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Lipid production by microalga *Scenedesmus* sp. AARL G022 in the cultivation with effluent from chicken manure biogas plant

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ABSTRACT

Microalgae are potential organisms in biooil production and they can accumulate higher lipid content than other oil plants. However, one of the major problems in algal oil production is its high cost. The aim of this study was to culture *Scenedesmus* sp. AARL G022 with the effluent from chicken manure biogas plant and evaluate its lipid accumulation. The effluent from the biogas plant was used directly or filtered through the water purifier at 6, 12, 25, 50, and 100% concentrations without additional nutrient supplementation. The highest level of growth was obtained when the microalga was cultivated with 12–25% of the non-filtered effluent. The highest level of biomass production involving *Scenedesmus* sp. AARL G022 was selected in order to upscale to a culture volume of 20 L using 12–25% of the non-filtered effluent. It was found that *Scenedesmus* sp. AARL G022 revealed the highest levels of growth as was observed using 25% of non-filtered effluent with chlorophyll *a* and a maximum biomass reading of 3,055.34 ± 331.86 μg mL^{-1} and 0.54 ± 0.06 g L^{-1}, respectively, and a maximum lipid reading of 44.50 ± 4.35 mg L^{-1}. Fatty acid profiles showed that palmitic acid (C16:0) and linoleic acid (C18:2) were the major fatty acids. This study will be useful for the purposes of increasing algal biomass and lipid production, as well as in the reduction of nutrient costs and the amounts of discharged wastewater.

Keywords: Microalgae; Fatty acid; Waste water; Biooil production; Chicken manure biogas

1. Introduction

Microalgae are conceivable as organisms for a lipid source for biodiesel production because of their high lipid content and the fact that the lipids are similar to those found in plants [1]. In addition, microalgae also show many other relevant benefits, for example, some microalgae can be cultivated in wastewater. Hence, they have been determined to be excellent in wastewater nutrient removal [2,3]. Recently, *Scenedesmus* sp. AARL G022 was found to be a promising strain possessing a high growth rate [4], it could also be cultivated under various trophic conditions, including photoautotrophic, chemoheterotrophic, and mixotrophic conditions [5].

However, cultivation of microalgae as feedstock for biodiesel still requires large amounts of water and a high cost of nutrients such as nitrogen, phosphorous, and other trace elements [6]. The US Department

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of Energy has also acknowledged the assistance of wastewater treatment in microalgal biofuel production. The use of wastewater can compensate for the commercial fertilizers required in the cultivation, which could offset high microalgal production costs [7].

Poultry farming is a main food production process. Over 50 billion chickens are cultured annually, for both their meat and their eggs. This industry generates large amounts of wastewater. The USA generates more than 10 million tons of poultry wastewater per year, while about 13 million tons of semi-solid chicken manure were generated annually [8]. Although chicken manure could be used as a substrate for biomethane production, the effluent from biogas production is still known to be rich in the nutrients found in microalgae. Hence, this study investigated the microalgal growth and lipid production from the cultivation of effluent from chicken manure from a biogas plant without other nutrient supplementation. The nutrient removal from this system was also evaluated.

2. Materials and methods

2.1. Preparation of effluent samples

Effluent samples were acquired from a biogas plant located in a chicken farm in Lamphun Province, Thailand. Wastewater used in this study was separated into two portions: non-filtered (NF) and filtered (F) using a three-step water purifier (Microfiltration (pure polypropylene), activated carbon, and 0.3-μm pore sized ceramic). The physicochemical properties of the effluent including color, odor, pH, and conductivity were examined using a multimeter (TI 9000, Trans Instruments, Singapore). Turbidity was determined using a spectrophotometer (DR/2010, HACH Company, USA). Alkalinity, COD, NO_3^--N, NH_4^+-N, and PO_4^{3-}-P were analyzed in accordance with the methods of the American Public Health Association (APHA) [9].

