



A comprehensive study of potential *Arthrospira platensis* cultivated in various manure-based media for biodiesel feedstock

Rachmawati Rusydi^{1,2,*}, Eva Ayuzar¹, Muliani^{1,2}, Saifuddin³

¹Aquaculture Department, Faculty of Agriculture, Universitas Malikussaleh, North Aceh, Indonesia

²Center of Excellence of Biodiesel Research and Innovation, Universitas Malikussaleh, Indonesia

³Antropology Department, Faculty of Social and Politics, Universitas Malikussaleh, North Aceh, Indonesia

*Corresponding author: rachmawati.rusydi@unimal.ac.id

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ABSTRACT *Arthrospira platensis* has emerged as a promising biodiesel feedstock due to its rapid growth and substantial biomass. In efforts to reduce production costs, researchers have explored alternative media derived from livestock waste to modify conventional mediums for *Arthrospira platensis* cultivation. The experimental design of this research employed a Completely Randomized Design, with treatments comprising inorganic fertilizer (A), chicken manure (B), cow manure (C), and goat manure (D). The livestock manures were macerated for seven days before being utilized as *A. platensis* medium. The results revealed significant ($p < 0.05$) impacts of different media on peak growth values and biomass production, reaching 2.03 ± 0.06 g/L and 1.76 ± 0.05 g/L, respectively for chicken manure. The highest peak lipid content was observed in *A. platensis* cultured in goat manure medium. This study recommends goat manure as the preferred medium for mass cultivation of *A. platensis*. Mass cultivation in goat manure medium yielded 1.53 kg of dried biomass, with a lipid content of 1.91% and a biodiesel yield of 1.65%. The predominant fatty acid in this biodiesel was heneicosane, constituting 26.4% of the total area.

KEYWORDS *Arthrospira platensis*; Biodiesel; Biomass; Lipid; Livestock manure

1. Introduction

Arthrospira platensis is a type of cyanobacteria belonging to microalgae, which has been recognized as a food source in the future (Costa et al. 2019) because of its good nutritional properties. The nutrients contained in *A. platensis* are very diverse (Braga et al. 2018) including protein, vitamins and various other minerals (Trivedi et al. 2015; Lupatini et al. 2017; Abdel-Moneim et al. 2022). *Arthrospira platensis* has a high lipid content which is consisted of essential fatty acids and lipid soluble antioxidants, so it can be used as a lipid source for many purposes (Bhakar et al. 2013; Vo et al. 2017; Kalsum et al. 2019; Hindarti and Ayuningtyas 2020).

Arthrospira platensis as lipid source already be explored for its potential biodiesel feedstock. For the example, *Arthrospira maxima*'s lipid can be converted into biodiesel with the biodiesel yield is 86.1%. The quality of the biodiesel based on *A. maxima* is within the standards limit of biodiesel (Rahman et al. 2017). Furthermore, *A. platensis* algae oil can be converted to 98.45% biodiesel through transesterification process with $[\text{Cu}(\text{H}_2\text{S})(\text{H}_2\text{O})_2]$ complex as a catalyst (Haghighi et al. 2022). The certain research revealed that in situ transesterification process applied to *Arthrospira platensis*'s

oil which could yield 84.7% of biodiesel (El-Shimi et al. 2013).

The engineering microalgae becomes the concerned biodiesel feedstock that could be cultivated in wastewater. This microalgae biodiesel refinery should be conducted along with development of wastewater proposal industry (Chen et al. 2020). Therefore, utilization of wastewater in microalgae cultivation can reduce cost production during cultivation and pollution in environment. One of wastewater that provides huge amount of nutrients is livestock manure.

Livestock manure is waste generated from livestock activities. Chicken, cow, and goat are livestock types that are commonly raised by people for consumption purpose. The nutrients content in those livestock manures are potential to fertilize the growth of *A. platensis* as biodiesel feedstock. However, the supplied nutrients are certainly different from each manure which is affected by each livestock feed. A study revealed that organic carbon, nitrogen, and kalium content were highest in goat manure among fertilizers from cow, chicken, and goat manures. Meanwhile, the chicken manure served highest phosphorus content. Then, cow manure contained higher amount of organic carbon, nitrogen, and potassium than chicken manure (Novitasari and Caroline 2021).

In general, the primary nutrients such as nitrogen and phosphorus in those manures are the main reason of their utilization as *A. platensis* media. A research showed that nitrogen content of cow manure, goat manure, and chicken manure are 1.12%, 2.09%, 2.09%, respectively. Nitrogen is primary nutrient that is important for microalgae growth and biomass production (Febtisuaharsi 2016). Furthermore, diminishing nitrogen concentration could affect to decreasing the cell growth, pigments and protein content of microalgae, specifically *Isochrysis galbana*. While that treatment could increase carbohydrate and poly unsaturated fatty acids contents in *I.s galbana* cells. Beside nitrogen, livestock manure also contains phosphorus and some trace elements (Zarrinmehr et al. 2020). Another research showed that phosphorus content in dairy manure and broiler litter were 7 and 14 g/kg of dry weight (Li et al. 2014). While the phosphorus content of goat manure ranged 350–500 mg/L (Mbatha et al. 2021).

