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Research article

Development of sustainable poultry waste management using integrated microalgae cultivation: Towards performance, resource recovery and environmental impact

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ABSTRACT

This study aimed at developing a sustainable waste management from poultry farm by integrating microalgae cultivation with the anaerobic digestion effluent of chicken wastes ($ADEC_W$). The analysis was focused on system performance, resource recovery and environmental impact of microalgal biomass-derived added value products. Laboratory-scale of three different systems, i.e. suspended microalgae, biofilm microalgae and the control as no microalgae seed added, was conducted under outdoor climatic conditions in Thailand. The results clearly showed that microalgae system was successfully developed with high treatment performance and potential renewable energy production for the $ADEC_W$. Compared to the control, it was demonstrated that most removals of nutrient and organic pollutants were achieved through microalgal assimilation. Biofilm microalgal system was capable for removing NH_4^+-N, PO_4^{3-}-P and dissolved COD of 97 %, 93 % and 75 %, respectively at the cultivation time of 14 days, while for suspended microalgal system these were 92 %, 87 % and 68 %, respectively. Biofilm microalgal system also showed advantages of higher biomass production and simple harvesting of biomass, due to it tightly attached on supporting material by the matrix of extracellular polymeric substances (EPS). Moreover, the analysis of potential electricity generation and environmental impact highlighted the promising sustainability of microalgae-based poultry wastes treatment as microalgae provided significant potentials for electricity generation and CO_2 reduction. The analysis showed that with nationwide egg-laying hen farms in Thailand, the total electricity generation can be as high as 72 GWh/year with the total CO_2 reduction capacity of 99 kton CO_2/year, while CO_2 emission from electricity generated by microalgal biomass is at least 29 % lower than conventional fuels. The study offers a promising waste management alternative with great potential to achieve efficient treatment and valuable resource recovery for poultry farms in the future.

Abbreviations: AD, anaerobic digestion; ADE, anaerobic digestion effluent; $ADEC_W$, anaerobic digestion effluent of chicken wastes; COD, chemical oxygen demand; DW, dry weight; EPS, extracellular polymeric substances; N, nitrogen; NH_4^+-N, ammonium-nitrogen; P, phosphorus; PO_4^{3-}-P, phosphate-phosphorus; SEM, scanning electron microscopy.

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1. Introduction

The continued increase of global population, which will approach 8.5 billion by 2030 and 10.4 billion by 2100, leads to a significant demand for food production including poultry products as high-quality protein sources [1,2]. Chickens are among the most widely distributed poultry worldwide, with the global annual production of chicken meat and egg in 2022 of 102 million tons and 1627 billion eggs, respectively [3,4]. Likewise, according to the USDA reported, Thailand becomes the top ten of global chicken products producers and top five of global exporters, and moreover continue to be key players in both domestic and international markets [5]. In 2022, there were over 8000 standardized chicken farms in Thailand with the total production of 3.3 million tons chicken meat and 15.6 billion chicken eggs, while is forecast a growing rate of more than 4 % annually [6,7].

Such high current and future expanded numbers of chicken farms will subsequently result in high production and accumulation of substantial quantities of chicken wastes comprised mainly of chicken manure, litter (e.g. straw bedding, sawdust and rice husks) and process-generated wastewater (e.g. from floor cleaning and gutter flushing) [8]. These chicken farm wastes generally contain high amounts of solids, organic pollutants and nutrients including nitrogen (N) and phosphorus (P), as well as potential source of opportunistic pathogens. Therefore, chicken wastes are not allowed to discharge directly into the receiving environment as can lead to severe problems, in particular the spread of pathogens, obnoxious odor, emission of greenhouse gases, deoxygenation and eutrophication of water bodies [9–11]. As environmental regulations becoming more stringent, appropriate management of chicken wastes treatment and disposal is required to maintain compliance. In recent decades, the treatment of chicken wastes by anaerobic digestion (AD) for the production of electricity has been successfully applied and continually developed in commercial application [12,13]. Although the AD of animal wastes is favored because of its advantages on the effectiveness of high-strength organic degradation, destruction of pathogens, recovery of renewable energy and sludge reduction, a post-treatment of anaerobic digestion effluent (ADE) is required to further remove organic pollutants and excess nutrients before discharging into the environment [14,15]. However, due to the potential high nutrients contained in the ADE of animal wastes, the concept of ADE treatment has shifted from being considered a waste to becoming valuable resources. Recently, the utilization of ADE of animal wastes including from chicken farm wastes as a rich alternative nutrient source for microalgal growth has been reported [11,16–19].

The microalgae-based wastewater treatment has gained significant attention as an economical and sustainable solution due to its efficient treatment, high growth rate, simple construction, easy and low-cost operation, CO_2 emission reduction and valuable resource recovery [20–22]. Theoretically, microalgae generally utilize inorganic N and P from wastewater in the forms of ammonium (NH_4^+) and orthophosphate (as PO_4^{3-}) by assimilating these nutrients into microalgal biomass [23]. Moreover, the commonly used systems for microalgae cultivation are suspended and non-suspended with the alternatives of open or closed environment. Although suspended microalgal system is widely used for convenient treatment of wastewater, the cost of microalgal biomass harvesting is high and thus poses a challenge for real-world application. While in non-suspended or biofilm system, a microalgal layer is formed on the supporting material, resulting in easy and low-cost for biomass harvesting prior to discharge [22]. In addition, previous studies reported that biofilm microalgal system was successfully treated different wastewaters varied from low nutrients content as used for treating domestic wastewater and polishing domestic wastewater effluent to high nutrients content of such as livestock wastes [24–26].

Until now, although there is overwhelming interest in the treatment of animal wastes by microalgae cultivation, limited information can be found on the integration of both efficient treatment and resource recovery perspectives for sustainable waste management of poultry farm. Especially, biofilm microalgal system has received little attention as post-treatment for ADE of chicken wastes with the results that the treatment performance, resource recovery potential and environmental impact of biofilm microalgae are unknown. Moreover, designs of the reactor and proper harvesting time to obtain optimal performance needs urgent attention for using the ADE of chicken wastes as microalgae growth medium. Therefore, this study aimed to investigate the treatment performance, as well as the potential of resource recovery and environmental impact of cultivating microalgae in the anaerobic digestion effluent of chicken wastes ($ADEC_W$) with subsequently utilized microalgal biomass for added value products. Two different microalgal reactors were cultivated with the $ADEC_W$ in outdoor climatic conditions of the dry season in northern of Thailand, where the removals of nutrient and organic pollutants with the biomass production were evaluated and compared with the control. Finally, the potential electricity generation from microalgal biomass and environmental impact of CO_2 reduction by microalgae and CO_2 emission factor for electricity generation were analyzed. The results obtained will be used to develop an integrated efficient and sustainable waste management from poultry farm, and also take advantage of valuable resource recovery from the microalgae-based treatment system.

Table 1
Chemical-physical characteristics of the anaerobic digestion effluent of chicken wastes.

