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The Crucial Impact of Microbial Growth and Bioenergy Conversion on Treating Livestock Manure and Antibiotics Using *Chlorella sorokiniana*

Hee-Jun Kim [1,2,†], Sangjun Jeong [1,3,†], YeonA Lee [4], Jae-Cheol Lee [5,6] and Hyun-Woo Kim [1,4,*]

[1] Department of Environmental Engineering, Soil Environment Research Center, Jeonbuk National University, 567 Baekje-daero, Deokjin-gu, Jeonju-si 54896, Republic of Korea; heejun.kim@kitox.re.kr (H.-J.K.); tkdwns1264@naver.com (S.J.)
[2] Environmental Fate and Exposure Research Group, Korea Institute of Toxicology, Jinju-si 52834, Republic of Korea
[3] Water Environmental Research Center, Jeonbuk National University, 109, Ballyong-ro, Deokjin-gu, Jeonju-si 54896, Republic of Korea
[4] Department of Environment and Energy (BK21 Four), Jeonbuk National University, 567 Baekje-daero, Deokjin-gu, Jeonju-si 54896, Republic of Korea; lee98@jbnu.ac.kr
[5] Division of Environmental Materials, Honam National Institute of Biological Resources (HNIBR), Mokpo 58762, Republic of Korea; jc.lee@hnibr.re.kr
[6] Department of Environmental Engineering, School of Architecture, Civil and Environmental Engineering, Mokpo National University, Mokpo 58554, Republic of Korea
* Correspondence: hyunwoo@jbnu.ac.kr; Tel.: +82-63-270-2446
† These authors contributed equally and are considered joint co-first authors.

Abstract: The residual antibiotics in livestock excreta (LE) have been regarded as a potential threat to the ecosystem and human society. Some photoautotrophic microalgae, however, were found to metabolize them during active biomass photosynthesis. This study investigates how the strength of the antibiotics impacts the overall biodiesel yield and composition of the harvested microalgal biomass grown from LE. The microalgal growth results demonstrate that increasing the concentration of residual antibiotics suppresses the microalgal growth rate from 0.87 d^{-1} to 0.34 d^{-1}. This 61% lower biomass production rate supports the proposition that the kinetic impact of antibiotics may slow lipid synthesis. Moreover, the analytical results of fatty acid methyl ester (FAME) demonstrate that amoxicillin substantially reduces the C16:0 content by over 96%. This study evidences that the functional group similarity of amoxicillin may competitively inhibit the esterification reaction by consuming methanol. This explanation further highlights that residual antibiotics interfere with microalgal lipid synthesis and its transesterification. Moreover, it was confirmed that the presence of residual antibiotics may not affect the major nutrient removal (total nitrogen: 74.5~78.0%, total phosphorus: 95.6~96.8%). This indicates that residual antibiotics inhibit the metabolism associated with carbon rather than those associated with nitrogen and phosphorus, which is connected to the decrease in the biodiesel yield. Overall, these results reveal that the frequent abuse of antibiotics in livestock may harm the eco-friendly conversion of waste-into-bioenergy strategy.

Keywords: livestock excreta; microalgae; antibiotics; biodiesel; bioenergy conversion

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1. Introduction

In recent decades, factory farming has caused various environmental problems in the development of the livestock industry because it is inevitably associated with the large-scale generation of livestock excreta (LE) [1,2]. In addition, the feed can influence not only the quality of the livestock but also the characteristics of the LE [3,4]. The LE contains not only well-known environmental pollutants but also residual pharmaceuticals that can be harmful to the ecosystem and human health [5]. Due to the continuous abuse of antibiotics,

they enter receiving water in the form of point or non-point source pathways [6]. Unlike other pollutants, they are present at very low concentrations, but even at the parts per billion (ppb) levels, they may cause bioaccumulation and toxicity in living organisms [6,7]. Moreover, LE treatment cannot be free from problems such as eutrophication, antibiotic-resistant bacteria, antibiotic-resistant gene transfer, greenhouse gases, and odor [8,9].

Antibiotics are commonly overused on most farms for excessive disease prevention, growth promotion, and efficient management of livestock's productivity [10,11]. Among the well-known antibiotics, amoxicillin (AMX) is a penicillin antibiotic with significant broad-spectrum and semi-synthetic characteristics [12]. AMX belongs to the beta-lactam group of antibiotics, which is active against a wide spectrum of Gram-positive bacteria [13]. AMX in the ecosystem, however, may increase the spread of antibacterial-resistant genes and ultimately result in the reproduction of beta-lactam-resistant bacteria [14]. Furthermore, long-time exposure to AMX may lead to liver injury, which is stimulated by amoxicillin-clavulanate-acid-secreted IFN-γ [15]. If AMX remains in the water environment, it poses a potential threat to both the ecosystem and human health [16–18]. Therefore, it is necessary to eliminate AMX in a highly efficient and sustainable way to minimize its potential hazard to humans. Additionally, antibiotics may potentially reduce the treatment efficiency of pollutants in the wastewater treatment process [19]. Moreover, due to their complex chemical structures, physico-chemical processes may be necessary for effective treatment [20].

From a viewpoint of waste-into-energy conversion and the circular economy, however, LE may be a valuable resource for renewable and sustainable energy that can contribute to replacing fossil fuels [21,22]. Thus, intensive research on biofuel (e.g., biodiesel, biogas, and bio-alcohols) generation from LE has been conducted internationally [23,24].