Investigation of appropriate livestock manure based media to support biomass and lipid production of *A. platensis* becomes interesting to be conducted for producing biodiesel. Then, identification fatty acids methyl ester of biodiesel from *A. platensis* feedstock cultivated in appropriate livestock medium is needed to fulfill the limited information of microalgal-based biodiesel. Based on this background, it is very important to do a study on *A. platensis* as potential biodiesel feedstock which is cultivated in livestock media in order to produce biodiesel inexpensively and effectively. This study aimed to determine the growth, peak population, harvested biomass of *A. platensis*, lipid content, biodiesel yield, and quality of biodiesel yield of *A. platensis*-biodiesel feedstock.

2. Materials and Methods

The study was conducted from August to October 2021 in Laboratory of Aquaculture Technology, Faculty of Agriculture, Malikussaleh University. *Arthrospira platensis* starter, as experimental object, was obtained from BBP-BAP Ujung Batee, Banda Aceh. Then, observation of biodiesel quality was done in Syiah Kuala University, Banda Aceh.

2.1. Preparation of inorganic fertilized medium

Inorganic fertilized medium was a medium enriched by inorganic fertilizers such as TSP, urea, and ZA. This medium was used to support the stock culture of *A. platensis* during the research. The compositions of inorganic fertilized medium were seawater 30 ppt, TSP fertilizer 30 ppm, urea fertilizer 80 ppm and ZA fertilizer 20 ppm, EDTA 5 ppm, and FeCl₃ 2 ppm, vitamin B1 0.001 ppm (Utomo et al. 2005).

2.2. Stock culture of *Arthrospira platensis*

Arthrospira platensis stock culture was carried out to increase *Arthrospira platensis* population continuously as the object of research. Stock culture was initialized by culturing 20% (600 mL) of *Arthrospira platensis* into 3,000

mL of inorganic fertilized medium. The culture was conducted for five days, whereas in day 5, the stock culture would be renewed into new inorganic fertilized medium in same volume.

2.3. Preparation and maceration of livestock manures media

The livestock manures used were chicken, cow, and goat manures obtained from livestock activity in North Aceh Regency. The sterilized livestock manures were grinded into fine granules and sieved by small pore sieve. The livestock manures media were made by dissolving 100 g of each livestock manure into 1,000 mL of seawater separately (Astiani et al. 2016). Furthermore, the maceration process of livestock manures in media was conducted by allowing the media to stand for one week (7 days) in closed containers and without aeration. Then, the media were filtered using cloth to separate the solution from its residue. The filtered livestock manure solutions in amount 150 mL were added into 2,850 mL of seawater 25 ppt. Furthermore, each livestock manure medium was aerated continuously before using as culture medium.

2.4. Cultivation of *Arthrospira platensis* in the livestock manure media

Cultivation of *A. platensis* in livestock manures media was started by providing 3,000 mL of livestock manure media with continuous aeration. A total of 600 mL (20% starter) of *A. platensis* inoculum was added into each livestock manure medium. Furthermore, *A. platensis* cultivation was maintained for 7 days with 24-hour lighting using a 20 watt TL lamp (intensity 2,000 lux). The containers of cultivation were closed with transparent cover to prevent contamination (Febtisuaharsi 2016).

Harvesting of *A. platensis* was carried out in day 7th of cultivation by flocculation method using 1 g/L of NaOH as flocculant agent. The flocculation process was carried out for 30 min then the flocs were filtered using filter papers. The filtered biomass of *A. platensis* was weighed as wet weight then it was dried for 24 h at 60 °C. After drying, the dried biomass was weighed again to determine its dry weight.

2.5. Lipid extraction of *Arthrospira platensis*

Arthrospira platensis lipid extraction was initiated by placing dried biomass into a Soxhlet flask and adding 200 mL of N-Hexane solvent. The lipid extraction process was carried out using the Soxhlet method with 4 h of immersion time. Furthermore, the results of the lipid extraction from the extraction were carried out by separating the solvent with the reactor for 30 min after that the lipid was put into the fume cupboard. The lipid content was calculated by formula:

$$Y(\%) = \frac{W_{lipid}}{W_{dried\ biomass}} \times 100\% \quad (1)$$

2.6. Transesterification process of biodiesel

The transesterification process of *A. platensis* lipids involved those several steps. Microalgae lipids were weighed and put into a 50 mL glass beaker. Furthermore, the KOH catalyst (1% of the pleated weight) was added to the erlenmeyer containing 1:6 methanol. Then, the mixture of catalyst and methanol was put into a test tube and homogenized with lipids. The transesterification process was carried out for 180 min at a temperature of 50–60 °C. After that, the reaction mixture was put back into the beaker glass while the glycerol was separated. Then, the beaker glass was put into the fume cupboard to evaporate the solvent. Biodiesel yield was measured through this formula:

$$\text{Yield} = \frac{\text{weight of crude biodiesel}}{\text{weight of microalgae lipid}} \times 100\% \quad (2)$$

2.7. Statistical Analysis

The analysis of variance was used a completely randomized design (CRD) with the significance level 0.05. The analysis of data was carried out by using SPSS 21 software. Furthermore, the further test used was Tukey test to determine the significant different among the treatments.