Parameters	Unit	Value/Concentration
pH	–	7.80 (0.09)
TKN-N	mg N/L	286 (15)
NH_4^+-N	mg N/L	207 (6)
PO_4^{3-}-P	mg P/L	72 (3)
Total COD	mg COD/L	379 (23)
Dissolved COD	mg COD/L	316 (12)
Total suspended solids	mg/L	179 (18)
Volatile suspended solids	mg/L	73 (4)

Note: Data are shown as the average of 3 samples with standard deviations in brackets.

2. Materials and methods

2.1. Characteristics of the anaerobic digestion effluent of chicken wastes

The anaerobically digested effluent of chicken wastes was collected from a local egg-laying hen farm in Chiang Mai, Thailand. This egg-laying hen farm was medium-scale poultry egg producers, which had 35000 laying hens and sold more than 10.2 million eggs annually. In total, 60 m^3 of the $ADEC_W$ was produced daily. In this study, microalgae were cultivated using the undiluted $ADEC_W$. The characteristics of the $ADEC_W$ are shown in Table 1.

2.2. Microalgae seed culture

A mixed-species of microalgae seed was obtained from the polishing pond at the egg-laying hen farm where the $ADEC_W$ was taken. Compared to single-species culture, the mixed-species microalgae reported advantages of higher nutrients assimilation and biomass production [27]. In this study, the collected microalgae seed was first concentrated by gravity settling to achieve 9.4 $\pm$ 0.1 g dry weight biomass/L before use [26].

2.3. Cultivation of microalgae

Mixed-species of microalgae-based treatment of the $ADEC_W$ using two different cultivation systems, i.e. (1) suspended and (2) biofilm, was compared with the control system for their treatment performances, potential of resource recovery and environmental impact. Each suspended microalgae, biofilm microalgae and the control system were cultivated using the $ADEC_W$ in an open outdoor photobioreactor in the dry season during December to January at Rajamangala University of Technology Lanna, Chiang Mai, Thailand (18°48′34.8″N, 98°57′10.2″E). The photobioreactors with a working volume of 40 L and had dimensions of 60 cm $\times$ 25 cm $\times$ 45 cm (top diameter $\times$ height $\times$ bottom diameter) were used in experiments, and were mixed continuously by an electro-magnetic air pump connected with a porous air diffuser. In suspended and biofilm microalgal systems, the mixed-species of microalgae seed with 1 % v/v was added and cultivated for 14 days, as used in the study of Khiewwijit et al. (2019) [26]. While the control system, no microalgae seed was added. For suspended system, the microalgae seed was directly added into the reactor and let the microalgal cells moved freely inside the culture area. Whereas for biofilm system, the microalgae seed was poured onto a flat patterned sheet, which was used as the supporting material for biofilm formation that could grow under approximately 2 cm-depth of water surface. This patterned sheet had a total area of 0.16 m^2 (40 cm length $\times$ 40 cm width) and placed at the center of the photobioreactor. Moreover, the patterned sheet comprised of three layers. A high-density polyethylene knitted shade net (90 % black) was used for the top and bottom layers, while a coarse scouring pad was used for the middle layer, as illustrated in Fig. 1.

All experiments were performed in triplicate. In addition, the removal efficiencies (*RE* in %) of nutrients and COD from the $ADEC_W$ by suspended microalgae, biofilm microalgae and the control system were calculated using Eq. (1) [28]:

$$RE_x\ (\%) = \left[\left(C_{x,in} - C_{x,out}\right) / C_{x,in}\right] \times 100\% \tag{1}$$

where RE_x is the removal efficiency of compound x (%), $C_{x,in}$ is the concentration of compound x in the influent (mg/L) and $C_{x,out}$ is the concentration of compound x after the treatment (mg/L). In this study, compound x refers to NH_4^+-N, PO_4^{3-}-P and dissolved COD.

2.4. Morphological characterization of microalgae

The production of microalgal cells after 14 days of cultivation both in suspended and biofilm microalgal systems were observed using a compound light microscope (Olympus CX23, Japan) for their morphological features, and moreover, the cells were further studied using a scanning electron microscopy (SEM) (Prisma E, Thermo Scientific, USA). Through a compound light microscope, the samples were prepared by simple wet mount method. In addition, prior to carrying out the observation under SEM, the samples were

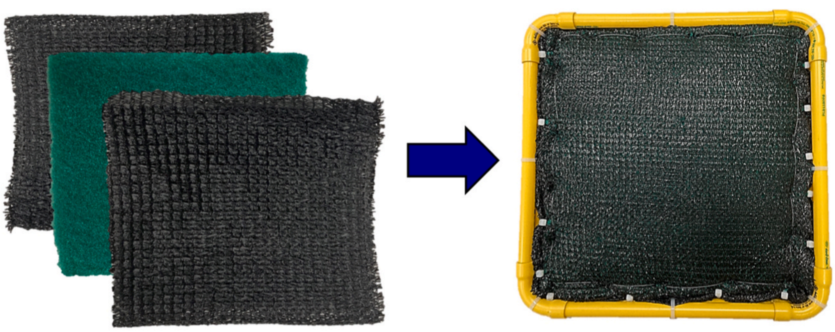

Fig. 1. Scheme of patterned sheet used for microalgae cultivation in biofilm system.

sputter-coated with gold/palladium for 45 s and 18–20 mA using an SC7620 mini-sputter coater (Quorum Technologies Ltd., UK) to decrease charging effects during observation.

2.5. Potential electricity generation and environmental impact analysis

In this study, the potential electricity generated by anaerobic microalgal biomass digestion was calculated using the model parameters given in the study of Mu et al. (2014) [29]. Moreover, the total CO_2 reduction capacity was also analyzed. The CO_2 reduction capacity was calculated from the mass flow and specific CO_2 uptake through the photosynthesis of microalgae for biomass production, which was 1.83 kg CO_2/kg dry weight of microalgal biomass [30].

Additionally, to further explore the environmental impact of CO_2 emission from the microalgal biomass power plant compared to other conventional fuels, CO_2 emission factor (CO_2 *EF*) was evaluated. The CO_2 *EF* was determined based on the 2006 IPCC guidelines for national greenhouse gas inventories, which expressed by Eq. (2) [31]:

$$CO_2\ EF = (C / NCV) \times (F) \times (44 / 12) \quad (2)$$

where CO_2 *EF* is the CO_2 emission factor (Ton CO_2/TJ), *C* is the ratio of carbon content of fuel source (%), *NCV* is the net calorific value (TJ/Mg of fuel), *F* is the carbon oxidation factor and 44/12 is the molecular weight ratio to convert kg of carbon to kg of CO_2. This study assumed a complete combustion process, which thereby the carbon oxidation factor was assumed to be 1. Moreover, 50 % carbon by dry weight of microalgal biomass was used [32].

