Pol. J. Environ. Stud. Vol. XX, No. X (XXXX), 1-9
DOI: *10.15244/pjoes/208927* ONLINE PUBLICATION DATE: 2025-12-29

Original Research

An Integrated Approach to Swine Wastewater Remediation and Indole-3-Acetic Acid Production using Cyanobacteria

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Received: 20 May 2025
Accepted: 3 August 2025

Abstract

This study investigated the growth, indole-3-acetic acid (IAA) production, and remediation capability of the cyanobacteria *Planktothrix spiroides* sp. in real swine wastewater under various influencing factors, including wastewater concentration, L-tryptophan supplement, and pH level. Similar effects were observed on the biomass growth, IAA production, and remediation performance of the microalgae under these factors, confirming the major role of *Planktothrix spiroides* sp. in swine wastewater treatment. The optimal conditions for growth, IAA synthesis, and remediation of the microalgae were obtained at a 1.3X dilution rate, 0.2 g L^{-1} of L-tryptophan addition, and a pH level of 9. Under these conditions, the highest biomass and IAA production were 64.1 ± 1.2 mg L^{-1} d^{-1} and 1.46 ± 0.05 μg mL^{-1}

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d^{-1}, respectively. The optimized growth corresponded to high remediation efficiencies of 79.8 ± 2.4 % for COD removal (COD removal rate at 46.7 ± 1.4 mg O_2 L^{-1} d^{-1}), 95.8 ± 0.1 % for TN removal (TN removal rate at 21.2 ± 0.01 mg N L^{-1} d^{-1}), and 87.1 ± 0.5 % for TP removal (TP removal rate at 4.0 ± 0.01 mg P L^{-1} d^{-1}). In contrast, the conditions, including undiluted swine wastewater, L-tryptophan concentration of up to 0.3 g L^{-1} or higher, and acidic pH levels (pH 5 and 6), showed inhibitory impacts on the growth of *Planktothrix spiroides* sp. and subsequently its remediation and IAA production capabilities. Results from this study confirmed the potential of cyanobacteria *Planktothrix spiroides* sp. in swine wastewater remediation combined with biomass and IAA production. Applications in larger-scale systems are required in the future, with a primary focus on enhancing the IAA production capability of microalgae.

Keywords: Cyanobacteria, swine wastewater, indole-3-acetic acid, nutrient recovery

Introduction

Overpopulation and economic growth have increased the demand for pork worldwide. The world consumed 110.5 million metric tons of pork every year during the 2019 – 2021 period, while the future pork demand was anticipated to be 128.9 million metric tons by 2031 [1].

Consequently, enormous amounts of resources are required to breed around 1.5 billion pigs annually, including 455 Mton of feed, 548.9 km^3 of water, and 115.5 Mha of agricultural area worldwide [2]. The swine production process, however, also generates wastewater consisting of urine and feces excreted by the pigs and wastewater from cleaning activities and/or runoff water, with high nitrogen and phosphorus contents of 10 – 80 g N pig^{-1} d^{-1} and 4.5 – 59.0 g P_2O_5 pig^{-1} d^{-1}, respectively [3]. Biogas digesters have been widely used as the preliminary treatment step for swine wastewater, especially in small-scale facilities, to remove the majority of solids and partly degrade the nutrient contents of the wastewater [4]. Nevertheless, the product biogas digestate still contains high concentrations of nutrients, up to 5800 mg N L^{-1} and 56.4 mg P L^{-1} [5], hence posing a huge burden to the centralized treatment system and subsequently the environment. Recently, under the context of sustainable development, the nutrients in swine wastewater have been considered as potential resources to be recovered, and one of the most promising solutions for remediating swine wastewater is via microalgae technology [6]. Microalgae have shown their capability of producing valuable products such as cosmetics, biofuels, fertilizers, pharmaceuticals, or feeds by utilizing nutrients from wastewater [7], of which the nutrient-rich swine wastewater is a promising resource.

Indole-3-acetic acid (IAA) is one of the most prevalent plant hormones in the auxin group, playing an indispensable role in plant growth. At appropriate concentrations, IAA contributes significantly to the development of stems, roots, and fruits, stimulates cell division, and enhances plant resistance to external stressors [8]. Similarly, IAA has been reported to promote the growth of various microorganisms and strengthen microbial cell defense mechanisms under adverse conditions such as drought and low temperatures [9]. IAA can be synthesized either by plants or by plant-associated microorganisms, including bacteria and fungi, through tryptophan-dependent or tryptophan-independent pathways, which respectively involve the presence or absence of tryptophan, a rare and energetically costly amino acid serving as a precursor for IAA biosynthesis [10]. Microbial IAA production predominantly occurs via the tryptophan-dependent route, particularly in the presence of L-tryptophan, and the extracted IAA has been utilized to promote the growth of plants and microorganisms or as a natural herbicide for weed control [11–13]. Consequently, microbial-derived IAA has emerged as an eco-friendly alternative to synthetic chemicals, aligning with sustainable agricultural practices [8].