3. Results and Discussion

3.1. Daily growth of *Arthrospira platensis* in different media

The daily growth of *A. platensis* cultivated in chicken, goat and cow manure media showed the better increment of growth than in inorganic fertilized medium. The daily growth of *A. platensis* cultured in different media had different peak value and life curve tendency. This life curve was determined by dried biomass harvested in each day for seven days of cultivation. The life curve of *A. platensis* cultured in different media is shown by Figure 1.

The result of experiment showed that *A. platensis* achieved its full life cycle. Most *A. platensis* in inorganic fertilizer, chicken manure, and goat manure media might suffer adaptation phase from day 0 until day 1st. Then, *A.*

platensis in inorganic fertilizer and chicken manure media achieved its exponential phase from day 1st until day 2nd, meanwhile *A. platensis* in goat manure medium needed longer time to get exponential phase than those two media, namely in day 3rd. Stationary phase of *A. platensis* in inorganic fertilizer and chicken manure media was achieved after day 2nd to day 3rd, while *A. platensis* in goat manure medium achieved its stationary phase after day 3rd to day 4th. Finally, the declining phase was reached in day 5th for cultivation in chicken and goat manure media and in day 6th for cultivation in inorganic fertilizer. However, the growth of *Arthrospira platensis*'s cells in cow manure medium was slight increase during experiment.

The life cycle of *A. platensis* in inorganic and chicken manure media showed abnormal growth tendency which suffered the decrease of cells after reaching lag phase in day 2nd. This condition might be caused by proportion of nitrogen and phosphorus in both of media could not support the growth of *A. platensis*. The data of nitrate as nitrogen source and phosphate as phosphorus source in each medium is shown by Table 1.

Figure 2 shows the peak value of *A. platensis*'s growth in this experiment. The peak value of growth of *A. platensis* cultured in chicken manure added medium became the highest value, namely 2.03 g/L in dried biomass. Then, *A. platensis* cultured in goat manure added medium resulted the second highest value of growth peak for seven days cultivation. Meanwhile, the lowest peak value of *A. platensis* growth was shown by cow manure added

TABLE 1 Nitrate and phosphate content of each medium for *Arthrospira platensis* cultivation.

Parameter	Initial				Final			
	A	B	C	D	A	B	C	D
Nitrate (mg/L)	1.3	12	1.9	2	0.7	1.8	1.5	1.8
Phosphate (mg/L)	1.9	32.2	7.45	7.65	1.3	25.25	4.75	4.95

Note:
 A : Inorganic medium
 B : Chicken manure medium
 C : Cow manure medium
 D : Goat manure medium

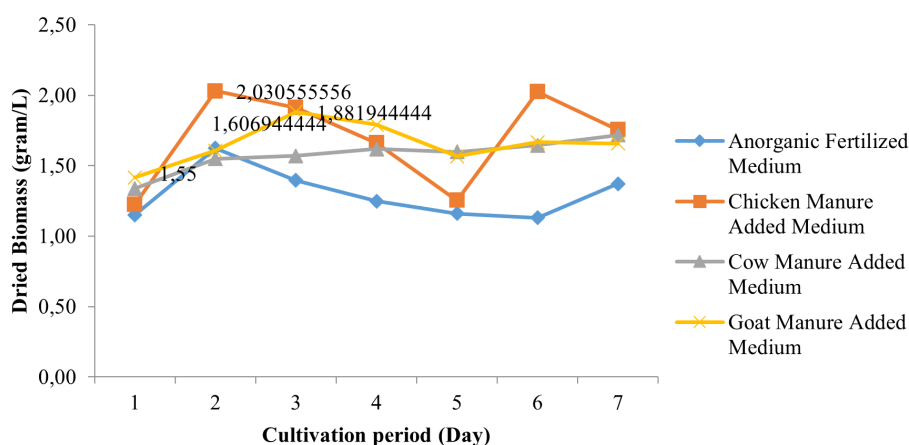


FIGURE 1 Daily growth of *Arthrospira platensis* in different media.