2.6. Analytical methods

Samples from suspended microalgae, biofilm microalgae and the control system were collected and measured pH by pH electrode (OHAUS Starter ST5000-B Bench pH Meter, USA). After that, the samples were centrifuged for 15 min at 2000 rpm and the concentrations of NH_4^+-N, PO_4^{3-}-P and COD were analyzed from the supernatant. Parameters of PO_4^{3-}-P and dissolved COD were measured using APHA standard methods [33], and NH_4^+-N was analyzed using Spectroquant test kit (Merck, Germany) with a detection range of 0.01–3.00 mg/L. Moreover, the yields of biomass production were analyzed with the same method used in the study of Boelee (2013) [34]. For suspended microalgae and the control, the biomass dry weight was measured at the beginning and the end of the experiment by filtration of the samples through pre-weighed glass fiber filters (Whatman GF/F, UK), followed by drying in a 105 °C oven at least 24 h. For the microalgal biofilm was scraped at the end of the experiment from the patterned sheet using plastic spoon and then blew off with air nozzle to ensure the entire biofilm formation was harvested. After that, the harvested microalgae were dried in a 105 °C oven at least 24 h for determining biomass dry weight.

3. Results and discussion

3.1. pH during microalgae cultivation

In this study, pH changes were monitored and used as a preliminary indicator of microalgal growth throughout the experiments. The pH usually increases during the growth of microalgae, which due to the photosynthetic CO_2 assimilation and consequently leading to accumulation of free hydroxide ions [21,35,36]. Fig. 2 shows the average pH during the cultivation in suspended and biofilm

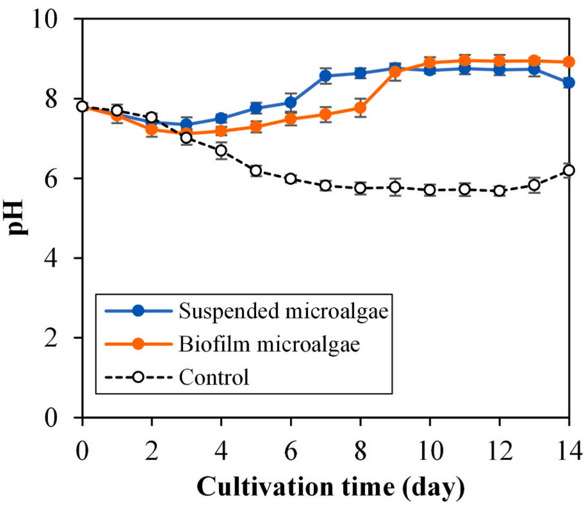

Fig. 2. pH during microalgae cultivation in suspended and biofilm systems compared to the control.

microalgal systems compared to the control.

As shown in Fig. 2, in the first 2 days of cultivation, the pH was slightly decreased for all three systems. This was likely due to the lag phase of microalgae where they adapted to the environment and the growth of heterotrophic bacterial populations in the $ADEC_W$, which resulted in less utilization of CO_2 by microalgae and release of CO_2 by bacteria [23,37]. Thereafter, for suspended and biofilm microalgal systems, the pH was increased gradually and reached the average pH of 8.57 at day 7 of cultivation for suspended microalgal system and 8.66 at day 9 for biofilm microalgal system. Similar results reported by Kumar et al. (2021) [38] also showed that pH values during the microalgae cultivation were increased with the increased photosynthetic activity of microalgae for biomass production. However, it was found that after day 7–13 of cultivation the pH in suspended microalgal system remained almost stable and then decreased to the average pH of 8.40 at day 14. Whereas for biofilm microalgal system, the pH was remained stable after day 9 until the end of experiment and the average pH reached 8.91 at day 14 of cultivation. These remaining or slightly decreasing pH values could occur when microalgae reached to the stationary and death phase due to a variety of environmental stresses, which will also explain later [39]. In contrast, in the control system, the pH was continually decreased from the beginning (average pH 7.80) to day 8 of cultivation (average pH 5.75) and then remained stable to day 12. After that the pH tended to increase, which probably caused by the presence microalgae in the $ADEC_W$ were growing, and ended up with the average pH of 6.19 at day 14 of cultivation. The decreasing pH observed in the control system confirmed the growth of heterotrophic bacteria in the $ADEC_W$, which known to reduce pH caused by the release of CO_2 from aerobic oxidation of organic matter [21].

3.2. Removal of nutrient pollutants from the anaerobic digestion effluent of chicken wastes

As shown in Fig. 3A and B, both suspended and biofilm microalgal systems could effectively remove nutrient pollutants from the $ADEC_W$. After the lag phase of microalgal growth in the first 2 days, the concentrations of NH_4^+-N and PO_4^{3-}-P were continually decreased both for suspended and biofilm microalgal systems. For suspended microalgal system, the average concentrations of NH_4^+-N

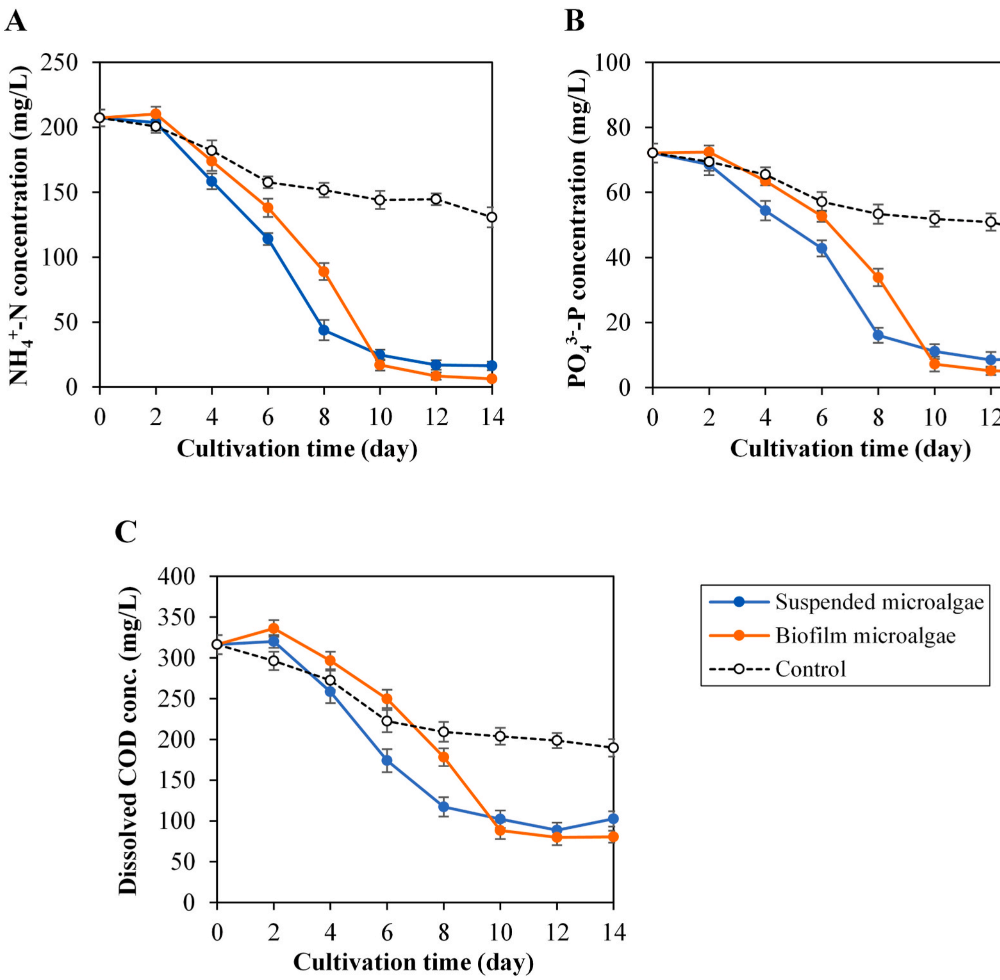

Fig. 3. The change of (A) NH_4^+-N, (B) PO_4^{3-}-P and (C) dissolved COD concentrations during microalgae cultivation in suspended and biofilm systems compared to the control.