Different microalgae species have been known to produce IAA, commonly with the supplement of tryptophan, suggesting their great potential in IAA mass production due to the high productivity [13–15]. Besides, as microalgal cells also contain high concentrations of valuable constituents, including carbohydrates, proteins, and lipids, microalgae could offer a dual-purpose application as both fertilizer and IAA supplement to promote plant growth [12]. Moreover, the use of wastewater as a nutrient source for microalgae production could provide additional value to the process via achieving nutrient recovery and wastewater remediation [16]. Various factors, including nutrient concentration, light intensity, irradiance regime, L-tryptophan dosage, pH, or CO_2 aeration, were reported to influence biomass growth and IAA production of microalgae [16–18]. Nevertheless, more studies should be conducted to provide further insights into the applicability of microalgae for wastewater remediation and IAA production [19]. This study aimed to assess the performance of microalgae *Planktothrix spiroides* sp. nov. (*Oscillatoriales*, Cyanobacteria) on swine wastewater remediation, biomass growth, and IAA production. The impacts of wastewater concentration, L-tryptophan supplement dose, and pH level were also investigated. The results of this study were then compared with previous works on IAA production by microalgae, and its future application was also discussed.

Materials and Methods

Microalgae Inoculum and Swine Wastewater

An axenic culture containing enriched biomass of the cyanobacterium *Planktothrix spiroides* sp. nov. (*Oscillatoriales*) was obtained from the Institute of Science and Technology for Energy and Environment, Vietnam Academy of Science and Technology, Hanoi, Vietnam. This strain was selected due to its ability to produce IAA [20].

The swine wastewater used in this study was the effluent of an underground biogas tank, which treated the mixture of pigs' urine and feces and the pig barn cleaning wastewater from a household pig farm in Hoai Duc District, Hanoi, Vietnam. The raw wastewater was allowed to settle overnight and subsequently filtered using filter paper with a pore size of 20 – 25 µm to remove large particulate matter. The filtered swine wastewater was then used as a cultural medium for microalgae cultivation.

Experimental Setup

In this study, the axenic culture of cyanobacteria *Planktothrix spiroides* was inoculated into swine wastewater, and the influences of initial concentrations of swine wastewater and L-tryptophan supplement and the pH of the culturing medium on treatment efficiencies, biomass growth, and IAA production of the cyanobacteria *P. spiroides* were assessed. Different concentrations of the swine wastewater were achieved by diluting with tap water at various dilution rates of 4 times (4X), 2 times (2X), 1.3 times (1.3X), and no dilution (1X). In another test, five initial L-tryptophan concentrations were applied, ranging from 0.1 to 0.5 g L^{-1}, to investigate the impacts of this precursor on microalgae growth, treatment, and IAA production. These L-tryptophan concentrations were in the same range (between 0.25 and 3.0 g L^{-1}) as applied in other studies [12, 17]. The impacts of five pH levels, including 5, 6, 7, 8, and 9, were also investigated, which was comparable to the pH range suggested by a previous study [17]. The pH levels were maintained daily by adding HCl or NaOH. The detailed experimental conditions are listed in Table 1.

All experiments were conducted in triplicate under controlled laboratory conditions for 8 days. Cultures were maintained under constant illumination at 5,000 lux using 10 W LED light bulbs (Vietnam) for 8 hours per day. Each test was conducted in a 1 L Erlenmeyer flask with a working volume of 500 mL. Continuous aeration was provided using a central ring blower pump (Model RB-200, Seiko, Vietnam) connected to each flask through sterile air tubing and a 0.2 µm air filter, ensuring proper mixing and effective air–liquid gas exchange.

Analytical Methods

A sample of the mixed liquor was collected in each flask every 2 days to measure the biomass as total suspended solids (TSS) (ISO 11923:1997). Other parameters, including chemical oxygen demand (COD) (colorimetric method, SMEWW 5220D:2012), total nitrogen (TN) (catalytic digestion after reduction with Devarda's alloy method, ISO 10048:1991), total phosphorus (TP) (ascorbic acid method, SMEWW 4500P C:2012), and IAA (TCVN 10784:2015) [21], were analyzed at the beginning and the end of the experiment.

Data Analysis

The performance of cyanobacteria *P. spiroides* in swine wastewater treatment was evaluated by the nutrient removal efficiency (%) = $(C_0 - C_t)/C_0 \times 100$ % and rate (mg L^{-1} d^{-1}) = $(C_0 - C_t)/(t - t_0)$ where C_0 and C_t are the concentrations in the mixed liquor expressed in mg L^{-1} at initial time t_0 and at the measurement time t (day).

