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Full Length Article

Use of Anthropic Acclimated *Spirulina platensis* (*Arthrospira platensis*) Bio-adsorption in the Treatment of Swine Farm Wastewater

Tzu-Yen Wang[1], Hsiu-Chuan Liu[2], and Yen Lee[3*]
[1]*Taitung Jiun-E Middle and Primary School, Taitung 950, Taiwan*
[2]*Taitung Ta-Wang Junior Middle School, Taitung 950, Taiwan*
[3]*Department of Life Science, National Taitung University, Taitung 950, Taiwan (R.O.C.)*
*For correspondence: yenlee@nttu.edu.tw

Abstract

The progressive acclimation method was adapted to enhance growth of the cyanobacterium *Spirulina platensis* in stepwise increasing concentrations of swine farm wastewater. Anthropically selected *S. platensis* decomposed and absorbed organic materials and metabolites remaining in the wastewater. Water quality checks and monitoring the concentration of *S. platensis* showed that by the fourth day, wastewater samples met governmentally approved emission standards. *S. platensis* lowered chemical oxygen demand and the concentrations of suspended solids, and also absorbed phosphorus compounds, nitrates, and ammonia, effectively removing bad odors. A swine farm in Taitung, Taiwan is growing *S. platensis* in large scale pools by applying this method. The de-polluting effect is significant, and *S. platensis* is recycled into pig feed. The *S. platensis* product market value is sufficient to cover all variable costs and contribute toward the cost of installation.

Keywords: Anthropic acclimation; *Spirulina platensis*; Bioreactor; Swine farm wastewater; Biosorption

Introduction

Wastewater emissions from pig farms are major sources of agricultural wastewater in Taiwan, and can have a significant negative impact on the environment. Conventionally, pig farmers in Taiwan treat wastewater using a three-step piggery wastewater treatment system—solid liquid separation, anaerobic reactor, and aerobic reactor (Su *et al*., 2003). However, small- and medium-size pig farmers cannot afford the high maintenance costs of such facilities. A number of farmers illegally discharge pig wastewater, resulting in eutrophication of rivers and ponds, which causes serious environmental pollution. Thus, finding a cost-effective wastewater treatment method is necessary to ameliorate this problem.

Microalgae have been applied to cultivate in many kinds of wastewater to improve water quality (Mulbry *et al*., 2006, 2008; de Godos *et al*., 2010; Li and Lee 2012) for years. Especially grow microalgae in agro-industrial wastewater, which rich in nitrogen and phosphorus pollutants meanwhile microalgae can be used to reduce the inorganic and organic load of these wastewaters (Markou and Georgakakis, 2011) at a minimal cost (Lee and Lee, 2002). For easily harvesting by microscopic size sieve and its high protein as well as other nutrients contents for food and production of other important metabolites (Khan *et al*., 2005; Nagaoka *et al*., 2005; Narasimha *et al*., 2006), this research used *S. platensis* for experiments.

Previous studies used stocked *S. platensis* or strains isolated from wastewater sites (de Godos *et al*., 2010; Cheunbarn and Peerapornpisal, 2010; González-Fernández *et al*., 2010), no strictly intended artificial *S. platensis* selection steps had been made. This was the first report that pig-farming wastewater had been bio-treated using anthropically acclimated cyanobacteria to reduce water pollution. The treated wastewater met Taiwan EPA (Environmental Protection administration) emission standards, and the *S. platensis* byproduct was used as pig fodder to reduce feed consumption. The wastewater treated by cyanobacteria added was obviously more effective than the control group hence the heterotrophic bacteria were not the significant factor.

Materials and Methods

Swine Wastewater Sampling

Wastewater was collected from Lai, Yung-Mei swine farm, located in Taitung County, Taiwan. This farm is approximately 1.5 hectares in area, and feeds 5,000 pigs. Farm workers are trained in SOP (standard operation procedures) for cleaning the farm, washing the animals, and for the discharge of pig waste. The first step in SOP wastewater treatment is solid-liquid separation; the separator

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removes part of the solid for composting, and the "raw wastewater" is separated for further treatment. The farm discharges up to 90 tons of wastewater into a 230-ton raw wastewater reservoir daily. Because the concentration of wastewater samples varies, the study involved routinely checking the COD (chemical oxygen demand), SS (suspension solid value), NH_4^+-N, PO_4^{3-}, and NO_3^- concentration elimination rates for three batch samples (including the control), which were collected at three various intervals to compare *S. platensis* bio-absorption efficiency.