Huai Nam Rin farm is a poultry farm, which generates significant amounts of agricultural wastewater from their farming activities. Although the wastewater was treated by the anaerobic fermentation process involved in biogas production, the discharged water from that process still contains high nutrient levels especially nitrates, ammonia, and phosphates, which are important nutrients for the growth of microalgae. Using wastewater as a nutrient source for microalgal cultivation always involves a concern for its stability, which may reflect the control of the productivity. However, from nearly one year of sample collection, the nutrient concentration levels found in the effluent were fairly stable because the farm production activity was constant over the single-year period of collection, thus this effluent is suitable for use as a nutrient source for microalgae production. Nevertheless, this wastewater showed a high level of turbidity (1202 FAU) from organic residue. The sediment may be a light barrier for microalgal cultivation. Thus, the wastewater was filtered and diluted before being applied to the cultivation process. After filtration, the turbidity was gradually reduced to 35 FAU. However, the filtration also reduced the nutrient contents in the effluent, which remained at approximately 3–9.5% of those in the original unfiltered effluent (Table 1).

2.2. Microalgae

Scenedesmus sp. AARL G022 was obtained from the Applied Algal Research Laboratory, Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand. It was maintained in algal medium (AM) [10], at room temperature under continuous 10.8 μmol photons m^{-2} s^{-1} fluorescent lamp illumination.

2.3. Cultivation of microalgae

Scenedesmus sp. AARL G022 was cultivated in non-filtered and filtered effluent without additional nutrient supplementation and compared with AM. The effluents were diluted with dechlorinated tap water at 6, 12, 25, 50, and 100% concentrations. The inoculum present in the linear growth phase was added to the diluted effluents or AM in 250 mL^{-1} conical flasks. The culture broths were mixed by air bubbles at indoor ambient temperatures under continuous illumination of 40.5 μmol photons m^{-2} s^{-1} with a fluorescent lamp. Samples were collected every 2 d for growth monitoring by the determination of chlorophyll *a* content [11,12].

2.4. Outdoor conditions

The appropriate effluent concentration levels were selected and processed in 20 L of batch cultivation. Outdoor ponds used in this study were made of clear glass and were 22-cm wide, 45-cm long, and 28-cm deep (Fig. 1). The experiment was run with an average daily solar radiation reading of 472.1 μmol photons m^{-2} s^{-1}. The average air temperature was 32.5 ± 2.9°C. Samples were collected every 2 d to monitor the growth in the same way as the indoor cultivation process. Nutrients including, NH_4^+-N, NO_3^--N, PO_4^{3-}-P, and COD were analyzed on the first day and the last day of the cultivation process.

Table 1
Physical and chemical properties of effluent from a chicken manure biogas plant

Properties of effluent	Non-filtered wastewater	Filtered wastewater
Color	Opaque brown	Clear yellow
Odor	Strong smell of hydrogen sulfide	Odor decrease
pH	6.75 ± 0.00	7.64 ± 0.00
COD (mg L^{-1})	1,031.47 ± 21.00	98.27 ± 6.53
NO_3^--N (mg L^{-1})	20.00 ± 8.16	0.60 ± 0.04
NH_4^+-N (mg L^{-1})	392 ± 3.27	26.00 ± 0.82
PO_4^{3-}-P (mg L^{-1})	207.67 ± 16.74	15.43 ± 0.43
Alkalinity (mg L^{-1})	3,273.33 ± 36.82	294.00 ± 16.33
Conductivity (μs cm^{-1})	4,383.33 ± 12.47	409.33 ± 5.91
Turbidity (FAU)	2,074.00 ± 11.43	26.00 ± 0.00

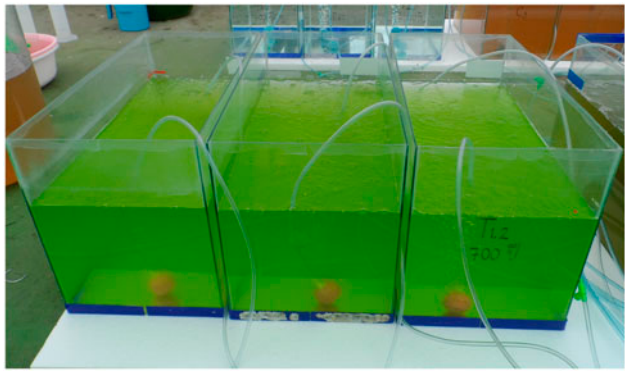

Fig. 1. Photograph of bench scale outdoor open ponds.