The growth of cyanobacteria *P. spiroides* and its capability of IAA production were assessed by the productivity = $(X_t - X_0)/(t - t_0)$ of biomass and IAA. Where the biomass productivity was reported as mg of biomass L^{-1} d^{-1}, while the unit of IAA productivity was µg mL^{-1} d^{-1}; X_t and X_0 are biomass concentration (mg biomass L^{-1}) and IAA concentration (µg mL^{-1}) at time t and initial time t_0, respectively.

The concentrations of different wastewater constituents, IAA, microalgae biomass, treatment efficiencies, and the productivities of microalgae and IAA were reported in average values and their corresponding standard deviations. Multiple comparisons between data sets that were not normally distributed were performed using the Kruskal–Wallis tests. Then, in case of any significant difference detected, the post-hoc Dunn tests were used subsequently for the pairwise comparisons. The significance level applied in this study was $p \leq 0.05$. Data analysis and visualization were conducted using Microsoft Excel version 2016 and R software - R version 4.4.1 (R Core Team, 2016).

Results and Discussion

Microalgal Swine Wastewater Remediation

Among the different dilution rates applied in this study, a high level of remediation performance, especially in terms of removal rate, was obtained in swine wastewater with a 1.3X dilution rate. Poorer results were observed with a 4X dilution rate ($p < 0.05$) (Figs. 1a, 1d, and 1g). No significant difference was found between COD removal efficiencies in all dilution rates. The cyanobacteria *P. spiroides* cultivated in the original swine wastewater (1X dilution rate) showed the lowest TN and TP removal efficiencies, statistically lower than the results at 1.3X and 2X dilution rates, respectively ($p < 0.05$). The cyanobacterium *P. spiroides* exhibited remarkable tolerance to high-strength swine wastewater, a significant advantage for practical applications, as it eliminated the need for substantial dilution. Interestingly, nutrient removal efficiency was inversely correlated with dilution rate, consistent with previous laboratory and field-scale studies demonstrating superior microalgal performance in concentrated waste streams [22, 23]. The diminished treatment capacity observed in undiluted wastewater was likely attributable to inhibitory factors such as elevated salinity and turbidity, which impair microalgal physiology through oxidative stress induction and light attenuation, respectively [24].

A clear impact of L-tryptophan supplementation on the swine wastewater remediation capability of cyanobacteria *P. spiroides* was observed in this study. Microalgal performance peaked at 0.2 g L^{-1} and significantly decreased as the L-tryptophan concentration increased, with the lowest results obtained at 0.5 g L^{-1} ($p < 0.05$) (Fig. 1b, 1e, and 1h). This suggests that higher L-tryptophan concentrations lead to increased IAA levels, which in turn negatively affect microalgal growth when the concentrations exceed the optimal dose [17, 25]. Cruz et al. (2024) [12] also reported growth reductions in *Chlorella fusca* LEB 111 and *Spirulina sp.* LEB at L-tryptophan concentrations of 2.0 and 3.0 g L^{-1}, respectively, possibly due to excessive IAA production.

In addition, the swine wastewater remediation performance by *P. spiroides* was generally improved under neutral to alkaline pH conditions (pH 7–9), compared to acidic pH conditions (pH 5 and 6) ($p < 0.05$) (Fig. 1c, 1f, and 1i). In particular, negligible COD reduction was observed in all tests under acidic conditions, resulting in undetectable COD removal efficiencies and rates. An optimal pH range of 7–9 was also recommended for *Chlorella sp.* HQ in wastewater treatment and biofuel production [26], while Bartley et al. (2014) [27] reported enhanced growth of *Nannochloropsis salina* at pH 8 and 9, compared to lower pH levels of 5–7. In microalgae-based wastewater treatment systems, the removal of COD was commonly achieved via a bacterial oxidation process that degrades organic matter by utilizing dissolved oxygen generated from microalgal photosynthesis [22]. Moreover, constant aeration was applied in all experiments of this study for proper mixing and thus satisfying a part of the oxygen demand from the bacterial oxidation process. This dissolved oxygen supplement could be particularly important in experiments with unfavorable conditions for microalgal growth, such as in undiluted swine wastewater or high L-tryptophan concentration, which maintained certain COD removal levels. Therefore, the undetectable COD removal obtained in acidic pH conditions suggests that both microalgae and bacteria in swine wastewater were inhibited. A similar conclusion was made by Kokina et al. (2022) that a low pH level of 6.5 showed adverse effects on the organic matter oxidation and the nitrification processes of bacteria in municipal wastewater [28].