Although using microalgae could significantly remove both the total nitrogen and total phosphorus from LE while recovering the resources of bioenergy and biomass [25,26], traditional wastewater treatment facilities have shown a limited capability to treat residual pharmaceuticals [27]. Previous reports on microalgal treatment suggest that bioremediation of antibiotics is possible as an ecologically broad and sustainable approach which is gaining scientific attention [28].

Among various microalgae, *Chlorella* spp. are suitable species that can treat LE and antibiotics; however, the previous focus has been to verify biomass productivity [29]. In contrast to the effect of antibiotics on biomass, their impact on the bioenergy yield or components remains largely unknown. Hence, this study tests a microalgal treatment using *C. sorokiniana* to reduce the contaminants in LE together with antibiotics. Experiments were designed to confirm how the strength of the antibiotics affects the photosynthesis of *C. sorokiniana* and what factors causes the deterioration in the biodiesel potential. Specifically, the experiments were designed to evaluate the growth kinetic constants of *C. sorokiniana* according to the amount of AMX, to reveal the inhibitory mechanisms associated with AMX affecting nutrient and antibiotic removal, and to identify the causes that change the biodiesel yield and its components according to the antibiotics dose.

2. Materials and Methods

2.1. Inoculum and Culture Conditions

This study used *C. sorokiniana* as the inoculum, which was obtained from the Korean Collection for Type Cultures (KCTC). It has been reported that *C. sorokiniana* easily adapts to various environmental conditions and reduces the total nitrogen and total phosphorus significantly in wastewater [30]. The medium used for culturing the microalgae was BG-11, which is an artificial medium commonly used for culturing microalgae [31]. The composition of BG-11 is as follows based on 1.0 L: 1 mg EDTA disodium salt, 40 mg $K_2HPO_4 \cdot 3H_2O$, 6 mg citric acid, 1.5 g $NaNO_3$, 36 mg $CaCl_2 \cdot 2H2O$, 75 mg $MgSO_4 \cdot 7H_2O$, 6 mg ferric ammonium citrate, 20 mg $NaCO_3$, and 1 mL mixed trace metal solution. Each liter of trace metal solution contained 49 mg $Co(NO_3)_2 \cdot 6H_2O$, 2.9 g H_3BO_3, 1.8 g $MnCl_2 \cdot 4H_2O$, 0.39 g $NaMoO_4 \cdot 2H_2O$, 79 mg $CuSO_4 \cdot 5H_2O$, and 0.22 g $ZnSO_4 \cdot 7H_2O$.

Th microalgae culture was carried out under the following conditions: the temperature was 28 °C, the light cycle ratio was light/dark = 16:8, the light intensity was 180 μmol/s·m^2, and it was incubated for a total of 6 days in a batch reaction. The cultured microalgae biomass was harvested using centrifugation at 8000 rpm for 10 min using a centrifuge. The harvested biomass was lyophilized for 96 h at −40 °C and 5 m Torr using lyophilization because the moisture content reached about 80 wt%.

2.2. Livestock Excreta Characteristics and Antibiotics

The LE used for the microalgae cultivation was obtained from a pig farm in K-City of Korea. The LE was sourced from a factory pig farming facility with a liquid manure handling system. We performed the collection of the livestock excreta (20 L) on a clear day with a temperature of approximately 25 °C. The initial characteristics of the LE are shown in Table 1. The antibiotic, used to achieve the purpose of this study, was ≥900 μg/mg AMX (Sigma-Aldrich, St. Louis, MO, USA). In this study, a large amount of amoxicillin (0.01 to 20 ppm) was tested to confirm its negative effects on the biological treatment of the LE according to the microalgal growth kinetics, biomass yield, and composition of biodiesel.

Table 1. Initial characteristics of LE.

Characteristics	Unit	Value
pH	-	7.5
CODcr (Chemical Oxygen Demand)	mg COD/L	280
T-N (Total Nitrogen)	mg N/L	120
T-P (Total Phosphorus)	mg P/L	1.5

2.3. Biodiesel Production from the Microalgae Biomass

The experimental method used for the biodiesel production in this study is direct transesterification (DT). First, lipids are extracted from the microalgae biomass by destroying the cell walls using physical, chemical, and biological methods [32]. Next, the extracted lipid is transesterified with alcohol in the presence of a catalyst to obtain methyl ester and glycerol. Finally, the final reactant is centrifuged to produce high-purity free fatty acid methyl ester (FAME).

In this study, two homogeneous catalysts (HCl and NaOH) were used to produce high-quality biodiesel of the same concentration (0.5 M based on methanol). The applied temperature varied based on the characteristics of the catalysts reported previously, meaning the acid catalyst (HCl) was optimized at a higher temperature (90 °C), while the base catalyst (NaOH) was optimized at a lower temperature (25 °C) [33]. The ratio of the biomass, catalysts, and n-hexane was adjusted to 1 g, 10 mL, and 10 mL, respectively. For the biomass, the microalgae were cultured using artificial growth medium (BG-11), the LE, and the LE containing antibiotics. At each temperature condition, the catalyst and the microalgal biomass were put into a test tube and mixed for 1 h for the DT reaction. After the reaction was completed, the FAME was extracted from the n-hexane layer. A flow chart of the overall DT process is shown in Figure 1.

