃ (gas) with the reaction (a) below, this mechanism has been shown to contribute greatly to ammonium remediation, as reported by Martinez *et al.* [25] for *Scenedesmus obliquus*:

$$NH_4^+ + OH^- \le NH_3 + H_2O$$
 (a)

Although the release of NH_3 from the medium makes the pH decreased, this effect is covered by changes caused by the bicarbonate reaction that mentioned earlier [25]. The decrease in ammonium levels also occurs in cultures that are not inoculated with microalgae, this indicates that there are other microorganisms that absorb ammonium from the ADDMW medium. Several groups of microorganisms that may have contributed to the reduction of NH_4^+ were in the Ammonium Oxidizing Bacteria (AOB) and Nitrite Oxidizing Bacteria (NOB) groups known as nitrifying bacteria [26, 27].

Ammonium oxidation will produce nitrite, and nitrite oxidation will produce nitrate [28], which then can be absorbed by phototrophic microorganisms, e.g. microalgae, this is evidenced by the end of cultivation of some non-inoculated culture bottles showing a greenish color. The nitrification process in aerated wastewater cultures was also observed in Delgadillo-Mirquez *et al.* [18].

und orthophosphate					
Treatment	Reduction rate $(mgL^{-1}h^{-1}) \pm SD$		Reduction efficiency \pm SD		
	$\mathrm{NH_4^+}$	PO4 ³⁻	$\mathrm{NH_4}^+$	PO4 ³⁻	
W 50	48.70 ± 0.098	6.49 ± 0.261	$92.80 \pm 0.58\%$	$94.48 \pm 6.26\%$	
WN 50	22.79 ± 2.857	4.75 ± 0.168	$92.88 \pm 0.73\%$	$89.71 \pm 3.51\%$	
W 100	48.55 ± 0.251	6.65 ± 0.098	$93.67 \pm 0.85\%$	$82.12 \pm 1.2\%$	
WN 100	15.01 ± 6.332	4.73 ± 0.219	$93.54 \pm 0.52\%$	$91.76 \pm 5.55\%$	
W S	36.49 ± 6.248	5.52 ± 1.199	$94.55 \pm 0.07\%$	$94.28\pm0.6\%$	
WN S	16.21 ± 3.259	4.34 ± 0.111	$93.19 \pm 0.55\%$	$89.71 \pm 2.39\%$	
	11 D I 11 I	1 1 1 9 9	11.1.		

Table 3 Reduction rate on the first three days of cultivation and reduction efficiency of ammonium and orthophosphate

*W = Wastewater, WN = Wastewater not inoculated, S = Sunlight

3.3. Reduction of orthophosphate (PO_4^{3-}) levels

It was found that cultures which inoculated with *C. vulgaris* and given light intensities of 50 and 100 μ mol/m²s (W 50 and W 100) gave the highest orthophosphate reduction rate of 6.49 \pm 0.261 and 6.65 \pm 0.098 mg L⁻¹h⁻¹ (Table 3 and Figure 3). In general, cultures inoculated with microalgae gave a higher rate of reduction compared to cultures that were not inoculated with microalgae with significant differences (p<0.05), while for reduction efficiency gave relatively similar results (p>0.05) (Table 3).

Just like ammonium, orthophosphate reduction can be also caused by indirect remediation mechanisms that occur due to pH increases due to the reasons that previously explained. Unlike NH₃ which can come out into gas, orthophosphate is precipitated by reacting with metal ions which occur at alkaline pH [6, 12].



*W = Wastewater, WN = Wastewater not inoculated, S = Sunlight

Figure 3 Orthophosphate reduction in ADDMW medium

Cultures that were not inoculated with microalgae also experience a decrease in orthophosphate levels (Figure 3), this indicates that there are microorganisms that consume orthophosphate so that the levels are reduced. Phosphorus is a key element for the synthesis of nucleic acids and phospholipids and is generally absorbed in the form of phosphates (PO_4^{2-}) [29]. Some heterotrophic bacteria can absorb dissolved phosphate by accumulating intracellularly in the form of polyphosphates. This absorption results in reducing the phosphate content in ADDMW [30]. If the levels of intracellular polyphosphate are excessive then microorganisms have a regulatory mechanism by being able to excrete phosphorus out of the cell [31], this is probably what causes in Figure 3 there are several points of increase in measured orthophosphate levels.