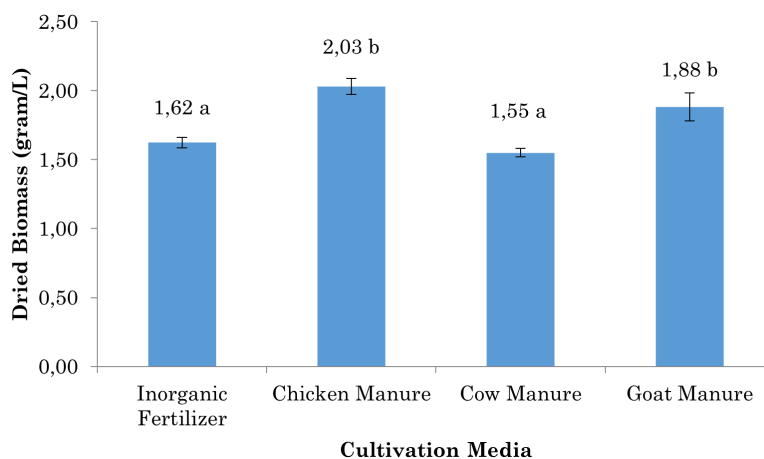


FIGURE 2 Peak value of *Arthrospira platensis*'s growth in different media.

medium which was not significant different from inorganic fertilized medium ($p > 0.05$). In general, different media affected significantly on peak value of *Arthrospira platensis*'s growth at confidence level of 95% ($p < 0.05$).

3.2. Biomass production of *Arthrospira platensis*

Based on the experiment, different media in cultivation of *A. platensis* also significantly affected the biomass production of *A. platensis* ($p < 0.05$). Biomass production of *A. platensis* cultured in inorganic fertilized medium was different from *A. platensis* cultured in livestock manure media. The highest biomass production was shown by chicken manure medium with the value 1.76 g/L (dried biomass). Then it was followed by *A. platensis* biomass production from cow manure medium. Meanwhile, the lowest biomass production of *A. platensis* was shown by inorganic fertilized medium which was 1.37 g/L (dried biomass). The biomass production of *A. platensis* cultured in different media can be seen on Figure 3.

3.3. Daily lipid content

Daily lipid content of *A. platensis* was evaluated after determining the potential media based on its growth and

biomass production. The measurement of daily lipid content purposed to determine the appropriate time of harvesting *A. platensis* that had the best lipid productivity. Daily lipid content of *Arthrospira platensis* cultivated in livestock manure media is shown by Figure 4.

Based on the graph, production of lipid content in *A. platensis*'s cells fluctuated during cultivation. In general, lipid content dropped in exponential phase until stationary phase, namely from day 2 to 5. Then it dramatically increased in phase of mortality of *A. platensis*'s life cycle, which was in day 6. This phenomenon was related to biomass production of *A. platensis*. According to its lipid content and biomass production, the day which showed the best lipid productivity could be determined.

Different from cow and goat media, chicken manure medium showed the peak lipid content in day 5th, then it decreased drastically after day 5th. This result implied that accumulation of lipid in *A. platensis*'s cells was used as energy to adapt for lack nutrition in medium and to regenerate its new cells in its exponential phase. Lipid content peak (Figure 4) showed different tendency from cell growth of *A. platensis* (Figure 1). During its reproduction from lag phase to exponential phase, the cells would con-

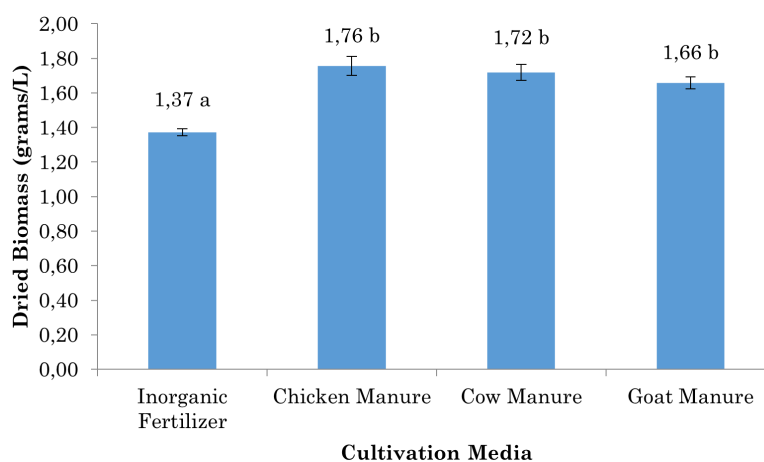


FIGURE 3 Biomass production of *Arthrospira platensis* in different media.