The yield of FAME was calculated by dividing the total mass of the FAME by the total mass of the dried microalgae biomass. The yield of FAME was calculated using Equation (1) below [34].

$$\text{FAME yield } (\%) = \frac{\text{Total mass of FAME } (\text{g})}{\text{Total mass of dried microalgae biomass } (\text{g})} \quad (1)$$

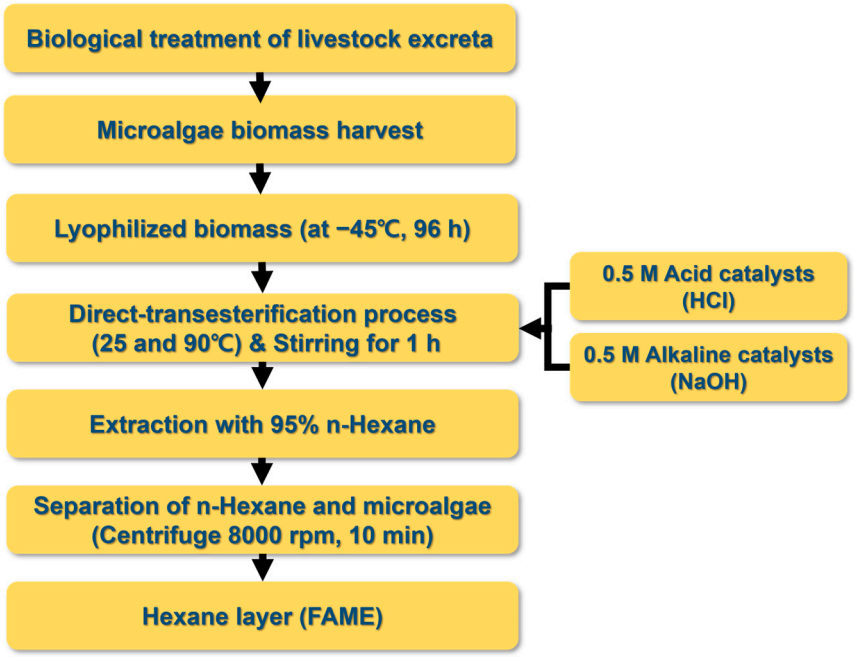

Figure 1. Experimental procedures of biodiesel production.

2.4. Analytical Methods for Water Quality and Fatty Acid Methyl Ester (FAME)

The water quality characteristics of the wastewater influent and effluent were analyzed following the standard methods. We employed the Standard Methods for the determination of total nitrogen (T-N) and total phosphorus (T-P): 4500 NC (persulfate digestion) and 4500 PE (ascorbic acid), respectively [35]. The chlorophyll-a (chl-a) concentrations were analyzed using a method based on acetone extraction using spectrophotometry.

The analytical parameters for the LC-ESI/MS/MS analysis of amoxicillin were established employing an LC-ESI/MS/MS Triple Quadrupole (6410 LC/MS/MS, Agilent, Santa Clara, CA, USA) equipped with HPLC and an electrospray ionization source (Agilent Technologies). The mobile phase comprised a blend of 0.1% formic acid in distilled water and 0.1% formic acid in acetonitrile (ACN), employed on a Synergi Hydro-RP 80 Å (150 × 2 mm) (97:3, *v*/*v*) and introduced into the system at a flow rate of 0.2 mL/min. The column oven temperature was 30 °C and the sample injection volume was 5.0 µL. Mass spectrometric detection was performed using a series 6410 LC-MS/MS Triple Quadrupole (Agilent Technologies) using multiple reaction monitoring.

The yield and composition of the extracted FAME were analyzed using a gas chromatograph (GC) (GC 2020, Shimadzu, Kyoto, Japan). The GC was equipped with a flame ionization detector (FID) and an SPTM-2330 capillary column (30 m × 0.25 mm × 0.20 µm; Sigma-Aldrich, USA). The methods used for the analysis are as follows: (1) Helium was used as the carrier gas (constantly 1 mL/min and the split ratio was 10:1); (2) The temperature of the FID detector and injector was set to 240 °C; (3) The oven temperature was programmed from 140 °C to 220 °C at a rate of 4 °C/min. The peak was interpreted based on the FAME mixture (CRM 18918, Supelco, Bellefonte, PA, USA) from C8:0 to C24:0.

3. Results and Discussion

3.1. Negative Effect on the Microalgae Growth of AMX

Figure 2 presents the change in chl-a, T-N, and T-P at the various concentrations of AMX while treating the LE. It was observed that the control experiment with no AMX showed an exponential growth pattern for chl-a. When the residual AMX concentration varied from 0.01 to 20 mg/L, the amount of chl-a was higher than the control for 4 days. On the fifth day, that of the control exceeded all the reactors due to the active exponential growth phase. This higher chl-a content in the initial startup periods implies that AMX

might stimulate the growth of *C. sorokiniana*, which uptakes AMX according to bioaccumulation and bioadsorption mechanisms [36], and the transferred AMX must have caused a hormetic effect [37]. It seems that the stimulation may not last longer than 5 days, possibly due to the increasing oxidative stress within algal cells induced by AMX, which leads to the overturn in the chl-a content [38,39].