were significantly decreased from 204 mg/L at day 2 of cultivation to 44 mg/L at day 8 and then slightly decreased to 16 mg/L at day 14, which corresponded to the NH_4^+-N removal efficiency of 92 %. A similar trend was also found for the PO_4^{3-}-P removal. The average concentrations of PO_4^{3-}-P were continually decreased from day 2 (69 mg/L) to day 8 of cultivation (16 mg/L) and followed by a slight decrease to 9 mg/L at day 14, resulting to 87 % removal efficiency of PO_4^{3-}-P. While for biofilm microalgal system, the average concentrations of NH_4^+-N were significantly decreased from 210 mg/L at day 2 of cultivation to 17 mg/L at day 10 and followed by a slight decrease to 6 mg/L at day 14, which corresponded to the PO_4^{3-}-P removal efficiency of 97 %. Besides, the average PO_4^{3-}-P concentrations were decreased from 72 to 7 mg/L at day 2 to day 10 of cultivation, respectively, after that remained almost stable and reached 5 mg/L at day 14, which translated to the PO_4^{3-}-P removal efficiency of 93 %. The N and P decreased through microalgae cultivation is caused by the photosynthetic activity and microalgal growth. Nitrogen is an essential element for microalgal growth and plays a fundamental role in the synthesis of proteins, lipids and carbohydrates. Whereas phosphorus is nutrient required for various mechanisms, for example energy transport, biosynthesis of nucleic acids and DNA [23]. In this study, the higher removal efficiencies of NH_4^+-N and PO_4^{3-}-P achieved in biofilm system is due to the optimal utilization of light and CO_2 for the microalgae growth and minimization of cell washouts in biofilm microalgal system [22,24,25].

In contrast, the control system showed much less removal efficiencies of both NH_4^+-N and PO_4^{3-}-P. The NH_4^+-N removal efficiency of 37 % was achieved with the average concentration of 131 mg/L in the effluent at day 14 of cultivation, while the removal efficiency of PO_4^{3-}-P was 35 % with the average concentration of 47 mg/L in the effluent. These N and P are also essential nutrients for the growth and metabolism of heterotrophic bacteria [37].

3.3. Removal of organic pollutants from the anaerobic digestion effluent of chicken wastes

The dissolved COD concentrations was monitored during microalgae cultivation both in suspended and biofilm systems, and compared to that of in the control system. Presumably, the capacity of heterotrophic microalgae to use organic carbon as carbon and energy source, as well as, the presence of heterotrophic bacterial populations in the $ADEC_W$ could be attributed to decrease in the dissolved organic pollutants for their growth of biomass [20,39].

As illustrated in Fig. 3C, after seeding the mixed species of microalgae into each cultivation system, the average concentrations of dissolved COD were increased within the first 2 days which reached to 320 mg/L for suspended microalgal system and 336 mg/L for biofilm microalgal system. The slight increase in COD concentrations may due to the accumulation of biodegradable organic matter related to cell death of microalgae seed as they could not adapt to changes during the lag phase. After that, as expected, the concentrations of dissolved COD were significantly decreased both in suspended and biofilm microalgal systems. For suspended microalgal system, dissolved COD was continually reduced during the cultivation from day 2 to day 12, which decreased to the average dissolved COD concentration of 89 mg/L at day 12 of cultivation. While thereafter, the average concentration of dissolved COD was increased and reached 102 mg/L at day 14 of cultivation, resulting in the removal efficiency of 68 %. These fluctuations of dissolved COD concentrations during the microalgae cultivation could be occurred when the cultures reach the death phase, which caused by various factors such as nutrient depletion, toxicity and cellular senescence [23,39]. Thus, a slight increase in dissolved COD concentration after day 12 of cultivation could be explained by the released of organic matter from dead microalgae back into the system, which coincided with decreasing in pH values at the end of cultivation in suspended microalgal system (Fig. 2). Similar results were observed by Nguyen et al. (2020) [40] in which the increasing COD in medium was found when microalgal cells reached the death phase. These results indicated that a harvesting frequency of every 14 days seems insufficient to remain the optimal microalgae growth in suspended system when using the $ADEC_W$ as a source of nutrients.

On the other hand, for biofilm microalgal system, the average concentrations of dissolved COD from day 2 to day 10 of cultivation were significantly decreased and then remained stable to the end of cultivation. At day 14 of cultivation, the average dissolved COD concentration was 80 mg/L, which translated to the COD removal efficiency of 75 %. These findings demonstrate the potential benefits from cultivating microalgae both in suspended and biofilm systems for the removal of organic pollutants from the $ADEC_W$. The results are consistent with Rajagopal et al. (2021) [11], who found the COD removal efficiency of nearly 45 % when using microalgae *Chlorella*

Table 2
Comparison of overall treatment performance and efficiency treating the $ADEC_W$ by microalgae cultivation compared to the control.

Cultivation system	Removal efficiency (%)	Concentrations after treating (mg/L)	
		Average	Standard deviation
1. Suspended microalgae			
NH_4^+-N	92	16	3
PO_4^{3-}-P	87	9	1
Dissolved COD	68	102	9
2. Biofilm microalgae			
NH_4^+-N	97	6	1
PO_4^{3-}-P	93	5	0.4
Dissolved COD	75	80	7
3. Control			
NH_4^+-N	37	131	8
PO_4^{3-}-P	35	47	2
Dissolved COD	40	189	11

vulgaris treating the ADE of chicken manure. Whereas for the control system, the average dissolved COD concentrations were gradually decreased from the beginning (316 mg/L) to 189 mg/L at day 14 of cultivation, resulting to the removal efficiency of 40 %. These organic carbon pollutants were used as an energy and carbon source for the formation of new biomass by heterotrophic bacteria [37].