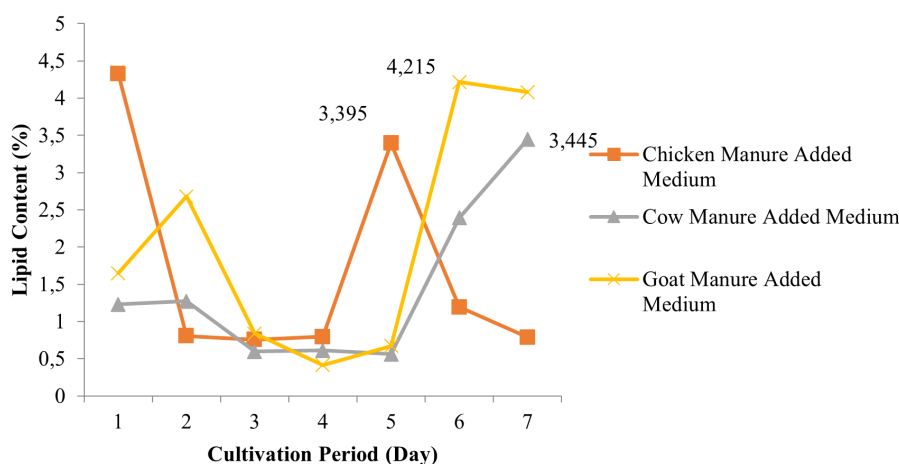


FIGURE 4 Daily lipid content of *Arthrospira platensis* in different media.

sume much energy, therefore energy accumulated in lipid form would be limited in the cells of *A. platensis*. It means that highest cells production in medium, lowest lipid content in cells. Therefore, lipid content pattern of *A. platensis* in chicken manure medium would adjust its life cycle pattern in that medium.

Beside of determining the appropriate harvesting time based on its lipid productivity, this result was also useful to determine the best cultivation medium used for huge cultivation purpose. The goat manure added medium was the good medium which supported lipid production at 4.215% in *A. platensis*'s cells. While, the chicken manure and cow manure showed less lipid content than goat manure. Therefore, the chosen medium was goat manure added medium.

3.4. Biodiesel production of *Arthrospira platensis* cultivated in goat manure added media

The experiment was continued to massive scale cultivation of *A. platensis* in goat manure added medium. The purpose of this step was to evaluate the biodiesel yield of *A. platensis* cultivated in goat manure added media. The result of this study was shown by Table 2.

A. platensis cultured in goat manure added medium in volume 1,000 L and harvested in day 4th could result 11,800 grams of wet biomass then it became 1,300 g of dried biomass. The water content of *Arthrospira platensis* in this study was 88.98%. Lipid content resulted by *A.*

platensis was 1.91% which was categorized as low lipid source. Then, biodiesel yield of *A. platensis* through transesterification process was 1.65% from its lipid content.

3.5. Quality of *Arthrospira platensis* based biodiesel

Biodiesel resulted from *A. platensis* biomass was measured to evaluate the fatty acids composition in the biodiesel. The result of biodiesel quality is shown by Figure 5.

The highest peak was achieved by fatty acid heneicosane with area 26.4%. Then, the second highest peak in biodiesel quality was achieved by 9-octadecenoic acid methyl ester (CAS) with area 24.92%. Furthermore, it was followed by linoleic acid ethyl ester with area 17.47%.

3.6. Discussion

This study showed that the livestock manures could affect *A. platensis* growth and biomass production which are shown by Figure 2 and Figure 3. Those impacts were better than was in inorganic fertilized medium that contained nutrients compounds in such as nitrogen, phosphorus, and some trace elements in inorganic form. The reason was that the total number of nutrients and their forms supplied by livestock manures and inorganic fertilizer were different. The restricting factor which was important for cyanobacteria's life was carbon (C), in where the proportion of carbon in inorganic fertilized medium was suspected lower than was in livestock manures media. These conditions actually could affect nutrients uptaking ability of *A. platensis*.

The growth of microalgae basically is affected by some factors. The important factors that should be considered are light intensity, salinity, pH, temperature, nutrients availability, CO₂, and dissolved oxygen concentration. Those factors can be optimized to gain the optimal biomass productivity of microalgae (Chowdury et al. 2020). The nutrients availability mentioned is the availability of inorganic nutrients mainly nitrogen and phosphorus, and also organic and inorganic carbon, which are absorbed by microalgae (Lage et al. 2021).

TABLE 2 Biomass, lipid, and biodiesel production of *Arthrospira platensis* cultured in goat manure added medium.

Number	Observed Parameters	Value
1	Wet Biomass of <i>Arthrospira platensis</i>	11,800 g
2	Dried Biomass of <i>Arthrospira platensis</i>	1,300 g
3	Lipid Weight	24.88 g
4	Lipid Content	1.91%
5	Biodiesel Weight	0.41 g
6	Biodiesel Yield	1.65%

