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Article

A low-cost method for purification of agricultural wastewater based on *S. platensis*

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SUMMARY

The contamination of water bodies by untreated agricultural wastewater is a major global issue. Traditional centralized treatment methods, including sedimentation tanks and photocatalytic degradation modules, which are commonly employed in economically developed areas such as Shanghai, present significant challenges for implementation in remote regions due to cost and technological limitations. Consequently, there is a pressing need for cost-effective alternatives in these less accessible areas. In this study, we hypothesized that *Spirulina platensis*, a species of cyanobacterial microalgae, has the potential to thrive in and purify agricultural wastewater, as has been previously reported for other cyanobacterial microalgae. Firstly, we conducted a comparative analysis of *S. platensis* growth in simulated agricultural wastewater (SAW) and a standard medium, Zarrouk's medium, to assess its adaptability to the growth conditions of SAW. Subsequently, utilizing total nitrogen, total phosphorus, and chemical oxygen demand as indicators for the purification of agricultural wastewater, we examined the efficacy of *S. platensis* in treating SAW. Our findings indicate that *S. platensis* is capable of reducing the concentration of each pollutant. However, the *S. platensis*–based treatment methods may be constrained by the potential toxicity of SAW with elevated nitrogen levels, necessitating additional research and development. This research offers a novel approach to cost-effective agricultural wastewater purification.

INTRODUCTION

Agricultural wastewater, originating from agricultural and livestock operations, is characterized by elevated levels of nitrogen, phosphorus, potassium, and organic substances (1). Upon introduction into aquatic ecosystems through natural runoff or anthropogenic discharge, this effluent can induce eutrophication and stimulate algal proliferation, leading to ecological degradation. For instance, in eutrophic waters, deceased algae settle in the benthic zone and undergo decomposition by microorganisms, which consume substantial amounts of dissolved oxygen. This process results in a significant reduction in dissolved oxygen levels, causing the mortality of aquatic organisms such as fish and a subsequent decline in biodiversity. According to the findings of the Second National Pollution Source Census Bulletin conducted by the Ministry of Ecology and Environment of the People's Republic of China in 2020, the chemical oxygen demand (COD), ammonia nitrogen (AN), total nitrogen (TN), and total phosphorus (TP) serve as crucial indicators for assessing chemical waste (2). In terms of total emissions from the agricultural sector in China in 2017, the quantities of these indicators were 10.6713 million tons, 216,200 tons, 1.4149 million tons, and 212,000 tons, respectively (2-4). The freshwater ecosystems of Taihu Lake and Chaohu Lake, which are among the top five freshwater lakes in China, are commonly impacted by water eutrophication, leading to algal blooms, underscoring the necessity of investigating effective treatment and reuse strategies for agricultural wastewater (5). Historically, agricultural wastewater treatment has predominantly utilized physical and chemical methods, which are complex and rely on large-scale treatment infrastructure (6-9). However, agricultural wastewater discharge sources are geographically dispersed, primarily located in rural and remote areas where the costs associated with centralized collection and transportation are expensive, making it unaffordable for farmers and enterprises, so sometimes they just directly discharge untreated water into farmland. Furthermore, the challenging economic conditions in these regions often deter individual farmers and small cooperative enterprises from shouldering the added financial burden of treating undeveloped soil and natural water bodies.

Microalgae have been widely used for wastewater management (10). *Spirulina platensis*, a species of cyanobacterial microalgae, exhibits a remarkably high efficiency in converting biomass into high-quality protein, thereby conferring upon it a substantial commercial value that significantly surpasses that of conventional crops (11). Additionally, various studies have highlighted the capacity of *S. platensis* to remediate eutrophic wastewater rich in organic compounds (12, 13). A particular study showed that ammonia, nitrite, and phosphate levels in aquaculture wastewater decreased by over 94.8% following treatment (13). The potential of *S. platensis* to efficiently purify eutrophic wastewater suggests its effectiveness for agricultural wastewater treatment, offering both purification and income generation in rural areas. However, previous studies have predominantly concentrated on the application of *S. platensis* for the treatment of industrial wastewater containing a singular contaminant component. Consequently, there remains a gap in our understanding regarding the efficacy of *S. platensis* in purifying a diverse array of eutrophic elements. Moreover, research indicates that elevated nitrogen concentrations can impede the growth of *S. platensis*, potentially diminishing the efficacy of *S. platensis* in wastewater purification and posing a challenge to the viability of this purification model (14).