2.5. Algal biomass production and lipid extraction

At the end day of cultivation, 20 mL of the sample was filtered through Whatman's GF/C glass fiber filters, dried at 60°C for 48 h, left in a vacuum desiccator to be cooled, and weighed to determine biomass levels. Total lipids were extracted according to a modified method of Bligh and Dyer [13]. Pre-weighted dry microalgal biomass was sonicated for 1 h in chloroform–methanol (2:1, v/v). The chloroform layer was collected and evaporated to complete dryness at room temperature. The percentage of lipid content was calculated according to the following equation (Eq. (1)):

$$\text{Lipid percentage } (\%) = \frac{(W_2 - W_1) \times 100}{S} \quad (1)$$

where W_1 is the weight of the centrifuge tube before lipid extraction, W_2 is the weight of the centrifuge tube after lipid extraction, and S is representative of the sample (g). The lipid productivity (mg L^{-1}) was obtained from the following equation (Eq. (2):

$$\text{Lipid production } (\text{mg L}^{-1}) = \frac{\%\ \text{LP} \times \text{DW}}{100} \quad (2)$$

where % LP represents lipid percentage and DW represents dry weight of biomass (g L^{-1})

2.6. Fatty acid composition analysis

Crude lipids obtained from the cultivation with 25% NF were analyzed using a gas chromatograph (GC 7890A, Agilent Technology, USA) that was equipped with a flame ionization detector. All operations were conducted under low light conditions and with the protection of helium. Separation was achieved on a HP-5MS capillary column (0.25-mm × 0.25-mm ID × 0.25-μm film thickness) with helium as the carrier gas at a flow speed of 0.1 mL min^{-1}. One microliter of sample solution was injected in split mode (split ratio = 25:1) for each analysis. The injector temperature of the machine was set to 250°C, and the column temperature program was set as follows: the initial temperature was 100°C; then it rose to 250°C at a rate of 3°C min^{-1}; the final column temperature was maintained at 250°C for 53 min. Fatty acid methyl ester was identified by chromatographic comparison with authentic standards (Sigma Chemical Co., St. Louis, MO).

3. Results and discussion

The cultivation of microalga in the different concentrations of filtered and non-filtered effluent showed that the highest levels of chlorophyll *a* were found in 25% of non-filtered effluent (NF 25%) (Fig. 2(A) and (B)). However, the maximum readings of dry weight of *Scenedesmus* sp. AARL G022 were obtained from

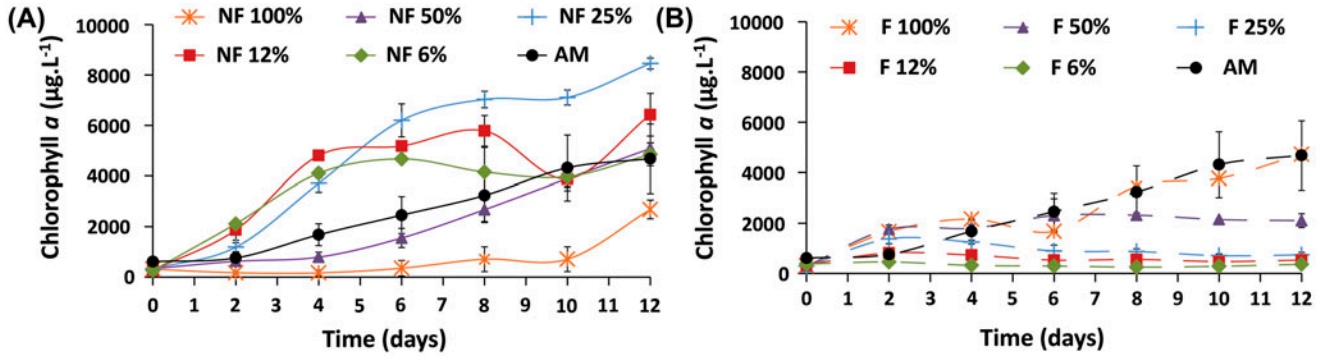

Fig. 2. Growth of *Scenedesmus* sp. AARL G022 in effluent acquired from a chicken manure biogas plant: (A) compared AM with a non-filtered wastewater (NF) and (B) compared AM with filtered wastewater (F).

the cultivation in NF 12–NF 25% (Fig. 3). This should be because the filtered effluent contained lesser nutrient concentration levels, while NF 50–NF 100% contained higher levels of both nutrients and turbidity. The excess of nutrients could suppress growth, while turbidity from the suspended solids caused the problem of light penetration for photosynthesis [14]. Thus, NF 12–NF 25% was selected to be studied in the outdoor large-scale cultivation study in order to optimize the suitable concentrations of effluent for *Scenedesmus* sp. AARL G022.