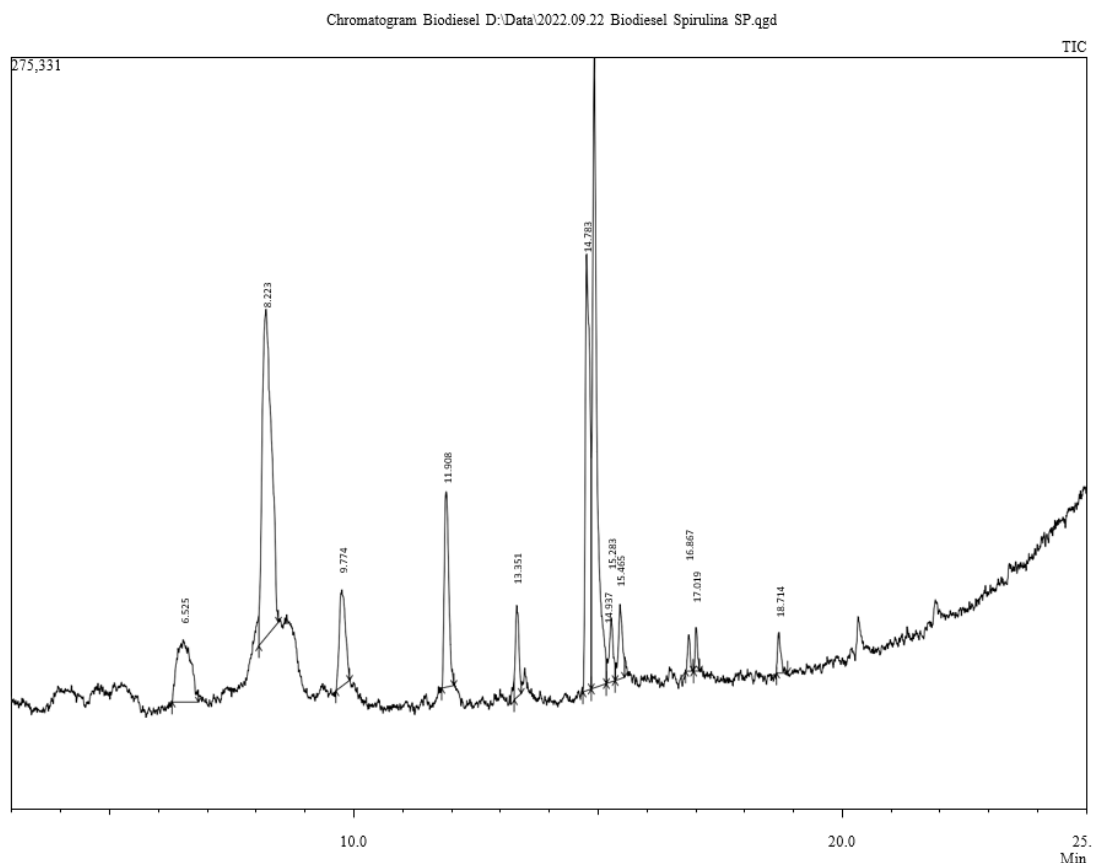


FIGURE 5 Fatty acids peaks contained in *Arthrospira platensis* based biodiesel using GC-MS.

TABLE 3 Fatty acids composition in *Arthrospira platensis* based biodiesel.

Peak#	R.Time	Area	Area%	Height	Height%	A/H	Name
1	6.525	449909	7.59	22935	2.98	19.62	1-Nonadecene
2	8.223	1565863	26.40	120155	15.60	13.03	Heneicosane
3	9.774	310945	5.24	35355	4.59	8.79	n-Pentadecanol
4	11.908	457919	7.72	71639	9.30	6.39	Hexadecanoic acid, methyl ester (CAS) Me
5	13.351	167705	2.83	33741	4.38	4.97	1-Heptacosanol
6	14.783	1036289	17.47	159987	20.77	6.48	Linoleic acid ethyl ester
7	14.937	1478276	24.92	231198	30.02	6.39	9-Octadecenoic acid, methyl ester (CAS) M
8	15.283	138031	2.33	23603	3.06	5.85	Pentadecane, 8-hexyl-
9	15.465	155913	2.63	27266	3.54	5.72	Methyl stearate
10	16.867	47618	0.80	13382	1.74	3.56	n-Tetracosanol-1
11	17.019	54284	0.92	16041	2.08	3.38	Eicosane
12	18.714	68288	1.15	14965	1.94	4.56	Hexatriacontane
		5931040	100.00	770267	100.00		

Arthrospira platensis cultured in inorganic fertilized and chicken manure added media had peak value of growth on day-2 of cultivation (Figure 1). While, *A. platensis* cultivated in goat manure added medium had a peak value of growth on day-3 of cultivation. However, the growth of *A. platensis* cultivated in cow manure added medium had a slight growth during seven days of cultivation. In general, the exponential phase of *A. platensis* occurred in day 2nd to day 3rd then it is continued in second cycle in day 6th

to day 7th during the experiment. Increment of life curve of *A. platensis* was a characteristic of exponential phase of *A. platensis*'s life. It meant that producing biomass was highest compared to other phases of life.

The life cycle of *Arthrospira platensis* in normal pH 8.0 showed the exponential phase on day 3 to day 5. Meanwhile, increment of medium pH would extend the lag phase of *A. platensis* (Putri 2019). Furthermore, *Arthrospira platensis* cultured in vannamei culture waste

water showed the exponential phase in day 3rd to day 6th of cultivation, while the stationary phase was on day 7th and the declining phase was on day 8th (Hartami et al. 2022). Generally, peak value of *Arthrospira platensis*'s biomass is started on day 3rd of cultivation which consider in the exponential phase.