Overall, the best performance was observed in the conditions of 1.3X dilution, 0.2 g L^{-1} of L-tryptophan concentration, and pH 9, at 79.8 ± 2.4 % and 46.7 ± 1.4 mg O_2 L^{-1} d^{-1} of COD removal efficiency and removal rates, respectively. Under these conditions, the cyanobacteria *Planktothrix spiroides* sp. could achieve 95.8 ± 0.1 % of TN removal efficiency (TN removal rate of 21.2 ± 0.01 mg N L^{-1} d^{-1}) and 87.1 ± 0.5 % of TP removal efficiency (TP removal rate of 4.0 ± 0.01 mg P L^{-1} d^{-1}). These results were at a high level compared to other similar studies employing microalgae for swine wastewater treatment, which commonly had ranges of 12.5 – 90 % of TN removal, 28 – 97 % of TP removal, and 21 – 80 % of COD removal [24]. Comparable results were reported in a study employing microalgae *Chlorella vulgaris* for swine wastewater remediation, showing 36.1 – 65.2 % (3.8 – 6.8 mg O_2 L^{-1} d^{-1}) of COD removal, 61.3 – 92.2 % (3.4 – 12.2 mg N L^{-1} d^{-1}) of TN removal, and 71.8 – 82.4 % (0.1 – 0.3 mg P L^{-1} d^{-1}) of TP removal [22].

Microalgal Growth and IAA Production

Good compatibility of cyanobacteria *Planktothrix spiroides* sp. in swine wastewater with low dilution rates was further confirmed via the high biomass concentrations obtained at the end of the 2X and 1.3X experiments, translating to high biomass productivity levels (Fig. 2a). The highest IAA productivity was also achieved in the 1.3X experiment ($p < 0.05$), associated with a high final IAA concentration at 5.0 ± 0.3 μg mL^{-1} (Fig. 2d). The lowest biomass and IAA productivities ($p < 0.05$) were observed in undiluted swine wastewater, which was consistent with the remediation performance, possibly due to the unfavorable conditions in this wastewater.

The biomass growth and IAA production of the microalgae under different L-tryptophan concentrations were also in line with its remediation performance, in that the highest biomass and IAA productivities were achieved at 0.2 g L^{-1} of L-tryptophan concentration. Besides, lower final biomass and IAA concentrations were obtained at higher L-tryptophan levels, resulting in lower productivities with the lowest values observed at the highest L-tryptophan concentration of 0.5 g L^{-1} ($p < 0.05$) (Fig. 2b and 2e). In comparison, Ahmed et al. (2014) [17] reported a continuous enhancement in IAA production of microalgae *Chroococcidiopsis* sp. MMG-5 and *Synechocystis* sp. MMG-8 by increasing the L-tryptophan supplement from 0.25 to 1.5 g L^{-1}. In another study, a high IAA concentration of 10 μg mL^{-1} was shown to inhibit the biofilm development of microalgae [30]. In this study, inhibitory effects on the growth of *Planktothrix spiroides* sp. started to occur with L-tryptophan concentrations higher than 0.3 g L^{-1}.

Acidic pH levels (pH 5 and 6) showed adverse effects on the growth and IAA production of cyanobacteria