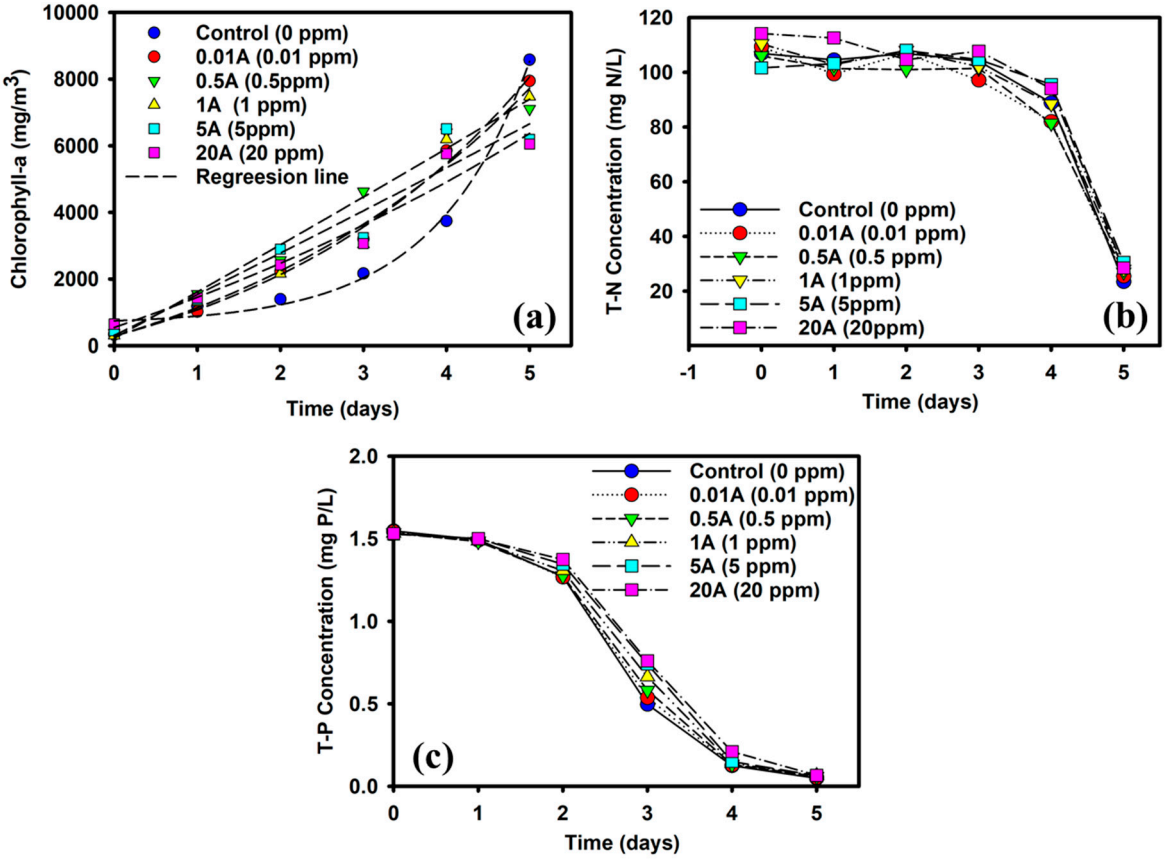

Figure 2. Variations in (**a**) chlorophyll-a, (**b**) T-N, and (**c**) T-P according to the change in antibiotic concentration in the microalgal treatment of LE.

Table 2 compares the final chl-a content, average daily growth rate, and specific growth rate of each experiment quantitatively after 5 days of culturing. The chl-a content of the control increased from 327.7 mg/m^3 to 8583 mg/m^3 while that of 0.01~20 ppm AMX increased to 7947~6052 mg/m^3. Compared to the control, the results evidence that the photosynthesis is inhibited more significantly by the end as the concentration of residual antibiotics increases. This is consistent with a previous study that revealed the metabolic inhibition of AMX in microalgae [40]. The specific growth rate obtained from a regression of growth pattern further supports the overall inhibitory effect of AMX. In the control, the specific growth rate was 0.87 days^{-1}. However, the rates gradually reduced to as low as 0.34 days^{-1} as the concentration of residual AMX increased from 0.01 to 20 ppm.

AMX, known as a beta-lactam antibiotic, can disrupt penicillin-binding proteins and interfere in the biosynthesis of the cell walls, causing osmotic rupture of the microalgae [41]. Also, beta-lactams significantly inhibit the growth and physiological processes of the algae by disturbing the primary photochemistry, photophosphorylation, electron transport, and carbon assimilation [42], which can lead to a reduction in biomass growth, a reduction in various syntheses [43,44], and even cell death [45]. Thus, the experimental results evidence that as the concentration of residual antibiotics increases, this reduces the microalgal growth rate by ~26.1% according to the prescribed inhibition mechanisms. These decreasing trends are consistent with those of other studies, which presented more significant inhibition by 25.6~79.9% in the biomass growth due to AMX [40,46].

Table 2. Chl-a concentration and specific growth rate according to residual AMX strength.

AMX Strength (mg/L)	Initial Chl-a (mg/m^3)	Final Chl-a (mg/m^3)	Average Daily Microalgae Growth (mg/m^3/Day)	Specific Growth Rate ($Days^{-1}$)
0	328 [1]	8583	1651	0.87
0.01		7947	1519	0.46
0.5		7108	1345	0.35
1		7475	1435	0.43
5		6194	1150	0.34
20		6052	1080	0.35

[1] Initial average chl-a value of control experiment. Standard deviation was ±56 mg/m^3.

Overall, despite the initial growth stimulation, these results indicate that residual AMX may deteriorate the treatment performance of LE due to unavoidable inhibition, leading to decreased photoautotrophic cell synthesis.