3.4. Protein levels of C. vulgaris biomass

The highest protein content was obtained by *C. vulgaris* which was cultivated at 100 μ mol/m²s (W 100) (Table 4), and it can be inferred from the result that the light intensity influenced protein content (p<0.05). According to Seyfabadi *et al.* [32] and Ogbonda *et al.* [33], protein production is directly proportional to the increase in light, and the maximum percentage of protein content is observed in high light irradiance and long photoperiodism. The intensity of sunlight is lower during the study period as previously mentioned.

Protein levels obtained in this study are relatively lower compared with the results obtained by Seyfabadi *et al.* [32] which is $46 \pm 3.7\%$ (Table 4) with the same intensity and photoperiodism conditions. This difference can be caused by several things, the first is extraction method that was used in this study which is not yet optimal, according to Slocombe et al. [21] the hot-TCA method can give different results for different species, this suggests that it is possible that the hot-TCA method is less suitable for *C. vulgaris*.

Table 4 Frotent of this study and comparison with interature				
Sample	Protein content (%DW) \pm SD	Source		
W 50	$8.84\pm0.96\%$			
W 100	$10.74 \pm 0.63\%$	This starley		
W S	$8.50\pm0.64\%$	This study		
BBM S	$7.44 \pm 1.2\%$			
Synthetic medium; 16:8 photoperiodism; light intensity 100 µmol/m ² s	$46\pm3.7\%$	Seyfabadi et al. [32]		
Synthethic domestic household wastewater medium; mixotrophic growth	40-50%	Miao <i>et al.</i> [14]		

Table 4 Protein content of this study and comparison with literature

*W = Wastewater, S = Sunlight, BBM = Bold Basal Medium

Another reason that can affect protein levels is the availability of Nitrogen sources from the growth medium. The growth medium used from the research by Seyfabadi *et al.* [32] is a synthetic medium based on Zehnder & Gorham [34] which has NO_3^- (nitrate) levels of 496 mg/L, more than twofold the NH_4^+ (ammonium) level owned by ADDMW medium, which is 153 and 116 mg/L (Figure 2) and half-strength BBM medium which is only around 125 mg/L. Protein synthesis is directly related to Nitrogen content from the medium [25], it is known that the synthesis of amino acids glutamate which is the precursor of other amino acids in microalgae starts from the reduction of nitrate to ammonium or direct ammonium assimilation and undergoes a series of sequential reactions facilitated by the enzyme glutamine synthetase (GS) and glutamine 2-oxoglutarate aminotransferase (GOGAT) [16]. Whereas the difference with Miao *et al.* [14], although both use wastewater medium, there are differences in the type of cultivation carried out, Miao et al. [14] performed mixotrophic cultivation which contained carbon sources other than carbon dioxide such as glucose or galactose in the medium, compared with the photoautotrophic growth that occurred in this study. Mixotrophic growth is known to increase protein and lipid yield, this is also reported by Abreu *et al.* [35].

4. Conclusion

It can be concluded that the intensity of light has an influence on the growth of *C. vulgaris* biomass in ADDMW medium, with the highest light intensity of 100 μ mol/m²s giving the highest biomass productivity and protein content and the culture which exposed to sunlight give the highest specific growth rate and shortest doubling time although not significantly differ from other treatment. In addition, cultures inoculated with *C. vulgaris* had a significantly different effect on the reduction rate of ammonium and orthophosphate but not on the reduction efficiency compared with cultures that were not inoculated with *C. vulgaris*. This indicates that *C. vulgaris* can be used as a phycoremediation agent of anaerobically digested dairy manure wastewater (ADDMW), and the wastewater can also function as an alternative growth medium for microalgae.

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References

 National Geographic Indonesia. (2016). Air Sungai di Indonesia Tercemar Berat. [Online]. Accessed: http://nationalgeographic.co.id/berita/2016/05/air-sungai-di-indonesia-tercemarberat. Accessed on December 17th, 2018.