In this study, we hypothesized that *S. platensis* may

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effectively decrease levels of COD, TN, and TP in simulated agricultural wastewater (SAW), at a lower cost compared to traditional physical and chemical treatment methods. In this context, we cultivated *S. platensis* in a SAW medium and it exhibited a typical growth pattern, suggesting its ability to survive in such conditions. Additionally, we observed that *S. platensis* was able to remove TN, TP and COD. However, when *S. platensis* was exposed to SAW with elevated nitrogen concentrations, which has also been observed in polluted water bodies, it failed to maintain normal growth, indicating limitations in its wastewater purification capacity under high nitrogen conditions. Finally, we conducted a cost analysis of utilizing *S. platensis* for the purification of agricultural wastewater and demonstrated its potential as an economically viable option for this purpose.

RESULTS

Growth kinetic measurements of *S. platensis*

We cultivated *S. platensis* in Erlenmeyer flasks and observed its morphology under a microscope. The body of *S. platensis* exhibited a blue-green coloration and consisted of spiral-like filaments (**Figure 1**).

It is difficult to collect large quantities of agricultural wastewater with general characteristics. Therefore, considering the efficiency and convenience of the experiment, we prepared an agricultural wastewater simulant based on the three indicators of the main components of agricultural wastewater in similar studies. The water quality indicators of the SAW were similar to those of the field-collected water (**Table 1**). Meanwhile, we also prepared a $NaHCO_3$ medium widely used in the large-scale production of platensis and cultured algae as a control group (**Table 2**).

To test whether *S. platensis* can grow in SAW, we inoculated *S. platensis* into the commonly used inorganic large-scale production medium $NaHCO_3$, and SAW medium, respectively, with the same initial optical density at a wavelength of 560nm (OD_{560}). The OD_{560} value is directly proportional to the concentration of the algal solution. During the first two days of culture, the OD_{560} value of *S. platensis* in SAW increased faster than that in control group, which grew in the inorganic medium, and then the growth rate decreased in the following two days. On day 7, the OD_{560} value in the SAW medium was 1.0, while the final OD_{560}value was 0.65 for the control group, revealing that *S. platensis* may grow better when cultivated in the SAW medium compared to the inorganic medium (**Figure 2**).

Determination of pollutant concentrations upon *S. platensis* treatment

We cultivated *S. platensis* in SAW for a duration of seven days and conducted pre- and post-culture measurements of the three main water quality indexes (TN, TP, and COD) to show the efficiency of removal of pollutants by *S. platensis* in agricultural wastewater (**Figure 3**). The average concentrations of TN, TP, and COD in SAW before and after treatment with spirulina were found to be 90.08 mg/L and 49.44 mg/L, 50.79 mg/L and 26.5 mg/L, 527.11 mg/L and 152.26 mg/L, respectively. The concentrations of TN, TP, and COD exhibited a declining trend following treatment with microalgae, indicating that *S. platensis* has the ability to purify the SAW against these pollutants.

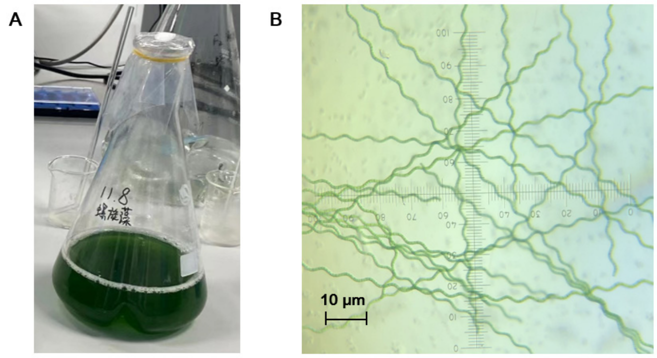

Figure 1. Morphological observation of *Spirulina platensis*. A) *S. platensis* cultured in the flasks. **B)** Micrograph of *S. platensis*. The *S. platensis* was cultured in Zarrouk's medium and observed with an optical microscope. The slide ruler and scale bar are equal to 100 µm and 10 µm respectively, and the magnification is 100x.

Water quality index	TN	TP	COD
Concentration (mg/L)	90.08	50.79	527.11

Table 1. Pollutant concentrations in simulated agricultural wastewater (SAW). The combination of total nitrogen (TN), total phosphorus (TP), and chemical oxygen demand (COD) was employed to simulate agricultural wastewater.