In order to summarize the performance of using microalgae for the treatment of $ADEC_W$, Table 2 provides an overall treatment performance of nutrient and organic pollutants by microalgae cultivation compared to the control system. The results clearly showed that microalgae could be used for efficient treatment from the $ADEC_W$, in particular biofilm microalgal system. In the present study, biofilm microalgae can successfully cultivate in the $ADEC_W$, which able to achieve efficient treatment of 97 % NH_4^+-N, 93 % PO_4^{3-}-P and 75 % dissolved COD removals at the cultivation time of 14 days. While suspended microalgal system gave the removal efficiencies of NH_4^+-N, PO_4^{3-}-P and dissolved COD of 92 %, 87 % and 68 %, respectively. Compared with the use of microalgae, much lower treatment efficiencies of 37 % NH_4^+-N, 35 % PO_4^{3-}-P and 40 % dissolved COD were obtained by the control system with no microalgae seed added. However, the contribution of nutrient and organic pollutants removal through microalgal assimilation or bacterial metabolism should be further explored and more fundamental knowledge about the integration of microalgae-bacteria consortium on the $ADEC_W$ is needed. In addition, although there are currently no effluent standard limits that regulate the treated effluent discharges from poultry farm in Thailand, such high nutrient and organic pollutants removals especially in biofilm microalgal system provided good effluent quality that could directly discharge into the environment as compared to the agro-industrial effluent standard limits [41].

3.4. Biomass production

Table 3 shows the results of biomass production in terms of net dry weight (DW) yield from suspended and biofilm microalgal systems compared to the control. As demonstrated by the data in Table 3, the results clearly showed that after 14 days of cultivation, both suspended and biofilm microalgal systems gave a higher biomass yield compared to the control. The average net DW biomass yields were 1383 and 1521 mg DW/L for biofilm microalgal system and suspended microalgal system, respectively. Besides, the control was obtained 115 g DW/L of the average net DW biomass yield. The higher biomass yield corresponded to higher nutrients uptake that potential increased the photosynthetic activity of microalgae for biomass production, which coincides with the results of the previous study [27]. However, it should be noted that the biomass harvested from suspended microalgal system was a mixture of microalgae and bacteria, which may result in difficulties for the resource utilization of harvested microalgae [35], whereas the biomass of attached microalgae was harvested from biofilm system.

In addition to the higher biomass production, biofilm microalgal system also has additional advantage of reducing the cost for biomass harvesting, as a simple harvesting method such as scraping could be used and no separation of microalgal biomass production required before discharging the treated effluent [22,25]. On the other hand, for suspended microalgal system, the production of biomass and treated effluent must be separated and thereby resulted in a higher operating cost for biomass harvesting. Examples of common separation methods for harvesting microalgae from aqueous solution are centrifugation, coagulation and flocculation, filtration and flotation [20,42]. Furthermore, as suggested by Boelee (2013) [34], it is essential to determine the suitable period for harvesting microalgal biomass that can maintain microalgae in the growth phase to ensure optimal nutrients uptake capacity, as well as to reduce cell death that would lead to the release of organic matter and nutrients from cell decomposition. The frequency of microalgal biomass harvesting is generally dependent on the biomass production rate, which most varies with the availability of light and nutrient concentrations [43]. Based on all the results obtained in this study, a harvesting frequency of 14 days for biofilm microalgal system was promising to maintain the optimal treatment for the $ADEC_W$. Whereas, the results of the decreased pH and increased dissolved COD at the end of the experiment in suspended microalgal system (Figs. 2 and 3) indicated that the suspended biomass should be harvested before 14 days, which suggested for example every 12 days.

3.5. Morphological identification via light and scanning electron microscopy

The microalgal suspended and biofilm grown on the $ADEC_W$ after 14 days of cultivation were observed under a compound light microscope (Fig. 4A–D) and SEM (Fig. 4E–H). Both suspended and biofilm systems showed a similar diversity of microalgae species and likely dominated by *Chlorella* sp. and *Scenedesmus* sp., which in accordance with other previous studies that proven capability of growing both microalgae species in the ADE of poultry wastes including from chicken farms [11,18,19]. Additionally, the results of

Table 3
Biomass production from suspended and biofilm microalgal systems compared to the control.

Cultivation system	Biomass production (mg DW/L)
Suspended microalgae[a]	1383 (93)
Biofilm microalgae[b]	1521 (78)
Control[a]	115 (9)

Note: Results are the average values with standard deviations in brackets.
[a] Net dry weight (DW) biomass yield = Final biomass density at the end - Initial biomass density.
[b] Net DW biomass yield was harvested microalgal biomass grown on the patterned sheet at the end of cultivation.

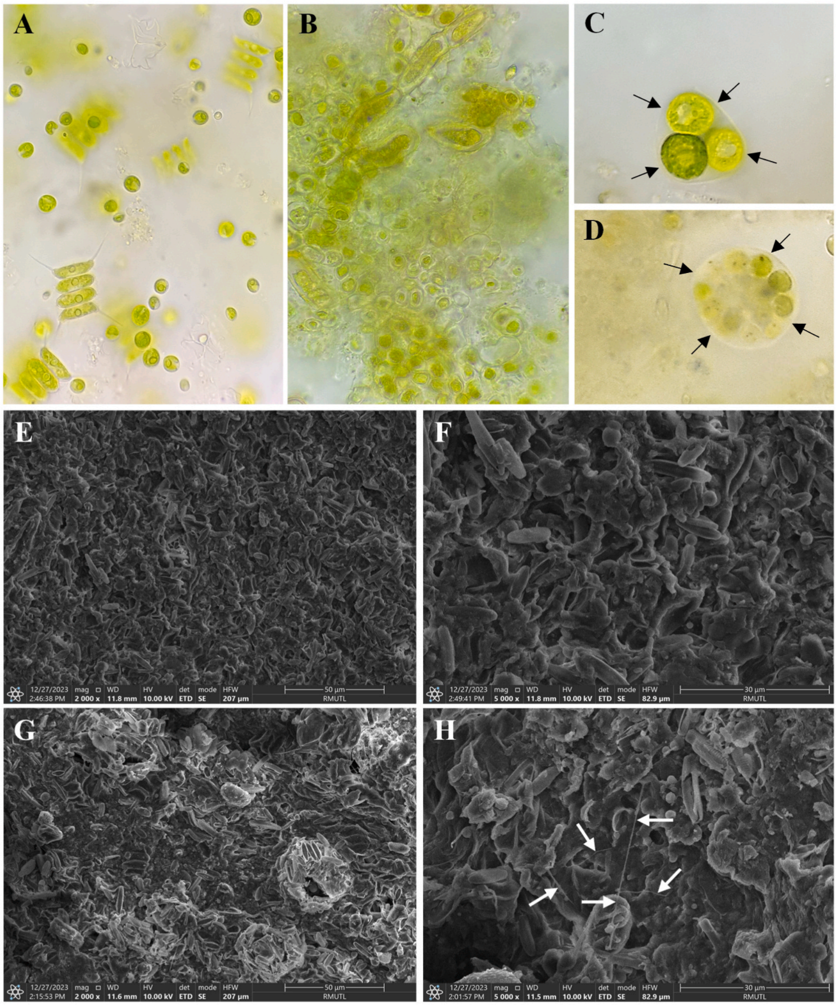

Fig. 4. Morphological images of microalgal production after 14 days of cultivation observed by a compound light microscope with 1000× magnification both in (A) suspended system and (B, C, D) biofilm system, and by a scanning electron microscopy (SEM) with white line indicating the size in μm both in (E, F) suspended system and (G, H) biofilm system. Arrows point at the EPS matrix in microalgal biofilm system.

microscopic images of microalgal biofilm compared to suspended system evidently demonstrates that microalgae were held together by extracellular polymeric substances (EPS) matrix (Fig. 4C, D, 4H). This finding is consistent with the previous studies, wherein the synthesis of EPS in biofilm formation could enhance the adhesion of microalgal cells or microalgae-bacteria consortium [34,44]. The production of EPS mainly comprises of polysaccharides and proteins, and other components like lipids, humic acids and nucleic acids. The EPS in biofilm has diverse functions, including as a glue-like substance to tightly attach microorganisms to the surface and the abilities to enhance settleability of biomass, as well as to bind metal ions that could potentially increase the removal of heavy metals from wastewater [45,46].