Source of *S. platensis* and Culture Conditions

S. platensis was purchased from Pingtung Fisheries Research Institute, Taiwan. The culture was maintained and dispensed from 20-L transparent plastic tanks with covers in the BG-11medium, pH 7.1 (Rippka *et al.*, 1979). An aquarium air pump (110 V, 4.5 W, 50 HZ, about 4400 mL air/min) was used to provide air to each tank through a plastic tube containing a sterilized cotton ball. The tube contacted the bottom of the tank, and a micropore gas diffuser connected to the end of the tube dispersed the air bubbles. The airflow also provided water circulation within the tank. Temperatures were at ambient outdoor levels, ranging between 20^{o}C and 29^{o}C. An incandescent light (1,500 lux) was suspended 10 cm above each tank to provide supplemental continuous illumination.

Determination of *S. platensis* Concentration

S. platensis dry weight was obtained by modifying a published method (Ratana *et al*., 2010). Samples (100 mL) were filtered through Whatman filter paper (110 mm diameter), and washed with 250 mL acidified water (pH 4) to prevent salt precipitation during filtration; filter papers were oven dried at 80^{o}C for 24 h before weighing. The pore size of the filter paper is large enough to leak out heterotrophic bacteria. *S. platensis* cell size is larger and kept on the filter paper. The acid water wash step might get rid some of the heterotrophic bacteria. Some of the heterotrophic bacteria should remain with cyanobacteria. Pure culture was not required in this system.

Anthropic Acclimation

S. platensis was progressively acclimated to growth in high concentrations of raw wastewater. Anthropic selection began with 5% v/v concentration of sterilized raw wastewater in 20-liter transparent plastic-covered tanks. The raw wastewater was diluted with distilled water before autoclaving. Aquarium air pumps, temperature conditions, and lighting were as described. The initial *S. platensis* seeding concentration was 0.2 g/L (dry weight). When the *S. platensis* concentration reached 1.0 g/L, it was harvested by being filtered through a sterilized sieve with 0.1 mm^2 pore size. Selected *S. platensis* samples were used to further seed 10% concentration sterilized raw wastewater samples. The anthropic selection procedure was repeated, and increased to 15% concentration sterilized raw wastewater, and the process continued until the cyanobacteria could grow in 20% concentration sterilized raw wastewater. *S. platensis* was then harvested and reseeded in unsterilized raw wastewater at 20% concentration; the final seeding amount was also 0.2 g/L. The high concentration was intended to allow *S. platensis* to form the dominant strain in the diluted raw wastewater.

COD, SS, NH_4^+-N, PO_4^{3-} and NO_3^- Concentrations

The growth of *S. platensis* in 20% concentration unsterilized raw wastewater was measured by collecting triplicate samples from each experimental tank. Prior to examination, each water sample was filtered through paper and the COD, NH_4^+-N, PO_4^{3-}, and NO_3^- concentrations were checked by a Hach DR/2010 spectrophotometer. The measuring bottle held 10 mL water sample. All steps were conducted according to the Hach DR/2010 manufacturer's instructions. The SS value was calculated by filtering 100 mL sample water through a piece of oven-dried glass fiber filter paper (Pall type A/E) before oven drying the filter paper, and measuring the difference in mass. When the wastewater SS concentrations were too high, dilutions were made by adding double distilled water. The SS concentrations were then checked and calculated by multiplying different dilution coefficient.

Treatments

Two experimental groups were assembled. The first group used a 20% of concentration unsterilized raw wastewater with aeration, and the second group used a 20% concentration of unsterilized raw wastewater with aeration, with *S. platensis* added. The statistical hypothesis is whether *S. platensis* could cause a significant difference in swine farm waste water quality, as compared to the control group (without cyanobacteria). Aeration is necessary for cyanobacteria survival in swine farm waste water. Hence, an aeration group was used to determine whether aeration would improve the waste water quality.

Three samples were collected from each of the controls, the first group, and the second group every day. All tests were conducted in triplicate, using the same batch of unsterilized raw wastewater as a control. JENCO 6173 pH meter was used to measure the pH of each tank. *S. platensis* growth was monitored by microscope each day during the experimental period.

Statistical Analysis

Data were reported by calculating the means and standard deviation. Independent sample *t*-tests were completed to independently compare control group averages with the first and second experimental groups, respectively. A confidence level of 95% was chosen.

Results

Anthropic Acclimation and *S. platensis* Growth Curve

The progressive acclimation method was adapted to enhance *S. platensis* tolerance to swine farm raw wastewater by artificial selection. *S. platensis* grew in 20% concentration unsterilized raw wastewater. For the first three days, cells entangled into clumps, and on the fourth day, cells dispersed out from the clumps. The growth curve is shown in Fig. 1. Acclimated *S. platensis* seeded in the 20% concentration unsterilized wastewater formed the dominant cyanobacterial strain. If the seeding quantity exceeded 0.2 g/L, the anthropic acclimated cyanobacteria formed a dominant strain, and not only grew well in 20% concentration unsterilized wastewater during the seven day duration of the experiment, but also reproduced successfully during later usage.