After the cultivation of this microalga using effluent from a biogas plant was determined to be successful on the laboratorial scale, the cultivation for lipid production was attempted in outdoor cultivation. The cultivation of *Scenedesmus* sp. AARL G022 in 20-L batch open ponds with 12–25% non-filtered effluent revealed that the highest chlorophyll *a* contents were recorded at NF 12–NF 25% and were not found to be significantly different (Fig. 4). However, the biomass and lipid productivity of *Scenedesmus* sp. AARL G022, when cultivated with NF 25%, were found to be significantly higher than those of NF 12% (Table 2). Since, the amount of nutrients in NF 25% (NO_3^- 25.33 mg L^{-1}, NH_4^+ 82.93 mg L^{-1}, PO_4^{3-} 32.6 mg L^{-1}) were close to those of the general medium for microalgae, such as AM, the amount of nutrients in NF 12% were only half of the NF 25%. In addition, although NF 12% also revealed less turbidity than NF 25%, in the outdoor cultivation, the illumination from sunlight was strong enough to penetrate the turbidity of NF 25%. It could provide sufficient energy to microalgal cells and result in higher biomass production and % lipid content. However, % lipid of *Scenedesmus* sp. AARL G022 in this study (7.32–8.43%) was not high when compared with that of other reports (19–35%) (Table 2). Several studies have reported on the cultivation of *Scenedesmus* spp. with various types of wastewater such as municipal wastewater [15–19], piggery wastewater [20,21], and food wastewater [22].

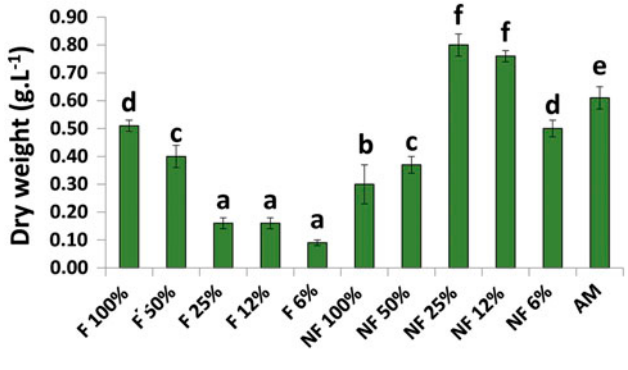

Fig. 3. Dry weight (g L^{-1}) of microalgal cultivated in filtered and non-filtered effluent at different concentrations compared with AM. Letters indicate statistical significance at $p < 0.05$ using one-way ANOVA with *post hoc* Duncan's multiple-range test.

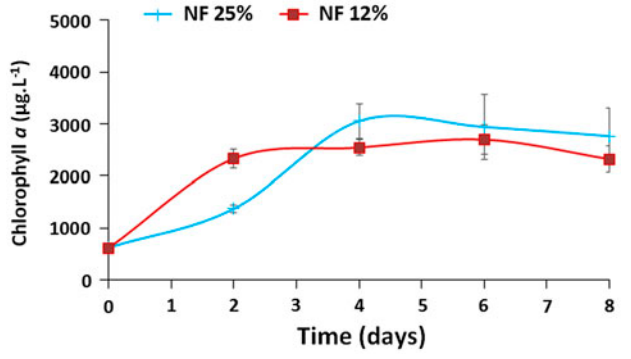

Fig. 4. Growth of *Scenedesmus* sp. AARL G022 in effluent acquired from a chicken manure biogas plant (NF 12–NF 25%).

Table 2
Dry weight (g L^{-1}), percentage of lipid, and total lipid (mg L^{-1}) from microalgal cultivated in non-filtered effluent at different concentrations, compared with other wastewaters