3.6. Potential electricity generation and environmental impact

In recent years, microalgae have attracted considerable attention worldwide because of the potential use of microalgal biomass for promising renewable energy sources and high-value bioproducts such as biogas, biofuels, biopolymer, electricity, animal feed and natural pigments [47], as well as a feasibility of CO_2 reduction capacity through the photosynthesis by microalgae [30]. Using the total number of laying hens nationwide in Thailand and expected annual growth from year 2019–2032 as an example [7,48], the potential electricity generation and total CO_2 reduction capacity utilizing the biomass from microalgal biofilm system cultivated with the $ADEC_W$ were presented in Fig. 5A. Furthermore, Fig. 5B reviews the CO_2 emission factor for electricity generation produced from microalgal biomass and compared to other conventional fuels.

As shown in Fig. 5A, utilizing the resources in the ADE of laying hen wastes nationwide in Thailand it is possible to cultivate 50 kton DW/year of microalgal biomass in 2023, which consequently could generate a total potential electricity of 66 GWh/year. Furthermore, the total potential electricity generation tended to increase yearly due to continually growing nationwide demand. In 2030, the total

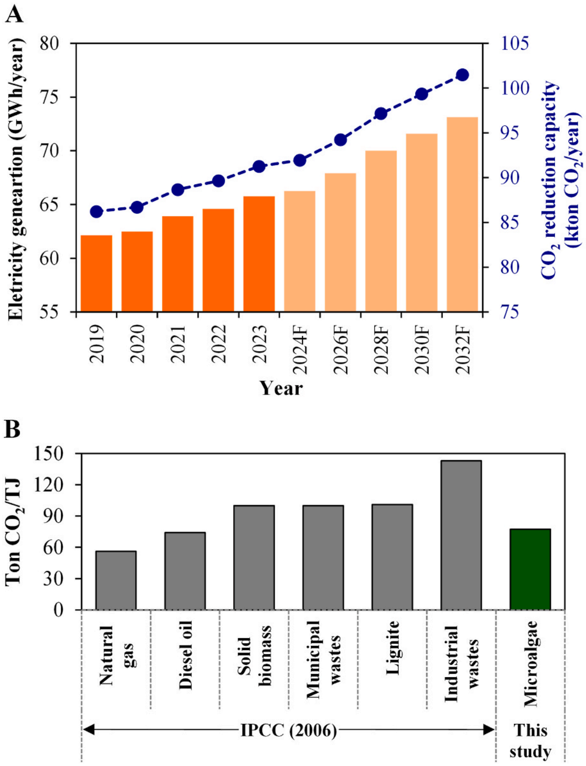

Fig. 5. The potential electricity generation and environmental impact utilizing microalgal biofilm biomass for the treatment of $ADEC_W$: (A) potential electricity generation and total CO_2 reduction capacity and (B) CO_2 emission factor for electricity generation from microalgal biomass compared to conventional fuels.

electricity generation is forecasted to reach as high as 72 GWh/year, which results in an annual benefit of over 7.4 million USD/year [49]. Although the potential electricity generated by laying hens nationwide accounted for less than 1 % of the total electricity demand in Thailand (197,271 GWh/year in 2022) [50], this provided a good starting point for substituting fossil fuels for electricity generation by renewable resources as well as electricity cost savings in egg production for poultry farms. These results indicated that the microalgae-based wastewater treatment is becoming attractive for sustainable development and circular economy concept by enhancing the utilization of renewable resources with innovative technology and thereby offers a further opportunity for achieving the Sustainable Development Goals (SDGs) at the global level [51,52].

Additionally, as further illustrated in Fig. 5A, microalgae cultivating using the ADE of laying hen wastes could reach the total CO_2 reduction capacity through the photosynthesis of 91 kton CO_2/year in 2023 and possible to reach as high as 99 kton CO_2/year by 2030, which equal to the CO_2 emission per capita for nearly 27000 people [50]. The results clearly showed that the treatment of poultry wastes by microalgae was a sustainable alternative to reduce the effect of global warming and climate change through the capability of CO_2 sequestration via photosynthesis process [30,53]. Besides, microalgae provide a positive environmental benefit to reduce the CO_2 emission when using microalgal biomass as energy substrate for electricity production compared to other conventional fuels. Fig. 5B shows that the CO_2 emission factor for electricity from microalgal biomass was 77 Ton CO_2/TJ, which lower than that of industrial wastes, lignite, solid biomass and municipal wastes by 85 %, 31 %, 29 % and 29 %, respectively (IPCC, 2006) [31].

4. Conclusions

This study provides evidence that the use of integrating microalgae cultivation with the $ADEC_W$ is an efficient and sustainable technology for poultry waste management. In particular, biofilm microalgal system has successfully developed with a high treatment performance, which gave average removal efficiencies of NH_4^+-N, PO_4^{3-}-P and dissolved COD of 97 %, 93 % and 75 %, respectively, at the cultivation time of 14 days. Whereas suspended microalgae system removed 92 % NH_4^+-N, 87 % PO_4^{3-}-P and 68 % dissolved COD. Compared to suspended microalgal system, biofilm system provides advantages of simple harvesting and high biomass production that implies substantial potential to use as sustainable source for value-added products and shows the positive environmental impact on CO_2 emission. The next step forward is to further explore and optimize electricity generation and CO_2 reduction utilizing the biomass from suspended and biofilm microalgal systems cultivated with the $ADEC_W$ for a practical implementation. This study shows that microalgal biofilm-based treatment of poultry wastes exhibited promising potentials for electricity generation and CO_2 reduction capacity through CO_2 sequestration and low electricity CO_2 emission factor. The study offers a promising alternative for the efficient treatment and valuable resource recovery of poultry wastes, which beneficial for poultry farmers and other stakeholders to improve sustainability of the waste management in the future.

CRediT authorship contribution statement

Rungnapha Khiewwijit: Writing – review & editing, Writing – original draft, Visualization, Resources, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Siraprapa Chainetr:** Writing – review & editing, Supervision, Project administration, Conceptualization. **Surasit Thiangchanta:** Writing – review & editing, Supervision, Conceptualization. **Khanchit Ngoenkhumkhong:** Supervision, Resources.