3.2. Nutrient and AMX Removal in Microalgae Treatment

3.2.1. Effect of AMX on T-N Removal

Figure 2b shows the T-N reduction according to the residual antibiotic concentration. Table 3 tabulates the initial and final contents of T-N and the corresponding T-N removal efficiency in each experiment. The monitored T-N removal efficiency ranges from 78.0 to 74.5%. It was revealed that variation in the residual AMX concentration demonstrated no significant change in the removal efficiency. This indicates that *C. sorokiniana* actively assimilates nitrogen via its photoautotrophic metabolism despite the existence of AMX [47]. This non-correlated T-N removal means that AMX has little impact on the microalgal absorption of T-N despite the restriction of microalgal growth, and the free ammonia level of this study seems much lower than that of the inhibition level. This is consistent with previous batch studies which demonstrated an approximate 98% nitrogen removal, a result achieved without the influence of specific antibiotic inhibition [48,49]. Previous studies have also indicated that existing co-substrates, which are plentiful in livestock excreta, may enhance the degradation of AMX and promote the synthesis of proteins associated with T-N absorption [41].

Table 3 compares the nitrogen removal efficiency according to the microalgal species and the existence of antibiotics. Despite the presence of antibiotics, the T-N removal efficiency of the microalgae was maintained higher than seen in the literature [50]. Other studies showed a high nitrogen treatment efficiency even when wastewater contained the antibiotics AMX and sulfamethoxazole (SMX) but showed a longer operation period (7~18 days), and the initial concentration (45~55.4 mg N/L) was also lower than our study [43,49]. Also, microalgae without antibiotics presented a relatively similar or lower removal efficiency (29.4~70.4%) although the treatment time was longer (7~10 days) [50–53]. Overall, T-N removal by *C. sorokiniana* is not significantly affected by AMX even at a concentration of 20 mg/L. It seems that the interactions related to the co-substrates influence the maintenance of photosynthetic activity if the free ammonia level is kept below the inhibition level [47,54].

3.2.2. Effect of AMX on T-P Removal

Figure 2c shows the T-P reduction for each AMX concentration. And Table 4 shows the initial, final, and corresponding removal efficiency according to the experimental conditions. The exact initial T-P concentrations were 1.53~1.55 mg P/L and the final concentrations were 0.04~0.07 mgP/L. The T-P removal efficiency reached as high as 95.6~96.8%. Although a slight decrease (about 1.2%) in the overall T-P removal efficiency was seen, AMX seems to have an effect on the T-P removal because the maximum removal rate of T-P at around day 2~3 decreased from 0.77 to 0.61 mg P/L/day. This seems to be associated with microalgae growth inhibition, but the consequences on the whole seem negligible.

Table 3. T-N concentration and removal efficiency according to residual antibiotic concentration.

Microalgae	Antibiotic Type	Antibiotic Strength (mg/L)	Initial T-N (mg N/L)	Effluent T-N (mg N/L)	Removal Efficiency (%)	Operation Period	Reference
Chlorella sorokiniana	AMX	0	106.9	23.5	78.0	5	This study
		0.01	109.4	25.5	77.0		
		0.5	106.0	27.0	76.0		
		1	110.4	28.0	75.2		
		5	101.7	30.5	74.6		
		20	114.2	28.5	74.5		
Chlorella vulgaris and *Scenedesmus dimorphus*	-	-	-	-	70.4	10	[51]
Chlorella vulgaris and *Scenedesmus dimorphus*	-	-	-	-	64.5	10	[52]
Chlorella sorokiniana	-	-	214.9	150.3	29.4	7	[50]
Chlorella vulgaris	-	-	113.3	51.8	54.3	7	[53]
Chlorella vulgaris	SMX	0.5	55.4	0.84	98.5	7	[49]
Chlorella regularis	AMX	3	45	7.92	82.4	18	[43]

Table 4 compares the T-P treatment results with previous studies to confirm the applicability of the microalgal leachate treatment. Including the results of this study, antibiotic application cases (95.6~98.7%) [43,49] show a better T-P removal efficiency than those without antibiotics (37.0~79.7%) [51,55–57]. The better removal of nutrients seems to be attributed to either the rapid absorption for the initial stimulation of growth or the enhanced photosynthesis of existing co-substrates, even though we admit that the microalgae, wastewater source, and antibiotics used were different.

Table 4. T-P concentration and removal efficiency according to residual antibiotic concentration.

Microalgae	Antibiotic Type	Antibiotic Strength (mg/L)	Initial T-P (mg P/L)	Effluent T-P (mg P/L)	Highest Removal Rate (mg P/L/day)	Removal Efficiency (%)	Operation Periods	Reference
Chlorella sorokiniana	AMX	0	1.55	0.04	0.77	96.8	5	This study
		0.01	1.54	0.05	0.73	96.5		
		0.5	1.54	0.06	0.69	96.1		
		1	1.53	0.06	0.64	95.8		
		5	1.53	0.06	0.60	95.8		
		20	1.53	0.07	0.61	95.6		
Chlorella sp.	-	-	57.3	18.1	0.89	68.4	45	[56]
Asterarcys quadricellulare	-	-	0.40	0.20	-	50.0	8	[55]
Neochloris aquatica	-	-	0.40	0.25	-	37.0		
Chlorella vulgaris and *Scenedesmus dimorphus*	-	-	-	-	-	79.7	10	[51]
Chlorella vulgaris and *Ganoderma lucidum*	-	-	-	-	-	70.3	10	[57]
Chlorella vulgaris	SMX	0.5	27.2	0.41	3.82	98.5	7	[49]
Chlorella regularis	AMX	3	9.1	0.12	-	98.7	18	[43]