Component	Concentration (g/L)
$NaHCO_3$	19.2
$CO(NH_2)_2$	0.2
$FeSO_4 \cdot 7H_2O$	0.01
Himalayan salt	35

Table 2. Composition of $NaHCO_3$ medium. The following are the components of the medium.

Impact of nitrogen content on *S. platensis* growth

We next sought to further evaluate the efficacy of *S. platensis* in purifying agricultural wastewater with high nitrogen content. We conducted separate inoculations of *S. platensis* into wastewater samples with 0.2 g/L and 1.0 g/L urea respectively, and subsequently monitored the growth dynamics of *S. platensis*. During the first three days of culture, the density of *S. platensis* measured by OD_{560} value was not notably different between the two groups with varying nitrogen contents. Nevertheless, the OD_{560} value of *S. platensis* grown in the SAW medium with additional nitrogen declined quickly on the fifth day, while the *S. platensis* in the simulated medium with normal nitrogen concentration grew normally and peaked on day seven (**Figure 4**). This observation suggests that the purification method based on *S. platensis* is constrained in high-nitrogen environments. The purification mechanism of *S. platensis* in wastewater depends on its ability to utilize the nutrients in wastewater to support its growth. If *S. platensis* is unable to grow normally, effective wastewater purification cannot be achieved.

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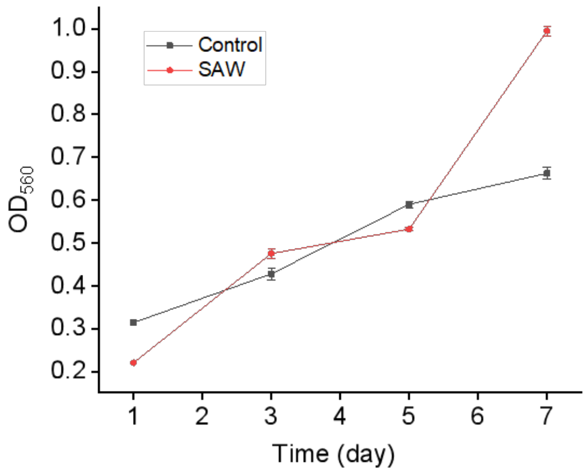

Figure 2. Growth of *S. platensis* under different culture conditions. Mean OD_{560} value of *S. platensis* measured every two days from control and simulated agricultural wastewater (SAW) media. All plotted points represent the average of two readings, and error bars represent standard deviation.

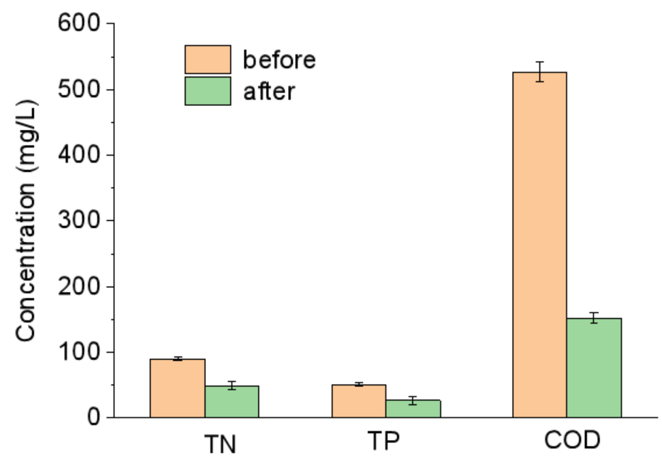

Figure 3. Three main water quality indexes prior and post to microalgae treatment for 7 days. Total nitrogen (TN), total phosphorus (TP), and chemical oxygen demand (COD) were measured from two different samplings. The error bars represent standard deviation.

DISCUSSION

In our study, we investigated the purifying capability of *S. platensis* on agricultural wastewater and calculated the cost-effectiveness of this purification method. Initially, *S. platensis* was cultivated in two different growth media: $NaHCO_3$ medium and a SAW medium. The results demonstrated that *S. platensis* exhibited faster growth in the SAW medium compared to the $NaHCO_3$ medium. Additionally, after seven days of treatment with *S. platensis*, decreased concentrations of TN, TP, and COD were observed within the SAW. Furthermore, by increasing nitrogen concentration levels in the simulated wastewater, we observed that high nitrogen levels impaired *S. platensis* growth based on OD_{560} measurements during cultivation experiments. Our findings provide valuable insights for developing an environmentally friendly, cost-effective approach to treating agricultural wastewater.