Data availability statement

Data will be made available on request.

Declaration of competing interest

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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References

[1] M. Miller, A. Gerval, J. Hansen, G. Grossen, Poultry expected to continue leading global meat imports as demand rises 1490–2022–955, United States Department of Agricultural (USDA) Economic Research Service, Amber Waves, 2022, pp. 1–9.
[2] UN, World Population Prospects 2022: Summary of Results, Technical Report, United Nations Department of Economic and Social Affairs, Population Division, New York, 2022.
[3] ChickenFans, Poultry industry statistics (2023): meat & egg production. https://www.chickenfans.com/poultry-industry-statistics/#Global-Egg-Production, 2023. (Accessed 17 April 2024).
[4] USDA Foreign Agricultural Service, Production – chicken meat. https://fas.usda.gov/data/production/commodity/0115000, 2024. (Accessed 17 April 2024).
[5] USDA Foreign Agricultural Service, Livestock and poultry: world markets and trade. United States Department of Agriculture Foreign Agricultural Service, United States Department of Agriculture, USA, 2024.
[6] Thailand's DLD, Report of Thai Livestock Standardized Farm, Department of Livestock Development, Bangkok, Thailand, 2023. http://predict.dld.go.th.
[7] Thailand's Regional Commercial Affairs Division, Agricultural Products Data (Fact Sheet), Ministry of Commerce, Bangkok, Thailand, 2024. https://regional.moc.go.th/th/content/category/index/id/3539.
[8] S. Sakar, K. Yetilmezsoy, E. Kocak, Anaerobic digestion technology in poultry and livestock waste treatment – a literature review, Waste Manag. Res. 27 (2009) 3–18, https://doi.org/10.1177/0734242X07079060.
[9] A. Błażejewska, M. Zalewska, A. Grudniak, M. Popowska, A comprehensive study of the microbiome, resistome, and physical and chemical characteristics of chicken waste from intensive farms, Biomolecules 12 (2022) 1132, https://doi.org/10.3390/biom12081132.
[10] A.J. Ashworth, J.P. Chastain, P.A. Moore Jr., Nutrient characteristics of poultry manure and litter, in: H.M. Waldrip, P.H. Pagliari, Z. He (Eds.), Animal Manure: Production, Characteristics, Environmental Concerns, and Management, ASA Special Publications, Madison, USA, 2020, pp. 63–87, https://doi.org/10.2134/asaspecpub67.c5.
[11] R. Rajagopal, S.E. Mousavi, B. Goyette, S. Adhikary, Coupling of microalgae cultivation with anaerobic digestion of poultry wastes: toward sustainable value added bioproducts, Bioengineering 8 (2021) 57, https://doi.org/10.3390/bioengineering8050057.
[12] A. Reza, L. Chen, X. Mao, Response surface methodology for process optimization in livestock wastewater treatment: a review, Heliyon 10 (2024) e30326, https://doi.org/10.1016/j.heliyon.2024.e30326.
[13] R. Kadam, S. Jo, J. Lee, K. Khanthong, H. Jang, J. Park, A review on anaerobic co-digestion of livestock manures in the context of sustainable waste management, Energies 17 (2024) 546, https://doi.org/10.3390/en17030546.
[14] G. Li, X. Bai, H. Li, Z. Lu, Y. Zhou, Y. Wang, J. Cao, Z. Huang, Nutrients removal and biomass production from anaerobic digested effluent by microalgae: a review, Int. J. Agric. Biol. Eng. 12 (2019) 8–13, https://doi.org/10.25165/j.ijabe.20191205.3630.
[15] Z. Zhang, Z. Xu, X. Song, B. Zhang, G. Li, N. Huda, W. Luo, Membrane processes for resource recovery from anaerobically digested livestock manure effluent: opportunities and challenges, Curr. Pollut. Rep. 6 (2020) 123–136, https://doi.org/10.1007/s40726-020-00143-7.
[16] C.M. Sichel-Crespo, E.Y. Ortiz-Montoya, N.H. Caicedo-Ortega, F. Machuca-Martínez, State of microalgae-based swine manure digestate treatment: an overview, Heliyon 8 (2022) e11256, https://doi.org/10.1016/j.heliyon.2022.e11256.
[17] G. Mohammadkhani, A. Mahboubi, M. Plöhn, C. Funk, P. Ylitervo, The potential of Nordic microalgae in nutrient removal from anaerobic digestion effluents, Physiol. Plantarum 176 (2024) e14153, https://doi.org/10.1111/ppl.14153.
[18] K. Reansuwan, S. Nitayavardhana, S. Koonaphapdeelert, Microalga *Chlorella* sp. in the cultivation with chicken farm biogas fermenter effluent and simultaneously nutrient removal, Biomass Convers. Biorefin. 13 (2023) 13553–13562, https://doi.org/10.1007/s13399-022-02670-3.
[19] M. Singh, D.L. Reynolds, K.C. Das, Microalgal system for treatment of effluent from poultry litter anaerobic digestion, Bioresour. Technol. 102 (2011) 10841–10848, https://doi.org/10.1016/j.biortech.2011.09.037.
[20] E. Geremia, M. Ripa, C.M. Catone, S. Ulgiati, A review about microalgae wastewater treatment for bioremediation and biomass production—a new challenge for Europe, Environments 8 (2021) 136, https://doi.org/10.3390/environments8120136.
[21] H. Jia, Q. Yuan, Removal of nitrogen from wastewater using microalgae and microalgae–bacteria consortia, Cogent Environ. Sci. 2 (2016) 1275089, https://doi.org/10.1080/23311843.2016.1275089.
[22] M. Plöhn, O. Spain, S. Sirin, M. Silva, C. Escudero-Oñate, L. Ferrando-Climent, Y. Allahverdiyeva, C. Funk, Wastewater treatment by microalgae, Physiol. Plantarum 173 (2021) 568–578, https://doi.org/10.1111/ppl.13427.
[23] A. Richmond, Q. Hu, Handbook of Microalgal Culture: Applied Phycology and Biotechnology, second ed., John Wiley & Sons, New Jersey, USA, 2013.
[24] B. Bisht, M. Verma, R. Sharma, P.K. Chauhan, K. Pant, H. Kim, M.S. Vlaskin, V. Kumar, Development of yeast and microalgae consortium biofilm growth system for biofuel production, Heliyon 9 (2023) e19353, https://doi.org/10.1016/j.heliyon.2023.e19353.