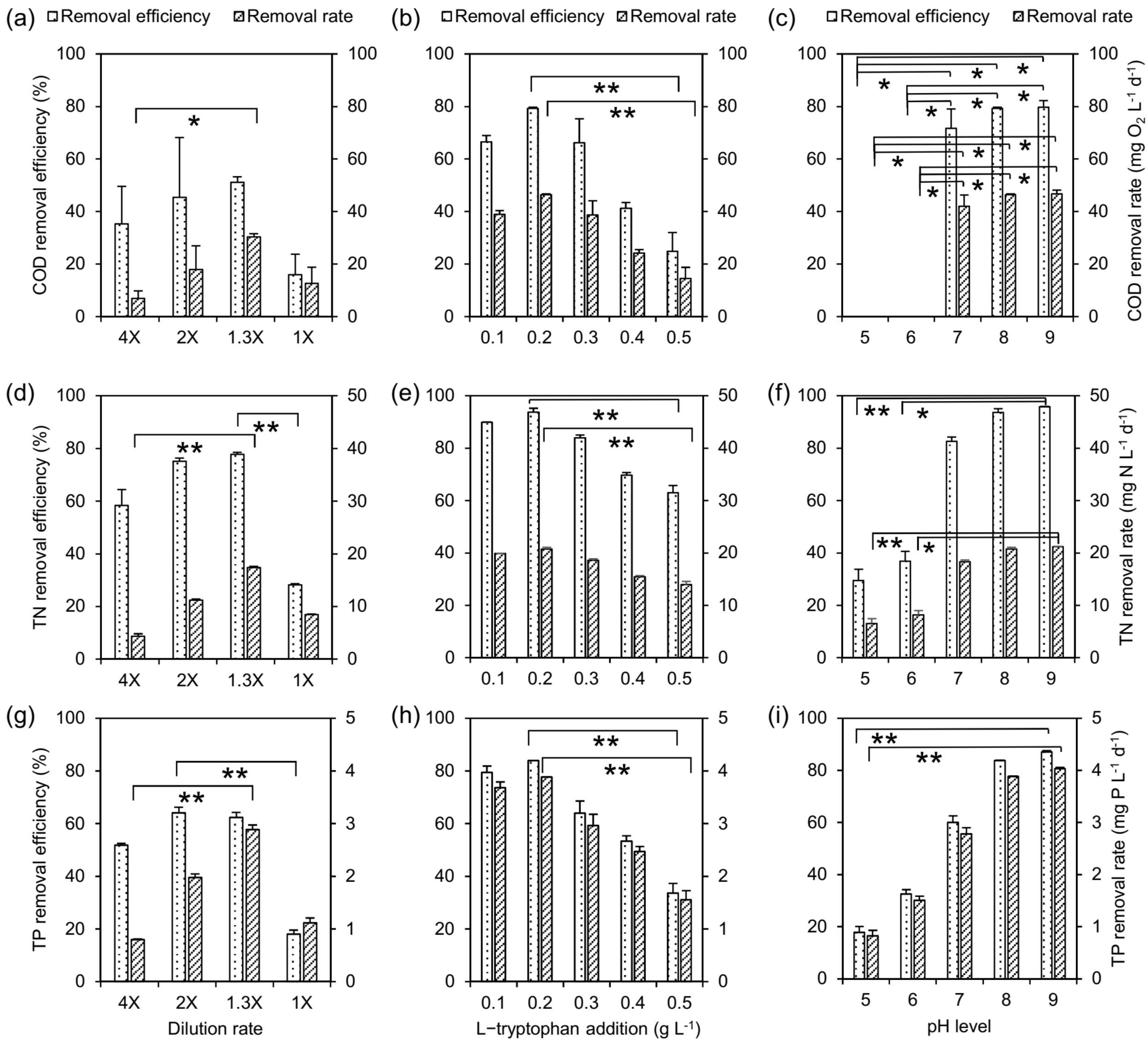

Fig. 1. Removal efficiencies (left axis) and removal rates (right axis) of COD: (a), (b), (c), TN: (d), (e), (f), and TP: (g), (h), (i) by the cyanobacteria *Planktothrix spiroides* sp. under different dilution rates: (a), (d), (g), L-tryptophan concentrations: (b), (e), (h), and pH levels: (c), (f), (i). The asterisks showed significant differences between the removal efficiencies or between removal rates, with *: $p < 0.05$ and **: $p < 0.01$.

Planktothrix spiroides sp. ($p < 0.05$). Higher levels of biomass and IAA were obtained at the end of the experiment with pH 7, 8, and 9 (Fig. 2c and 2f). Similar to the remediation performance, the highest biomass productivity was achieved at pH 9 ($p < 0.05$), at 64.1 ± 1.2 mg L^{-1} d^{-1}. A higher IAA concentration was also obtained under pH 9 conditions, reaching 12.3 ± 0.4 µg mL^{-1} at the end of the experiment, corresponding to the highest IAA productivity of 1.46 ± 0.05 µg mL^{-1} d^{-1} ($p < 0.05$). These outcomes are consistent with previous findings by López-Pacheco et al. (2019) [29], who emphasized the beneficial role of alkaline conditions in promoting nutrient uptake and photosynthetic efficiency in microalgal cultures, including in swine wastewater environments.

In general, similar impacts of swine wastewater concentration, L-tryptophan supplement, and pH level were observed on the growth, IAA production, and remediation performance of the cyanobacteria *Planktothrix spiroides* sp., suggesting the major role of the microalgae in swine wastewater treatment, especially in the removal of nutrients and providing excessive dissolved oxygen for organic matter oxidation by bacteria [22], as well as producing IAA. The biomass production and IAA synthesis capabilities achieved in this study were comparable to those of other studies employing microalgae for biomass and IAA production (Table 2). These works confirmed the potential of IAA production using microalgae, especially in combination with wastewater remediation [12, 16]. However, most of them were conducted on a small scale under laboratory conditions; thus, the applications of IAA production by microalgae in wastewater in larger-scale systems were required. Bunsangiam et al. (2021) [11] studied