In this study, we aimed to cultivate *S. platensis* in agricultural wastewater with a focus on its heterotrophic growth potential induced by the presence of organic matter, as previously reported (12, 13, 15). A previous study has suggested *S. platensis* could be cultivated in digested sago starch factory wastewater, in which the average specific growth rate of *S. platensis* was 0.51 per day (12). Additionally, other studies demonstrated the rapid proliferation of *S. platensis* in undiluted digested piggery wastewater, with a high protein content observed in the algal biomass (15). Our findings are consistent with available data. In our setup, the OD_{560}value of *S. platensis* in the wastewater reached 1.0 after seven days of cultivation, whereas it only reached 0.65 in the inorganic medium, indicating that the presence of nutrients in the wastewater facilitated heterotrophic growth of the algae (**Figure 2**).

Moreover, other studies have demonstrated the remarkable efficacy of *S. platensis* in purifying eutrophic wastewater (13). In line with these findings, our research further supported the specific purification capabilities of *S. platensis* in agricultural wastewater, which typically contains high levels of TN, TP, and COD and leads to eutrophication. Compared to the pre-treatment period, there was a trend toward reduction in TN, TP, and COD levels in treated wastewater after *S. platensis* treatment (**Figure 3**). However, it is important to note that this method exhibits certain limitations. When the nitrogen content in the medium was increased, *S. platensis* experienced rapid mortality by the third day. On the fifth day, there was a rapid increase in *S. platensis* OD_{560} value in SAW, while the OD560 value in SAW containing high nitrogen decreased rapidly (**Figure 4**). This observation suggests that while low concentrations of urea serve as a viable nitrogen source for algae growth, high concentrations of urea may exert toxic effects on algal populations. This result conforms with the previous study, which revealed a lower cell density of *S. platensis* with higher total urea treatment in culture (14).

Furthermore, our purification method based on *S. platensis* showed higher convenience of operation in rural areas than traditional physical and chemical methods. Traditional physical and chemical methods require specific equipment like membrane reactors or electrolytic cells, which are difficult to obtain in rural areas. Furthermore, previous literature reported the operating expense of one small membrane bioreactor being approximately 0.43 USD per 1 m3 wastewater, while using *S. platensis* as treatment generates only 0.162 USD per 1 m^3 of wastewater treated, showing relative convenience in rural areas (**Table 3**) (18). In our method, the estimation of *S. platensis* production expenses predominantly revolves around two aspects: the expense of growth medium and the *S. platensis* seedstock. As *S. platensis* can constantly divide to produce large numbers of offspring, we believe that the cost of microalgae can be dismissed. After determining the specific chemicals and their quantities required to treat a cubic meter of agricultural wastewater, the cost calculation of medium can be estimated more accurately (**Table 3**). The costs were assessed by considering the composition of the culture medium commonly employed in industrial production and the prevailing market prices (19). This evaluation was conducted by multiplying the per-gram cost of the raw materials by the required quantity for the treatment.

Further research could focus on the establishment of various gradients through additional experiments to determine

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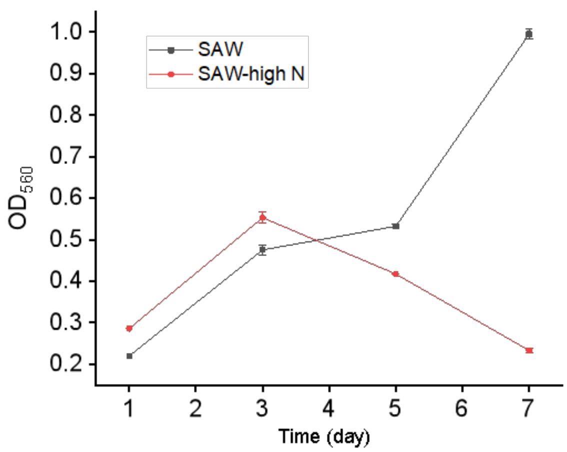

Figure 4. Growth of *S. platensis* grown in simulated agricultural wastewater (SAW) and SAW with additional nitrogen. Mean OD_{560} value of *S. platensis* measured every two days from two different media. All samplings were performed twice, error bars represent standard deviation.