[25] N.C. Boelee, H. Temmink, M. Janssen, C.J.N. Buisman, R.H. Wijffels, Nitrogen and phosphorus removal from municipal wastewater effluent using microalgal biofilms, Water Res. 45 (2011) 5925–5933, https://doi.org/10.1016/j.watres.2011.08.044.
[26] R. Khiewwijit, K. Panyaping, P. Wongpankamol, Nutrient removal by suspended and biofilm microalgae for treating the wastewater of agro-industrial pig farm, Walailak J. Sci. Technol. 16 (2019) 791–803, https://doi.org/10.48048/wjst.2019.4252.
[27] M. Stockenreiter, F. Haupt, J. Seppälä, T. Tamminen, K. Spilling, Nutrient uptake and lipid yield in diverse microalgal communities grown in wastewater, Algal Res. 15 (2016) 77–82, https://doi.org/10.1016/j.algal.2016.02.013.
[28] S.K. Prajapati, P. Kumar, A. Malik, V.K. Vijay, Bioconversion of algae to methane and subsequent utilization of digestate for algae cultivation: a closed loop bioenergy generation process, Bioresour. Technol. 158 (2014) 174–180, https://doi.org/10.1016/j.biortech.2014.02.023.
[29] D. Mu, M. Min, B. Krohn, K.A. Mullins, R. Ruan, J. Hil, Life cycle environmental impacts of wastewater-based algal biofuels, Environ. Sci. Technol. 48 (2014) 11696–11704, https://doi.org/10.1021/es5027689.
[30] V. Anguselvi, R.E. Masto, A. Mukherjee, P.K. Singh, CO_2 capture for industries by algae, in: Y.K. Wong (Ed.), Algae, IntechOpen, London, UK, 2019, pp. 1–11, https://doi.org/10.5772/intechopen.81800.
[31] IPCC, in: S. Eggleston, L. Buendia, K. Miwa, T. Ngara, K. Tanabe (Eds.), IPCC Guidelines for National Greenhouse Gas Inventories, Prepared by the National Greenhouse Gas Inventories Programme, Institute for Global Environmental Strategies (IGES), Hayama, Japan, 2006.
[32] R. Raja, H. Shanmugam, V. Ganesan, I.S. Carvalho, Biomass from microalgae: an overview, J. Oceanogr. Mar. Sci. 2 (2014) 1–7, https://doi.org/10.4172/2332-2632.1000118.
[33] APHA, Standard Methods for the Examination of Water and Wastewater, American Public Health Association, American Water Works Association, Water Pollution Control Federation, 2012.
[34] N.C. Boelee, Microalgal Biofilms for Wastewater Treatment, Wageningen University, The Netherlands, 2013. Doctoral Thesis.
[35] J. Sátiro, A. Cunha, A.P. Gomes, R. Simões, A. Albuquerque, Optimization of microalgae–bacteria consortium in the treatment of paper pulp wastewater, Appl. Sci. 12 (2022) 5799, https://doi.org/10.3390/app12125799.
[36] K. Larsdotter, Wastewater treatment with microalgae - a literature review, Vatten 62 (2006) 31–38.
[37] Metcalf and Eddy, Wastewater Engineering, Treatment and Resource Recovery, fifth ed., McGraw-Hill, USA, 2013.
[38] N. Kumar, C. Banerjee, S. Jagadevan, Identification, characterization, and lipid profiling of microalgae *Scenedesmus* sp. NC1, isolated from coal mine effluent with potential for biofuel production, Biotechnol. Rep. 30 (2021) 00621, https://doi.org/10.1016/j.btre.2021.e00621.
[39] S.-K. Kim, Handbook of Marine Microalgae: Biotechnology Advances, Academic Press, USA, 2015, https://doi.org/10.1016/C2013-0-19117-9.
[40] T.T. Nguyen, Q.A. Binh, X.T. Bui, H.H. Ngo, H.N. Vo, K.Y. Lin, W. Guo, C. Lin, F. Breider, Co-culture of microalgae-activated sludge for wastewater treatment and biomass production: exploring their role under different inoculation ratios, Bioresour. Technol. 314 (2020) 123754, https://doi.org/10.1016/j.biortech.2020.123754.
[41] S. Chainetr, R. Khiewwijit, N. Maneetien, S. Chaiwongsar, Chicken slaughterhouse wastewater characteristics, current treatment and future challenges: a review, RMUTL. Eng. J. 5 (2020) 41–55, https://doi.org/10.14456/rmutlengj.2020.6.
[42] M. Hladíková, R. Šulc, Selection of a separation method used for harvesting of microalgae from aqueous solutions, Chem. Eng. Trans. 86 (2021) 157–162, https://doi.org/10.3303/CET2186027.
[43] D.L. Sutherland, J. Burke, P.J. Ralph, Increased harvest frequency improves biomass yields and nutrient removal on a filamentous algae nutrient scrubber, Algal Res. 51 (2020) 102073, https://doi.org/10.1016/j.algal.2020.102073.
[44] P. Choudhary, S.K. Prajapati, P. Kumar, A. Malik, K.K. Pant, Development and performance evaluation of an algal biofilm reactor for treatment of multiple, Bioresour. Technol. 224 (2017) 276–284, https://doi.org/10.1016/j.biortech.2016.10.078.
[45] L. Huang, Y. Jin, D. Zhou, L. Liu, S. Huang, Y. Zhao, Y. Chen, A review of the role of extracellular polymeric substances (EPS) in wastewater treatment systems, Int. J. Environ. Res. Publ. Health 19 (2022) 12191, https://doi.org/10.3390/ijerph191912191.
[46] W. Babiak, I. Krzemińska, Extracellular polymeric substances (EPS) as microalgal bioproducts: a review of factors affecting EPS synthesis and application in flocculation processes, Energies 14 (2021) 4007, https://doi.org/10.3390/en14134007.
[47] P. Srimongkol, P. Sangtanoo, P. Songserm, W. Watsuntorn, A. Karnchanatat, Microalgae-based wastewater treatment for developing economic and environmental sustainability: current status and future prospects, Front. Bioeng. Biotechnol. 10 (2022) 904046, https://doi.org/10.3389/fbioe.2022.904046.
[48] OECD/FAO, OECD-FAO Agricultural Outlook 2023-2032, Organisation for Economic Co-operation and Development (OECD) and Food and Agriculture Organization of the United Nations (FAO), OECD Publishing, Paris, 2023, https://doi.org/10.1787/08801ab7-en.
[49] Thailand's PEA, Electricity Tariffs, Provincial electricity authority, Bangkok, Thailand. https://www.pea.co.th/electricity-tariffs, 2024.
[50] EPPO, Energy Statistics of Thailand 2023, Energy Policy and Planning Office, 2023. Bangkok, Thailand.
[51] C. Kettner, D. Kletzan-Slamanig, A. Köppl, B. Littig, I. Zielinska, Monitoring sustainable development: climate and energy policy indicators, J. Sustain. Res. 2 (2020) e200027, https://doi.org/10.20900/jsr20200027.
[52] L. Lamprinakis, Circular Regulations (CR) for bioeconomy development, J. Sustain. Res. 2 (2020) e200021, https://doi.org/10.20900/jsr20200021.
[53] M. Adamczyk, J. Lasek, A. Skawińska, CO_2 biofixation and growth kinetics of *Chlorella vulgaris* and *Nannochloropsis gaditana*, Appl. Biochem. Biotechnol. 179 (2016) 1248–1261, https://doi.org/10.1007/s12010-016-2062-3.