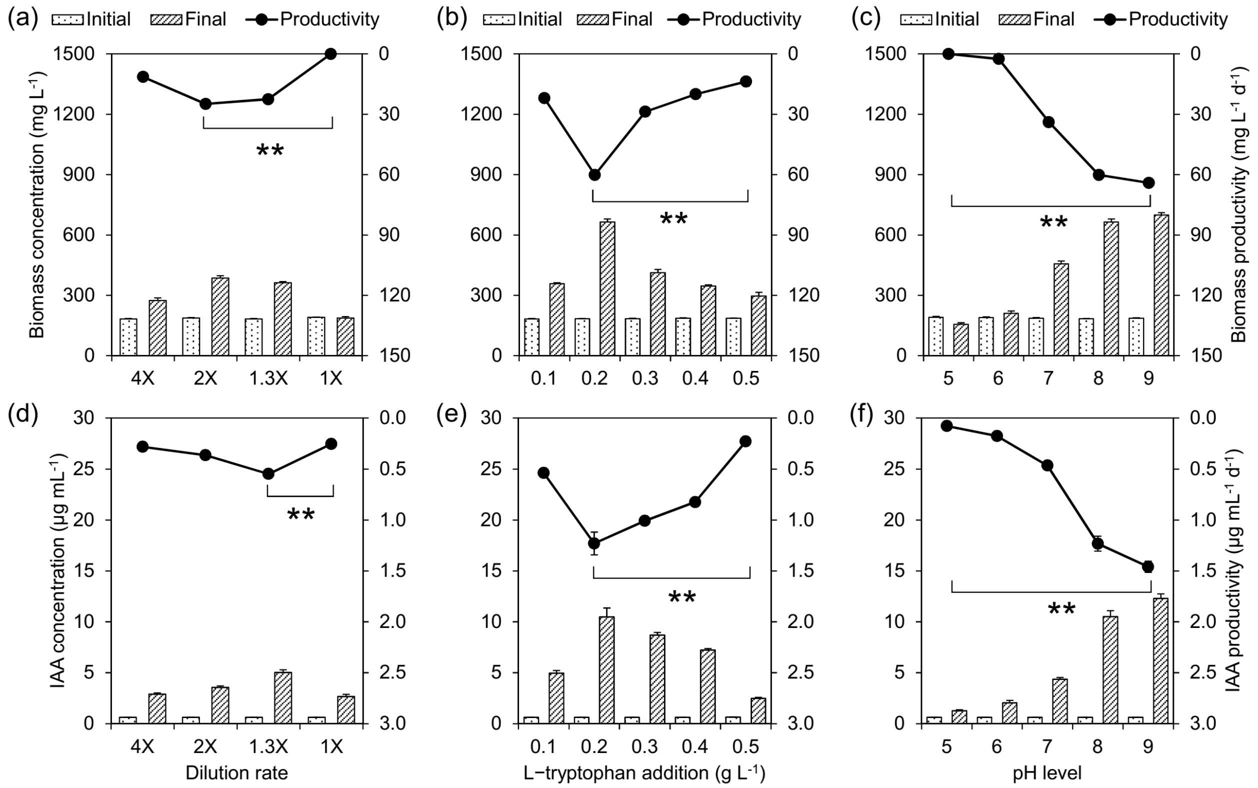

Fig. 2. Initial, final concentrations (left axis) and the productivities (right axis) of biomass (a), (b), (c), and IAA (d), (e), (f) achieved by the cyanobacteria *Planktothrix spiroides* under different dilution rates: (a), (d), L-tryptophan concentrations: (b), (e), and pH levels: (c), (f). Significant differences between biomass productivities or between IAA productivities were shown by the asterisks with **: $p < 0.01$.

the upscaling effects on IAA production of the yeast *Rhodosporidiobolus fluvialis* DMKU-CP293. They obtained an increase in IAA concentration from 2870.15 µg mL^{-1} to 3569.3 µg mL^{-1} by upscaling from a 2 L to a 100 L bioreactor, with a cost reduction from 0.74 to 0.6 USD g IAA^{-1}.

The Potential of Microalgae For Wastewater Remediation, Combined With Biomass and IAA Production