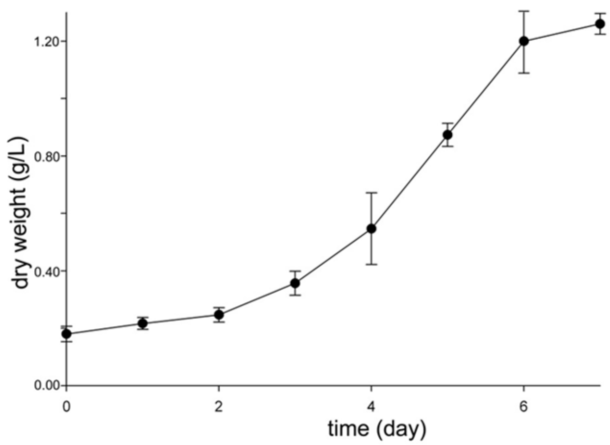

Fig. 1: *S. platensis* growth curve (grew in 20% concentration non-sterilized raw wastewater). Calculated generation time was approximately 55.6 h

COD Values

The initial COD for 20% unsterilized raw wastewater was 1,073 mg/L. By the fourth day, second experimental group tank (with aeration and cyanobacteria) achieved an effluent loading of 546 mg/L, meeting the Taiwan EPA (2010) pig waste water effluent standards of < 600 mg/L. Microscopic observation showed that as the COD value decreased, cyanobacteria intertwining was reduced. Experimental group 1 (with aeration and no cyanobacteria) also showed a decreasing COD, albeit at a lower rate than that of the second group. Aeration provided oxygen to competing aerobic bacteria, and enhanced their growth as well as increased nutrient consumption. The control group did not show a highly significant COD decrease (Fig. 2). By the seventh day, the second experimental group showed 85% COD removal. This revealed that *S. platensis* could effectively lower pig farm wastewater COD values; *S. platensis* photosynthesis releases oxygen into water to reduce COD. The control groups showed less COD reduction than the treatment groups, indicating that oxygen is a critical factor for these bioreactors.

The average COD value of the initial wastewater samples was about 1074.3 mg/L. At the 7th day, the second experimental group had an average COD concentration of 158 mg/L. The first experimental group had an average COD concentration of 632 mg/L, and the control group had an average phosphate concentration of 791 mg/L.

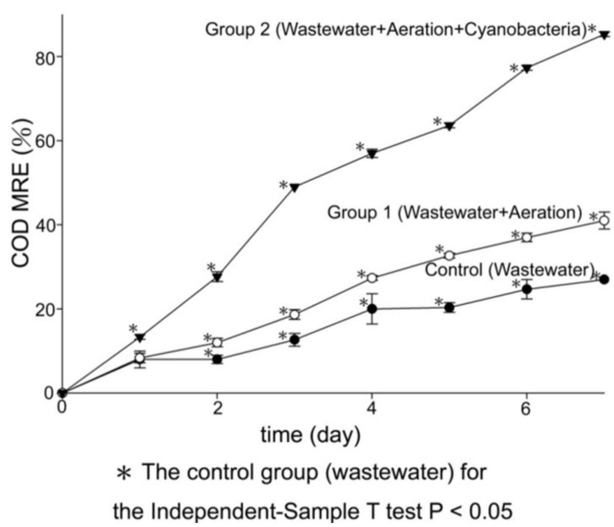

Fig. 2: *S. platensis* COD mass removal efficiency (MRE) that grew in 20% concentration unsterilized raw wastewater. The independent sample *t*-test indicated whether both aeration and aeration accompanied with *S. platensis* treatments were significant (figures below all had the same characteristics)

Suspension Solid Values

On the second day, the water quality for the second experimental group improved sufficiently to meet pig wastewater effluent standards (134.00 mg/L < 150mg/L). Experimental Group 1 also showed a significant decrease in the SS value. The raw wastewater contained gel-like materials, which could block the filter. The obstruction prevented measurements from being made, and breaking up the clumps using sonication was necessary. After two days of aeration and cyanobacteria bio-treatment, the gel-like materials began to disappear. On the seventh day, Aeration Group 1 also met wastewater discharge standards (Fig. 3).