3.2.3. Reduction of Amoxicillin

Figure 3 shows the variation in AMX according to different initial concentrations. Regardless of the concentrations, the AMX content rapidly decreased down to the detection limit (0.001 mg/L) within a day. It is well known that antibiotics can be removed by microalgae via various mechanisms, which include adsorption [58], bioaccumulation [59], biodegradation [60], photolysis [61], and hydrolysis [62]. Not only is the main removal

mechanism known as adsorption [37] but also *Chlorella* spp. are famous for the effective removal of various antibiotics, including AMX [28]. The results of *C. sorokiniana* are consistent with other *Chlorella* studies demonstrating an almost complete removal of AMX.

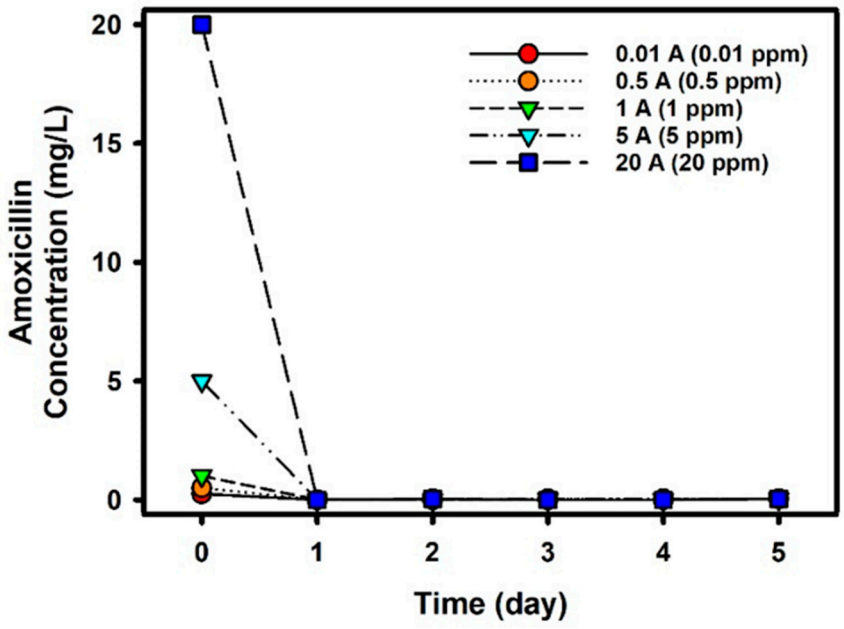

Figure 3. Antibiotic reduction according to different AMX concentrations in LE by microalgae.

In addition, because natural photoautotrophic growth uses light as an energy source, this could make photolysis contribute to AMX removal because intensive light can increase the dissolved oxygen and pH, which creates favorable conditions for photolysis. These reaction conditions may be able to induce reactive oxygen species that can support antibiotic clearance [61]. Overall, the results demonstrate that the microalgae can remove antibiotics from the water body appropriately. However, the statistical correlation and degradation mechanisms should be further demonstrated.

3.3. Inhibitory Effect of AMX on Biodiesel Production

3.3.1. Negative Effect of AMX Inhibition in Transesterification

In this study, biodiesel (FAME) was recovered from the microalgae biomass that was grown while treating LE with residual AMX according to the experimental design. Figure 4 presents the FAME yield obtained from the direct transesterification of the microalgal biomass using HCl and NaOH catalysts. It was observed that the yield decreases as the concentration of antibiotics increases. This negative correlation was more significant in the case of the HCl catalyst. The FAME yield reduced from 4.3% to 1.5%, while that of NaOH catalyst decreased from 4.3% to 2.9%. The cause of the decrease in the FAME yield must be associated with AMX's inhibition of the microalgal growth. A previous study confirmed that the higher the concentration of antibiotics, the more the microalgal photosynthesis was inhibited [28]. The results of this study were consistent with this and verify that the base catalyst might be preferable to prevent severe losses in the overall biodiesel recovery.

3.3.2. Changes in FAME Composition of Biodiesel Due to AMX

Figure 5 illustrates the variation in the FAME composition according to the residual AMX concentration and catalyst type. As a fuel, important FAME components are palmitic acid (C16:0) and stearic acid (C18:0) [63].