Based on the Figure 1, livestock manures added into media gave better performance of *A. platensis*'s growth than inorganic fertilized medium. This result was caused by micro nutrients of livestock manures which were more complete than inorganic fertilizer. Using livestock manure could help forming ion particles in medium so that *A. platensis* could absorb the nutrients easily. The growth performance of *A. platensis* in this study was similar to the experiment conducted by Astiani et al. (2016). Those experiment showed that the different livestock manures, namely chicken manure, bird manure, buffalo manure and cow manure could give significantly to affect (p -value < 0.05) on growth rate and biomass production of *A. platensis*.

The difference of daily growth of *A. platensis* related to its nutrients requirement, specifically nitrogen (N) compound. The study of Soni et al. (2019) found that *Spirulina platensis* uses nitrogen from urea more efficiently than nitrogen from KNO_3 for its growth, biomass productivity, and chlorophyll. Then, a study showed that chicken manure has abundant nutrients such as nitrogen 3.21%, P_2O_5 3.21%, K_2O 1.57%, calcium 1.57%, magnesium 1.44%, manganese 250 ppm and Zn 315 ppm. Meanwhile, goat manure has nutrients such as nitrogen 2.10%, P_2O_5 0.66%, K_2O 1.97%, calcium 1.64%, magnesium 0.6%, manganese 233 ppm and zinc 90.8 ppm (Andayani and Sarido 2013).

The growth of *A. platensis* in the inorganic fertilized medium and chicken manure added medium increased in the first day to second days, then decreased on the third to fifth day. Meanwhile, for goat manure added medium, the daily growth of *A. platensis* increased on the first up to third days, then decreased on the fourth and fifth days. This life cycle showed that *A. platensis* cultured in inorganic fertilized medium and livestock manure added media needed five days for its life period. Life cycle of *A. platensis* related to the activity of *A. platensis* cell division phase. Whereas the cell division phase indicated the availability of nutrients in the media fulfilling the *Arthrospira platensis*'s life needs.

Muliani et al. (2018) stated that the growth of microalgae is strongly influenced by several factors, including nutrient content in the media, water quality, quantity and quality of microalgae starter. Another study got the similar results to this study, in where the exponential phase of *Arthrospira platensis* occurred on the second day. Furthermore, for the stationary phase, *Arthrospira platensis*'s growth decreased on the third to fifth day which indicated that the nutrients contained in the media had begun to decrease and caused the cell division process to be hampered (Astiani et al. 2016).

Rusydi (2018) revealed that nitrogen and phosphate

contained in animal manure can stimulate the growth kinetics of microalgae and cyanobacteria (including *A. platensis*). Then nitrogen, phosphorus and potassium in livestock manure are very important nutrients for growth of microalgae (Febtisuharsi 2016). The experiment suggests that the use of waste especially livestock waste becomes potential to be explored in microalgae and cyanobacteria development as biodiesel feedstock.

The experiment revealed that different media in cultivation of *A. platensis* significantly affected the biomass production of *A. platensis* ($p < 0.05$) (Figure 3). *Arthrospira platensis* cultured in inorganic fertilized medium produced a distinct biomass compared to that which was cultivated in livestock manure media. The medium composed of poultry manure exhibited the greatest biomass production, measuring 1.76 g/L (dry biomass). The production of biomass by *A. platensis* in a medium containing bovine manure followed. Conversely, inorganic fertilized medium exhibited the least biomass production of *A. platensis*, measuring 1.37 g/L (dried biomass).

The highest biomass production was reached by *Arthrospira platensis* in chicken manure was not different significantly ($p > 0.05$) from *Arthrospira platensis*'s biomass from cow and goat manure added media. In general, livestock manure media could support the biomass production better than inorganic fertilizer medium. Utilization of livestock manures known as poultry waste exactly could optimize the biomass production of *Arthrospira platensis*.

Utilization of wastewater as *Arthrospira platensis*'s medium was also done by Zhai et al. (2017). Their experiment showed that using synthetic municipal wastewater (SWW) could enhance the biomass production of *Arthrospira platensis* in total of number 262.5 mg/L. This strategy was a good choice for optimizing the microalgal production and treating the wastewater in controlled environment Zhai et al. (2017).

The growth of *Arthrospira platensis* in various media during experiment was evaluated for seven days of cultivation. Generally, *Arthrospira platensis* needed five days for its full life cycle and continued to conduct the next life cycle along the availability of nutrients in media. Therefore, limited nutrients in those used media affected the biomass production which was harvested on day 7th. However, chicken manure medium still supplied the enough nutrients supporting the biomass production of *Arthrospira platensis*.