- [2] Rao P, Kumar RR, Raghavan BG, Subramanian VV and Sivasubramanian V 2011 *Water* Sa 37(1).
- Badan Pusat Statistik. (2017). Statistik Sektoral Peternakan. [Online]. Accessed: https://www.bps.go.id/subject/24/peternakan.html#subjekViewTab3. Accessed on January 9th, 2018.
- [4] Hena S, Fatimah S and Tabassum S 2015 *Water Resources and Industry* 10 1.
- [5] Labbé JI, Ramos-Suárez JL, Hernández-Pérez A, Baeza A and Hansen F 2017 Journal of Environmental Chemical Engineering 5(1) 635.
- [6] Whitton R, Ometto F, Pidou M, Jarvis P, Villa R and Jefferson B 2015 *Environmental Technology Reviews* **4(1)** 133.
- [7] Chevalier P and De la Noüe J 1985 *Biotechnology letters* 7(6) 395.
- [8] Tang EP, Vincent WF, Proulx D, Lessard P and De La Noüe J 1997 *Journal of Applied Phycology* **9(4)** 371.
- [9] Daliry S, Hallajisani A, Roshandeh JM, Nouri H and Golzary A 2017 Global Journal of Environmental Science and Management **3(2)** 217.
- [10] Becker EW 1994 Microalgae: Biotechnology and Microbiology (Vol. 10). Cambridge: Cambridge University Press.
- [11] Liu J, Yuan C, Hu G and Li F 2012 Applied Biochemistry and Biotechnology 166(8) 2127.
- [12] Ding J, Zhao F, Cao Y, Xing L, Liu W, Mei S and Li S 2015 International Journal of *Phytoremediation* **17(3)** 222.
- [13] Wang L, Li Y, Chen P, Min M, Chen Y, Zhu J and Ruan RR 2010 Bioresource Technology 101(8) 2623.
- [14] Miao MS, Yao XD, Shu L, Yan YJ, Wang Z Li N and Kong Q 2016 International Biodeterioration & Biodegradation 113 120.
- [15] Ahn YD, Bae S and Kang SJ 2017 *Energies* **10(10)** 1607.
- [16] Barsanti L and Gualtieri P 2014 Algae: Anatomy, Biochemistry, and Biotechnology. USA: CRC press.
- [17] Blair MF, Kokabian B and Gude VG 2014 Journal of Environmental Chemical Engineering 2(1) 665.
- [18] Delgadillo-Mirquez L, Lopes F, Taidi B and Pareau D 2016 Biotechnology Reports 11 18.
- [19] da Fontoura JT, Rolim GS, Farenzena M and Gutterres M 2017 *Process Safety and Environmental Protection* **111** 355.
- [20] Cataldo DA, Maroon M, Schrader LE and Youngs VL 1975 Communications in Soil Science and Plant Analysis 6(1) 71.
- [21] Slocombe SP, Ross M, Thomas N, McNeill, S and Stanley MS 2013 *Bioresource Technology* 129 51.
- [22] Kruger NJ 2009 The Bradford method for protein quantitation. *The Protein Protocols Handbook*, pp.17-24.
- [23] Borowitzka MA, Beardall J and Raven JA (Eds.) 2016 The Physiology of Microalgae Vol. 6, New York: Springer.
- [24] Wang L, Li Y, Chen P, Min M, Chen Y, Zhu J and Ruan RR 2010 Bioresource Technology 101(8) 2623.
- [25] Martinez ME, Sánchez S, Jimenez JM, El Yousfi F and Munoz L 2000 Bioresource Technology 73(3) 263.
- [26] Cydzik-Kwiatkowska A and Zielińska M 2016 World Journal of Microbiology and Biotechnology 32(4) 66.
- [27] Siripong S and Rittmann BE 2007 Water Research 41(5) 1110.
- [28] Prinčič A, Mahne I, Megušar F, Paul EA and Tiedje JM 1998 Applied and Environmental Microbiolog 64(10) 3584.
- [29] Madigan MT, Martinko JM, Stahl DA and Clark DP 2012 *Brock Biology of Microorganism*. USA: Pearson Education.

- [30] Krishnaswamy U, Muthusamy M and Perumalsamy L 2009 Eur. J. Appl. Sci 1(1) 6.
- [31] Jansson M 1988 *Hydrobiologia* **170** 177.
- [32] Seyfabadi J, Ramezanpour Z and Khoeyi ZA 2011 Journal of Applied Phycology 23(4) 721.
- [33] Ogbonda KH, Aminigo RE and Abu G O 2007 African Journal of Biotechnology 6(22) 2596.
- [34] Zehnder A and Gorham PR 1960 Canadian Journal of Microbiology 6(6) 645.
- [35] Abreu AP, Fernandes B, Vicente AA, Teixeira J and Dragone G 2012 *Bioresource Technology* 118 61.