Raw material	Price (USD/g)	Quantity required for treatment (g)	Cost (USD)
$NaHCO_3$	2.2×10^{-3}	4.50	1.0×10^{-2}
$MgSO_4$	3.8×10^{-3}	0.20	8.0×10^{-3}
$NaNO_3$	7.56×10^{-2}	1.50	1.13×10^{-1}
$CaCl_2$	3.3×10^{-3}	0.04	1.0×10^{-3}
K_2HPO_4	4.95×10^{-2}	0.50	2.4×10^{-2}
$FeSO_4$	2.7×10^{-3}	0.01	2.7×10^{-5}
$K2SO_4$	2.9×10^{-3}	1.00	2.9×10^{-3}
NaCl	2.7×10^{-3}	1.00	2.7×10^{-3}
Microalgae	1.4×10^{-3}	0.50	7.0×10^{-4}
		Total	0.162

Table 3. Estimated cost of S. platensis treatment for 1 m^3 agricultural wastewater. The cost of ingredients of medium and microalgae were calculated.

the optimal concentration of harmful substances in wastewater that allows for normal growth of *S. platensis,* so as to provide more evidence for microbial treatment of wastewater.

Overall, we have shown that *S. platensis* can be cultivated in a SAW medium and was able to remove TN, TP and COD, providing a proof-of-concept for the use of microalgae for wastewater treatment. Our study presents an innovative strategy for achieving affordable purification of agricultural wastewater, which could revolutionize wastewater treatment in rural areas.

MATERIALS AND METHODS

Experimental set-up

The *S. platensis* FACHB-314 strains were obtained from the Freshwater Algae Culture Collection at the Institute of Hydrobiology, National Aquatic Biological Resource Center of China. The SAW and the $NaHCO_3$ media were prepared as outlined (**Tables 1 and 2**). Before the exposure experiment, *S. platensis* was pre-cultivated at 25 ± 1°C under a lighting level of 4000 lux and with a cultivation cycle of 14 days in Zarrouk's medium (Guide Chem, Cat# ZY6AMP272). The light was provided from one side of the flask using six 6-watt LED lights at a distance of 60 cm, with a light cycle of 12 hours per day. The stock culture was grown in 1000 mL Erlenmeyer flasks placed on a shaking platform at 140 rpm.

All experiments were conducted in batches. First, a 400 mL solution of 25% (v/v) algal solution in medium was prepared for each medium tested and mixed with the remaining 75% medium (v/v). After that, the initial value for optical density at the wavelength of 560nm (OD_{560}) of each flask was measured as a baseline for the cultures in simulating agricultural waste medium and $NaHCO_3$ medium, respectively. Two replicates were prepared for each medium.

Growth kinetic measurements of *S. platensis*

To reflect the cell growth of *S. platensis*, the OD_{560} value was measured once every two days for a total of seven days (on days 1, 3, 5, 7), using a 721G spectrophotometer at the wavelength of 560 nm. This OD_{560} value represents the algae concentration in the fluid (17).

Determination of pollutant concentrations

The experimental group utilized SAW as the medium, while the control group employed $NaHCO_3$ as the medium. *S. platensis* was cultivated in each respective medium at the same initial OD_{560} value (0.2) for a duration of seven days. Subsequently, the TN, TP, and COD levels of the SAW were measured before and after treatment to evaluate pollutant removal efficiency. The TN level was determined using the alkaline potassium persulfate digestion UV spectrophotometric method, which involves digesting samples with alkaline potassium persulfate under high temperature (120-124°C), followed by measuring absorbance of the resulting solution with a UV spectrophotometer at a wavelength of 220-275nm (17). The TP level was measured using the ammonium molybdate method. Samples were digested with potassium peroxydisulfate at 120°C. Absorbance was measured at 700 nm, and TP was quantified via a calibration curve (17). The COD level was determined using the dichromate method, which uses acid-dichromate solution to digest oxidizable substances in the presence of silver sulfate catalyst and determines the COD concentration spectrometrically by measuring the absorbance of the Cr^{3+} formed at a wavelength of 600-610nm (17).

Impact of nitrogen content on *S. platensis* growth

To test the limitation of the purification method of *S. platensis*, additional nitrogen sources in the form of urea (Sigma-Aldrich, catalog number U1250) were also added to the SAW, increasing the urea concentration from 0.2 to 1.0 g/L. The microalgae were cultured in SAW with different concentrations of urea, respectively, and the OD_{560} value was measured every two days with repeated investigation.

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