At harvested time (day 7th), *Arthrospira platensis* generally already reached the stationary phase in its life. The absorbance of nutrients during stationary phase influenced the forming new cells of *Arthrospira platensis* to form biomass. This statement was similar to certain study, in where the longer the stationary phase needed by *Arthrospira platensis* in absorbing the nutrient content in the media, the more the amount of biomass will increase (Jati et al. 2012).

Beside of determining the biomass production of

Arthrospira platensis, the evaluation of lipid content during cultivation was also important to be considered for lipid productivity of *Arthrospira platensis*. Analysis of the lipid content was conducted on *Arthrospira platensis* cultured in livestock manures media. In general, daily lipid content of *Arthrospira platensis* suffered fluctuation during seven days of cultivation. The goat manure added medium reached a peak at 4.215% in day 6th and became the highest lipid content among the media else. Lipid content of those *Arthrospira platensis* can be seen on Figure 4.

The potential of goat manure as material for microalgae medium is also revealed by Sopandi et al. (2020). Adding goat compost give an effect to biomass production, in where 25 g/L of goat manure certainly result low biomass and high lipid content of *Arthrospira platensis*. Meanwhile, 100 g/L of goat manure encourage biomass and protein production of *Arthrospira platensis* but its lipid content was lower than was in 25 g/L of goat manure.

Different livestock manures added media resulted different daily lipid content of *Arthrospira platensis*. Daily lipid content curve showed that the lowest lipid content occurred on day 2 until day 5 whereas on those days *Arthrospira platensis* started to grow fastly called exponential phase. Energy obtained from absorbed nutrients was used to form new cells of *Arthrospira platensis* and yielded huge biomass during exponential phase and stationary phase. Availability of abundant nitrogen and other nutrients led growth of cells of *Arthrospira platensis*. Therefore, forming lipid content was less during that time.

In contrast, lipid content was growing up after stationary phase ended, namely on day 5 to day 7. Tendency of forming new cells in stationary phase was low, so energy was accumulated as lipid content in its cells. Rusydi et al. (2015) revealed that the relation of both nitrogen consumption rate and specific growth rate on *Nostoc muscorum* was a linear positive. The decreasing of total lipid in *N. muscorum* implied that there was a good growth of cells of *Nostoc muscorum*. The previous study of Mata et al. (2016) also showed contrast tendency of both biomass and lipid content. Their study resulted that stress condition could induce lipid accumulation in *A. platensis* and *C. vulgaris* but it was also followed by decreasing the biomass productivity of both microalgae.

The next investigation was determining biodiesel yield of *A. platensis* cultured in goat manure added medium in large scale. The experimental result showed that biodiesel yield of *A. platensis* was very low, namely 1.65% from its lipid yield. This result could be caused by low lipid content extracted from *Arthrospira platensis*, therefore fatty acids that could be converted into biodiesel were also low. This result is not in same line with the result revealed by El-Shimi et al. (2013). That study showed that the biodiesel yield of *A. platensis* by in situ transesterification is 84.7%.

Biodiesel yield resulted through transesterification process is actually affected by molar ratio of alcohol, presence of water and free fatty acid content, reaction temperature, catalyst concentration and agitation speed (Mathiyazhagan and Ganapathi 2011). Therefore, low

yield of biodiesel from feedstock *Arthrospira platensis* could be caused by low free fatty acid content in *Arthrospira platensis*'s lipid and presence of hexane as lipid extraction solvent.

Characteristics of fatty acid methyl ester in biodiesel is one of important measurement for suitable biodiesel. Based on this findings, heneicosane became the dominant fatty acid in *A. platensis* based biodiesel with the area 26.4%. Then, it was followed by 9-octadecenoic acid methyl ester (CAS) and linoleic acid ethyl ester with each area 24.92% and 17.47%.

This result was not similar to the findings of El-Shimi et al. (2013). The biodiesel from *A. platensis* had high palmitic acid (C16:0) with percentage 48.35%. Then, the study of (Pradana et al. 2020), the composition of fatty acids in biodiesel produced from *A. platensis* were consisted of high methyl oleate, methyl palmitate, and low methyl stearate.

4. Conclusions

Different media influenced significantly (p -value < 0.05) on peak value of growth and biomass production of *A. platensis*. The livestock manures media supported the better daily growth of *A. platensis* than inorganic fertilized medium. The highest peak value of growth and biomass production were produced by chicken manure added medium. The daily lipid content of *A. platensis* cultivated in livestock manures media showed different day of peak lipid content. The highest peak value of lipid content was showed by *A. platensis* cultured in goat manure added medium. However, the biodiesel yield of *A. platensis* cultivated in goat manure added medium was low. Heneicosane was the most predominant fatty acid in this biodiesel derived from *Arthrospira platensis*.

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Authors' contributions

RR, EA, M designed the study and carried out the experiment. RR and S analyzed the data. RR wrote the manuscript. All authors read and approved the final version of the manuscript.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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