This work showed that the application of cyanobacteria *Planktothrix spiroides* sp. in IAA production, combined with swine wastewater remediation, is promising. The microalgae achieved high remediation performance in high-strength real swine wastewater, suggesting their potential for application on a larger scale. The unfavorable conditions of undiluted swine wastewater could be overcome by applying simple pretreatment steps, including large suspended matter removal and minimum dilution, which can be commonly achieved in reality by integrating with a simple stabilization pond [31]. The compatibility of the microalgae with high-strength swine wastewater also resulted in biomass productivity of up to 64.1 ± 1.2 mg L^{-1} d^{-1}, equivalent to an annual productivity of 23.4 kg m^{-3} yr^{-1}. In terms of IAA production, the highest productivity was achieved by cyanobacteria *P. spiroides* at an L-tryptophan supplement concentration of 0.2 g L^{-1}, at 1.46 ± 0.05 µg mL^{-1} d^{-1} or 532.9 g m^{-3} yr^{-1}. However, the IAA production capability of microalgae was lower compared to other microorganisms such as endophytic fungi, bacteria, or yeast, which could achieve high values of 24.2, 70, or 3569.3 µg mL^{-1}, respectively [11, 32, 33]. The highest IAA yield achieved by the cyanobacteria in this study was 4.2 ± 0.3 mg IAA g^{-1} L-tryptophan, potentially resulting in an estimated amount of 9.1 tons of L-tryptophan required for every m^3 annually. In comparison, the calculated IAA yield obtained by endophytic fungi *Aspergillus awamori* W11 was around 60 mg IAA g^{-1} L-tryptophan, while an extremely high yield of 680 mg IAA g^{-1} L-tryptophan was obtained from the yeast *Rhodosporidiobolus fluvialis* DMKU-CP293 [11, 32, 33]. These differences highlight the importance of yield efficiency in assessing the economic viability of tryptophan supplementation. Future research should focus on improving yield, reducing dosage, or using alternative, low-cost precursors.

Besides optimization of the growing conditions, such as pH level, L-tryptophan concentration, or upscaling, it was indicated that IAA production could be increased by applying genetic engineering, which was evident in some bacterial strains [8]. Genetically altered microalgae were applied for improving the production of valuable substances such as lipids, carotenoids, ethanol, or various amino acids [34], yet studies on how they

Table 1. Initial conditions of different experiments in this study.

Experiments		COD (mg COD L^{-1})	TN (mg N L^{-1})	TP (mg P L^{-1})	L-tryptophan (g L^{-1})	pH	Biomass (mg L^{-1})
Wastewater dilution rates	4X	158.3 ± 15.8	59.7 ± 6.0	12.4 ± 1.2	0.1	8	183.1 ± 2.0
	2X	316.5 ± 31.7	119.5 ± 11.9	24.7 ± 2.5	0.1	8	187.3 ± 1.5
	1.3X	474.8 ± 47.5	179.2 ± 17.9	37.1 ± 3.7	0.1	8	183.1 ± 1.0
	1X	633.0 ± 63.3	239.0 ± 23.9	49.4 ± 4.9	0.1	8	190.9 ± 1.1
L-tryptophan levels (g L^{-1})	0.1	468.6 ± 46.9	177.2 ± 17.7	37.1 ± 3.7	0.1	8	182.7 ± 1.5
	0.2				0.2	8	183.4 ± 1.5
	0.3				0.3	8	183.3 ± 2.3
	0.4				0.4	8	186.1 ± 2.0
	0.5				0.5	8	186.2 ± 0.7
pH levels	5	468.6 ± 46.9	177.2 ± 17.7	37.1 ± 3.7	0.2	5	191.0 ± 4.1
	6				0.2	6	190.2 ± 2.4
	7				0.2	7	185.7 ± 3.1
	8				0.2	8	183.4 ± 1.5
	9				0.2	9	186.6 ± 1.5

Table 2. Biomass growth and IAA production of different microalgae in various culture medium.

Species	Medium	Biomass productivity (mg L^{-1} d^{-1})	IAA concentration	References
Planktothricoides raciborskii	BG-11, Bold's Basal and Z8 mediums	Specific growth rate: 0.1 – 0.23 d^{-1}	5.5 ± 2.4 μg g^{-1} fresh weight	[19]
Chroococcidiopsis sp. MMG-5	BG-11 medium	n.a.*	extracellular: 45 μg g^{-1} chl-a intracellular: 27 μg g^{-1} chl-a	[17]
Synechocystis sp. MMG-8	BG-11 medium	n.a.	extracellular: 60 μg g^{-1} chl-a intracellular: 31 μg g^{-1} chl-a	
Scenedesmus obliquus (UTEX 393)	Modified Bold 3N Medium	Specific growth rate: 0.25 – 0.65 d^{-1}	1.1 – 4.0 μmol L^{-1} (0.2 – 0.7 μg mL^{-1})	[18]
Synechocystis sp.	urban secondary effluent	49.0 – 73.1	72.15 ng g^{-1}	[16]
Phormidium sp.	urban secondary effluent	35.2 – 47.8	60.57 ng g^{-1}	
Scenedesmus sp.	urban secondary effluent	35.4	63.25 ng g^{-1}	
Chlorella fusca LEB 111	mixture of BG-11 medium and filtered dairy effluents	71.2 – 83.6	< 0.2 – 17 μg mL^{-1}	[12]
Spirulina sp. LEB 18	mixture of Zarrouk medium and filtered dairy effluents	144.4 – 202.4	< 0.2 – 18 μg mL^{-1}	
Rhodosporidiobolus fluvialis DMKU-CP293	Glucose-based medium	n.a.	3569.3 μg mL^{-1}	[11]
Chlorella vulgaris	Synthetic wastewater	50 – 80	3 – 7 μg mL^{-1}	[29]
Arthrospira maxima	Mixture of nejayote and swine wastewater	40 – 60	5 – 9 μg mL^{-1}	
Planktothrix spiroides sp. nov.	swine wastewater	0.0 – 64.1 ± 1.2	1.3 ± 0.1 – 10.5 ± 0.6 μg mL^{-1} Productivity: 0.08 ± 0.01 – 1.46 ± 0.05 μg $mL^{-1}d^{-1}$	This study