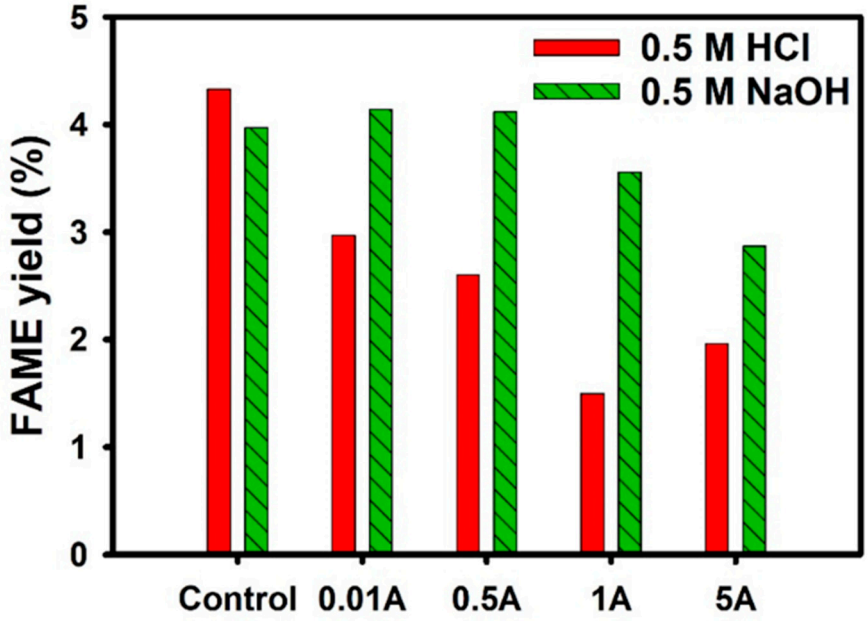

Figure 4. Effect of antibiotic concentration on FAME yield.

In the case of transesterification using HCl, the palmitic acid (C16:0) presented a drastic decrease as the residual AMX increased, while little change was observed in the case of the base catalyst. For the HCl catalyst, major elements of the FAME composition were transitioning from C16:0 to C18:2 (Figure 5b–e) and C18:3 (Figure 5b–d).

The cause of this change might be found in the esterification reaction. As shown in Equation (2), free fatty acids react with methanol and produce water through the esterification reaction.

$$\text{HOOC-R (free fatty acid)} + CH_3OH \leftrightarrow \text{R-COOCH}_3\ \text{(FAME)} + H_2O \quad (2)$$

Figure 6 compares the molecular structure of AMX with those of free fatty acids. It was observed that both AMX and free fatty acids similarly contain a carboxylic functional group (RCOOH). The carboxylic functional group of AMX also can be esterified by methanol [64]. This fact can reasonably explain why the FAME yields decrease in this study as the residual AMX concentration increases. Moreover, transesterification using an acid catalyst usually uses a temperature of 90 °C for optimal FAME recovery [65]. This high temperature may accelerate the reaction rate between the methanol and AMX, which results in methanol consumption due to this unnecessary reaction. Furthermore, Equation (2) also shows the production of water. Because the presence of water and the remaining free fatty acids may trigger soap formation, which consumes the catalyst, water also results in a low conversion rate. From these analyses, it can be confirmed that the presence of antibiotics in the transesterification process deteriorates the biodiesel yield and composition.

3.4. Limitations and Implications

This study primarily focuses on the effects of AMX in LE on microalgal growth, as well as the yield and composition of the biodiesel generated. Cultivating microalgae using LE is an eco-friendly method of converting waste into biofuel, effectively removing N and P. Additionally, the AMX in LE is degraded by microalgal metabolism, indicating that microalgae-based LE treatment can manage not only the nutrient levels but also the levels of antibiotics such as AMX.

With a continuous increase in antibiotic usage in the livestock industry, consideration of the type and concentration of antibiotics used is important. Not only AMX but various other antibiotics are used, including tetracyclines, quinolones, penicillin, cephems, ionophores, and sulfonamide [66–68]. Investigating the detailed interactions between these

antibiotics and microalgal growth, as well as optimizing the conditions of cultivation, is essential. Additionally, evaluating the efficiency of continuous cultivation is essential for the industrial application of LE treatment.