Microalgae	Type of wastewater	Dry weight (g L^{-1})	Lipid (%)	Total lipid (mg L^{-1})	Cultivation reactors	Refs.
Scenedesmus obliquus HM103382	Municipal wastewater	0.26 ± 0.03	19	50 ± 10	200-mL serum bottles	Abou-Shanab et al. [15]
Scenedesmus acutus	Municipal wastewater	1.179	28.3	333.66	1,000-mL flask	Sacristán de Alva et al. [16]
Scenedesmus bijuga	Municipal wastewater + 1/10 diluted food wastewater effluent, Centrifuged + (mixed, filtrated + autoclaved)	1.49	35.06	522.39	500-mL Flask	Shin et al. [17]
Scenedesmus obliquus	Municipal wastewater, filtrated + 15% CO_2	0.31	27	83.7	200-mL serum bottles	Ji et al. [18]
Scenedesmus obliquus	Municipal wastewater + 2% food wastewater filtrated + 10% CO_2	0.31	23.3	72.23	300-mL serum bottle	Ji et al. [19]
Scenedesmus obliquus YSW-14	Piggery wastewater	0.24	27 ± 3	64.8	400-mL flask	Ji et al. [20]
Scenedesmus spp.	10% Bold basal medium + 3% swine wastewater	0.197 ± 4	0.9	1.773	1,000 mL Flask	Kim et al. [21]
Scenedesmus obliquus	Bold basal medium + 3% food wastewater + 5.1% CO_2	0.2	20.8	41.6	300-mL serum bottles	Ji et al. [22]
Scenedesmus sp. AARL G022	NF 25%	0.54 ± 0.06*	8.43 ± 0.51	44.5 ± 4.35*	20,000-mL outdoor pond	This study
Scenedesmus sp. AARL G022	NF 12%	0.42 ± 0.05*	7.55 ± 0.80	31.38 ± 1.71*	20,000-mL outdoor pond	This study

However, those reports provided higher % lipid of *Scenedesmus* spp., whereas they are only a small-trail laboratorial system. Rather than nutrients, physical factors such as the mixing rate and illumination also affect the productivity when up-scaling [23]. In addition, CO_2 supplementation could support the high growth of *Scenedesmus* spp. as well as high % lipid [18,19,22]. Thus, to improve the level of productivity of the cultivation with effluent from chicken manure biogas plant, large-scale cultivation system designs and cheap source CO_2 supplementation should be of concern.

To evaluate the feasibility of producing biodiesel from *Scenedesmus* sp. AARL G022 grown in NF 25%, the lipids from this microalga were examined for its fatty acid profile using GC compared with those of AM. It was found that the major fatty acids in both samples were in the range of C16–C18 with slight diversity (Table 3). The major fatty acids in NF 25% were C16:0, followed by C18:2, while those of AM were C16:0, followed by C18:1, and C18:2, respectively. In addition, the % of unsaturated fatty acids (USFA) in AM was higher than that of NF 25%. This may be due to the different nutrient levels in each medium. Fatty acid compositions such as length and % USFA were influenced from nutrient alteration [24]. Oleic acid (C18:1) is one of the preferred ingredients in the biodiesel production for its oxidative stability and low temperature operability [25]. Although AM showed higher oleic acid than NF 25%, other fatty acids also affected the biodiesel quality and should be concerned. For biodiesel production, a high ratio of saturated fatty acids (SFA) is favored because it makes product higher oxidative stability, higher thermal stability, and higher ignition quality (cetane number) [26]. While USFAs were used to determine oxidative stability of the biodiesel fuels product, higher USFAs indicate to the lesser oxidative stability [27]. On the other hand, kinematic viscosity, cold filter plugging point, and density are largely influenced by the amount of USFA [28]. Therefore, fatty acid of NF 25% which showed equivalent ratio of SFA and USFA may have the best characteristic of both oxidative stability and low temperature fluidity. These results were

Table 3
Fatty acid profile derived from cultivation of microalgae, *Scenedesmus* sp. AARL G022 with NF 25% and AM

Fatty acid	NF 25% (Crude lipid %)	AM (Crude lipid %)
C16:0	42.8	34.1
C16:1	nd	1.97
C18:0	nd	3.28
C18:1	1.18	33.9
C18:2	36.61	12.68
C18:3	–	0.48
Others	19.41	4.67
Saturated	42.8	39.23
Unsaturated	37.79	60.59