Note: *n.a: not available.

could enhance the IAA production are still lacking. In this context, the investigation on metabolic or enzymatic pathways of IAA synthesis by microalgae in wastewater could also be beneficial for improving the IAA yield and the adaptability of microalgae in wastewater.

In addition, swine wastewater could also contain toxic contaminants such as heavy metals, antibiotics, or hormones [6] that may adversely influence the growth of microalgae [24], as shown by this study in the test using undiluted swine wastewater. Moreover, since L-tryptophan is used as a precursor for IAA synthesis, excessive supplementation of this substance could result in secondary pollution and require treatment. Although the primary objective of this study was to evaluate the remediation performance of *P. spiroides* in swine wastewater and its capacity for IAA and biomass production, the quality of the final effluent was not directly assessed against specific discharge standards or reuse guidelines. Nevertheless, the observed reductions in nutrient concentrations and organic matter suggest that the treated effluent may hold potential for beneficial reuse, such as in agricultural irrigation or environmentally safe discharge. One practical solution to enhance applicability is to integrate the microalgae-based system with other wastewater treatment technologies, such as waste stabilization ponds [31] or conventional biological treatment systems [35]. Such hybrid systems could handle a broader range of wastewater strengths while maintaining stable and reliable treatment performance. Besides, the impacts of photobioreactor design, operational mode, and seasonal variation on microalgal performance in such large-scale systems must be assessed over a long period to obtain optimized operational conditions. These investigations could also allow for an accurate estimation of the operational cost, sustainability, and validity of the technology. Moreover, besides IAA, microalgae were well-known as a sustainable solution for coupling wastewater remediation and production of different valuable products such as biodiesel, cosmetics, fertilizers, animal feeds, or pharmaceuticals [7]. Hence, the application of microalgae-based systems that integrate wastewater remediation and production of valuable substances, including IAA, could be advantageous by decreasing the operation cost while increasing the value from the biomass produced.

Conclusions

This study investigated the effects of wastewater concentration, L-tryptophan supplementation, and pH levels on biomass production, indole-3-acetic acid (IAA) synthesis, and swine wastewater remediation efficiency in the cyanobacterium *P. spiroides*. The strain demonstrated robust adaptation to high-strength wastewater, with optimal performance observed at 1.3× dilution of raw swine wastewater. Maximum growth occurred at pH 9 with 0.2 g L^{-1} L-tryptophan supplementation, yielding biomass productivity of 64.1 ± 1.2 mg L^{-1} d^{-1} and IAA production of 1.46 ± 0.05 µg mL^{-1} d^{-1}. Under these conditions, *P. spiroides* achieved removal efficiencies of 79.8 ± 2.4% COD (46.7 ± 1.4 mg O_2 L^{-1} d^{-1}), 95.8 ± 0.1% TN (21.2 ± 0.01 mg N L^{-1} d^{-1}), and 87.1 ± 0.5% TP (4.0 ± 0.01 mg P L^{-1} d^{-1}). Several factors inhibited growth and remediation performance, including undiluted wastewater, L-tryptophan concentrations ≥0.3 g L^{-1}, and acidic pH (5-6). These results highlight *P. spiroides*' dual potential for simultaneous wastewater treatment and bioproduct generation. However, system optimization requires further investigation through: (1) genetic modification to enhance IAA biosynthesis pathways, (2) metabolic studies of IAA production mechanisms, (3) integration with conventional treatment systems, and (4) pilot-scale validation of technical and economic feasibility. Long-term assessments of system stability and sustainability remain crucial for practical implementation.

Acknowledgments

This research was funded by the Ministry of Science and Technology (MOST) under grant number: ĐTĐL.CN-46/21 and the Vietnam Academy of Science and Technology (VAST) under grant number: NVCC31.06/24-25.

Conflict of Interest

The authors declare no conflict of interest.

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