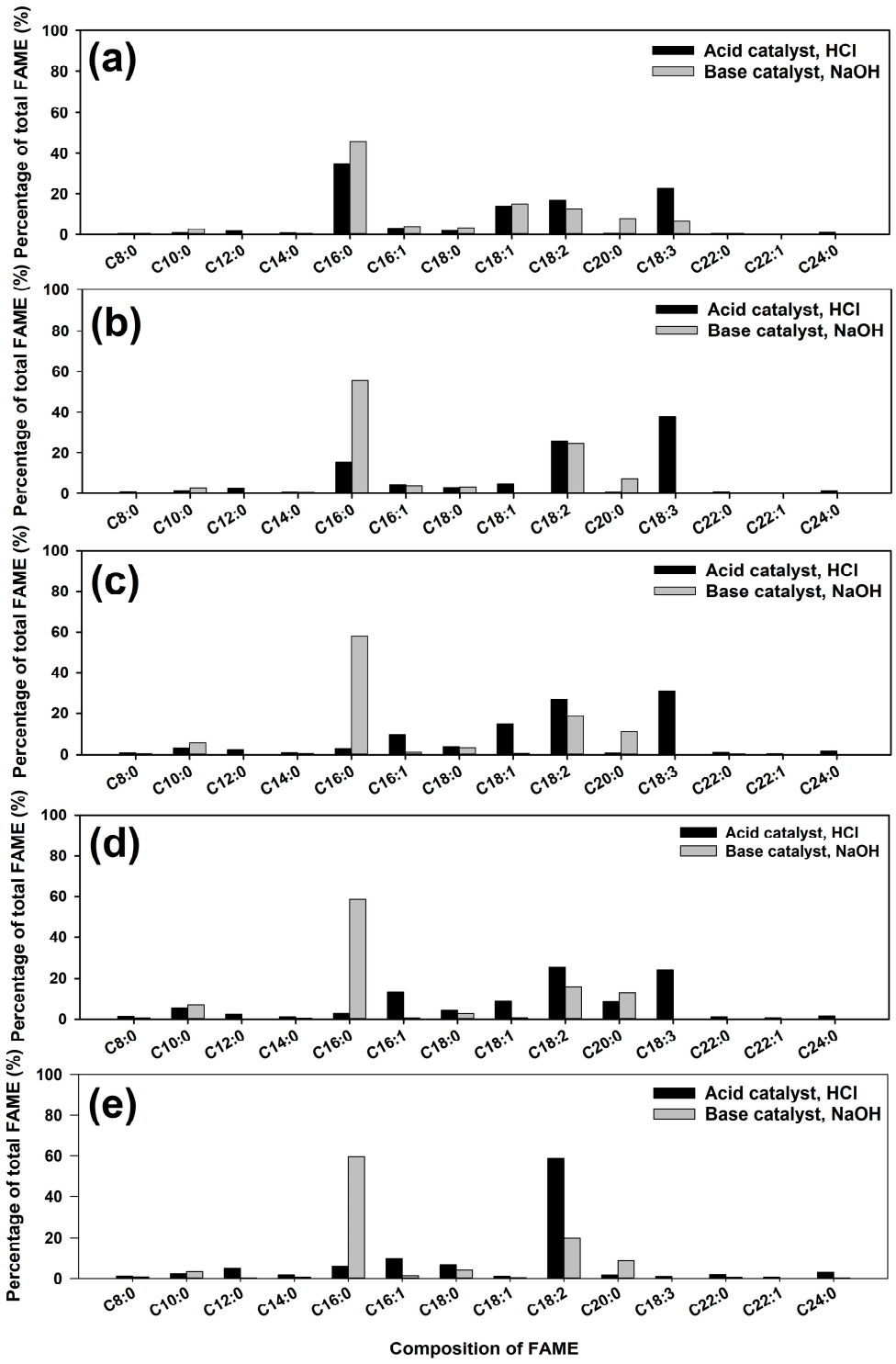

Figure 5. Change in FAME compositions according to antibiotic concentration: (**a**) control, (**b**) 0.01 ppm, (**c**) 0.5 ppm, (**d**) 1 ppm, (**e**) 5 ppm.

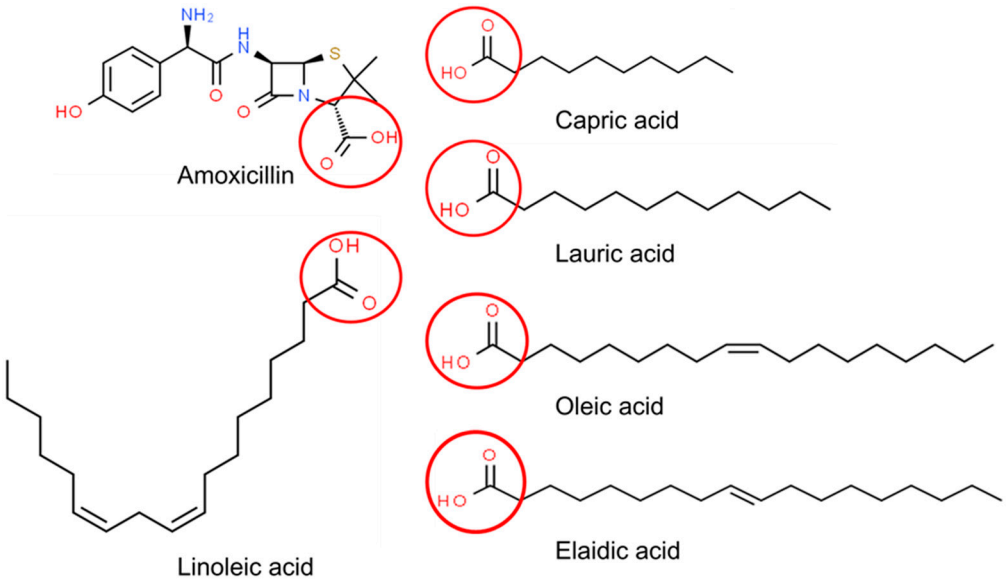

Figure 6. Comparative analysis of functional groups similarities between AMX and free fatty acids. Red circles denotes carboxylic functional group (RCOOH).

4. Conclusions

This study confirms that the presence of AMX while treating LE with microalgae leads to negative consequences in terms of the microalgal growth kinetics, biomass yield, and biodiesel composition. Specifically, an increase in the AMX concentration from 0.01 to 20 ppm resulted in a reduction in the average microalgal productivity from 1651 mg/m^3/day to 1080 mg/m^3/day and the growth kinetics from 0.87 days^{-1} to 0.35 days^{-1}. The overall biodiesel yield from the produced biomass significantly decreased from 4.3% to 1.5% with the acid catalyst and 4.0% to 2.9% with the base catalyst. The similarity in molecular structure between AMX and free fatty acids explains how methanol is consumed unnecessarily, leading to a low biodiesel yield while altering the FAME composition, in transesterification. The results show that residual AMX is strongly associated with a decrease in the C16:0 content in the FAME composition, which is an important constituent of biodiesel. Importantly, the findings of this study can be utilized to manage LE in animal breeding facilities using effective AMX controls. The harvested microalgae biomass resulting from this process can be effectively converted into biodiesel, offering an eco-friendly solution in waste-into-bioenergy conversion strategies.

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Abbreviations

LE	Livestock excreta
FAME	Fatty acid methyl ester
ppb	Parts per billion
AMX	Amoxicillin
COD	Chemical oxygen demand
T-N	Total nitrogen
T-P	Total phosphorus
DT	Direct transesterification
Chl-a	Chlorophyll-a

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