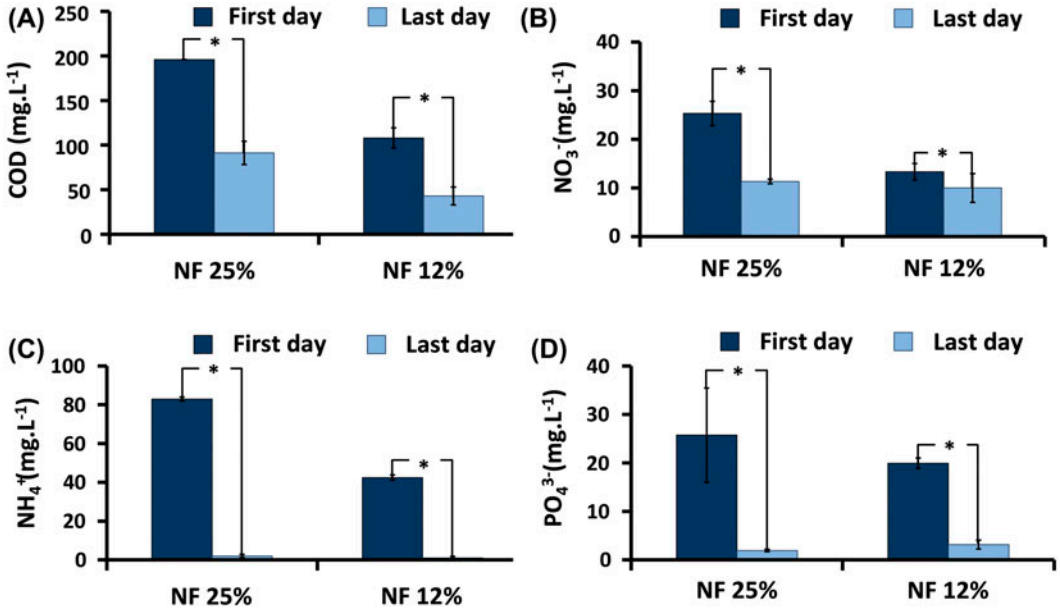

Fig. 5. The nutrient concentration: (A) COD, (B) NO_3^--N, (C) NH_4^+-N, and (D) PO_4^{3-}-P on the first day and last day of the cultivation process.
Note: *indicates statistical significance at $p < 0.05$ using *t*-test.

somewhat similar with those of the research of Zhou et al. [29] who cultivated *Scenedesmus* UM258 and UM284 in municipal wastewater, and who found that the major fatty acids present in UM258 were 18:1, 16:0, and 18:2, and in UM284 were 18:3, 16:0, and 18:2, respectively. The major fatty acids found in *Scenedesmus* sp. AARL G022 were similar to those present at other biodiesel raw material plants such as those involved with palm oil [30] and Jatropha oil production [31]. Hence, lipids from *Scenedesmus* sp. AARL G022 may have a considerable potential to be applied as a raw material for biodiesel production.

The purpose of using the effluent from the biogas plant was not only done to reduce the cultivation costs, but also to reduce the amount of discharged pollutants that occurred in the process. The cultivation of *Scenedesmus* sp. AARL G022 with NF 12% and NF 25% could remove COD by about 53–60% (Fig. 5(A)). The remaining CODs are lower than the legal values allowed by Thailand for discharge into the environment, which should not be over 300 mg L^{-1}. When considering the process that is involved with nutrient removal, nitrates (NO_3^--N) were removed by 25–55% of their initial level from the cultivation with NF 12–25%, respectively (Fig. 5(B)), while ammonium (NH_4^+-N) removal was found to be over 90% regardless of its initial concentration level (Fig. 5(C)), which is similar to the findings presented in other research

studies such as those by Martinez et al. [32] and Woertz et al. [33]. Orthophosphate (PO_4^{3-}-P) removal reached 84–92% (Fig. 5(D)), which was also observed by Woertz [34] who achieved around 76% phosphate reduction when treating digested dairy manure with microalgae. However, the wastewater in this study was not sterilized. Therefore, the wastewater could be tainted by the contamination of other micro-organisms such as ammonium-oxidizing bacteria and nitrite-oxidizing bacteria, along with other micro-organisms which may have been involved in the overall nutrient reduction that occurred in the wastewater [35].

4. Conclusion

This work presents the high potential for the cultivation of *Scenedesmus* sp. AARL G022 in the effluent from chicken manure in a biogas plant without other nutrient supplementation for lipid production. The high levels of growth and lipid productivity, and lower cost of nutrients were found when the substance was cultivated with 25% of non-filtered wastewater. This study will be useful for the purposes of increasing algal biomass volume and reducing production costs, and the discharge of wastewater. The acquired knowledge from this research study could be useful for the future development of microalgal cultivation with regard to alternative energy sources.

Acknowledgments

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