The average SS concentration of the initial wastewater samples was approximately 612.9mg/L. At the seventh day, the second experimental group had an average SS

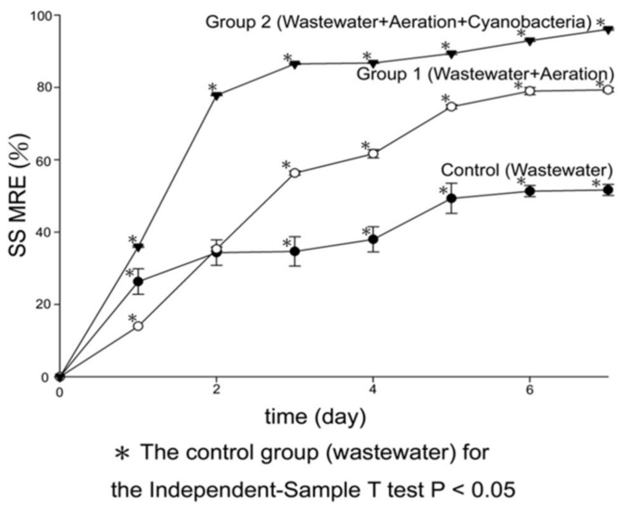

Fig. 3: *S. platensis* SS mass removal efficiency (MRE) that grew in 20% concentration unsterilized raw wastewater

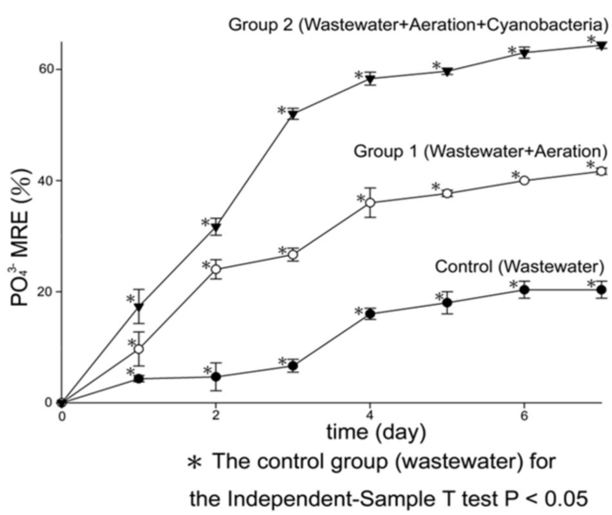

Fig. 4: *S. platensis* phosphate removal efficiency (MRE) that grew in 20 % concentration unsterilized raw wastewater

concentration of 23.8 mg/L. The first experimental group had an average SS concentration of 124.4 mg/L, and control group had an average SS concentration of 301.6 mg/L.

Phosphate (PO_4^{3-}) Concentration

The absorption of PO_4^{3-} by *S. platensis* was apparent from the rapid decrease in PO_4^{3-} concentration (Fig. 4). By the seventh day, Group 2 had removed 64.5% of the sample's original PO_4^{3-} content. Group 1 also showed a significant decrease in PO_4^{3-} concentration, but was less efficient. The control group showed a removal rate of less than 20% by the seventh day.

The average PO_4^{3-} concentration of the initial

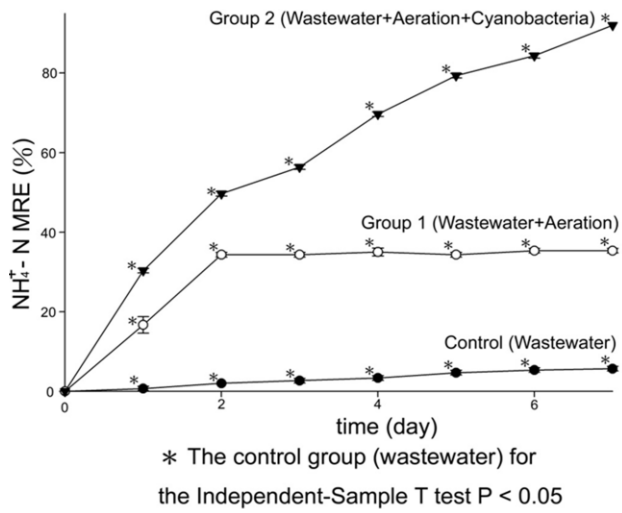

Fig. 5: *S. platensis* NH_4^+-N removal efficiency (MRE) that grew in 20 % concentration unsterilized raw wastewater

wastewater samples was approximately 32.6 mg/L. At the seventh day, the second experimental group had an average phosphate concentration of 11.5 mg/L. The first experimental group had an average PO_4^{3-} concentration of 19.0 mg/L, and the control group had an average PO_4^{3-} concentration of 26.9 mg/L.

NH_4^+-N Concentration

Fig. 5 shows that the second experimental group NH_4^+-N concentration decreased rapidly. By the seventh day, the NH_4^+-N concentration was 6.6 mg/L and its removal was 91.8% complete. The aeration group's NH_4^+-N removal reached 34% on the second day, and the NH_4^+-N concentration did not decline further. The control group did not show a decreasing NH_4-N concentration. The NH_4^+-N is a major source of malodor from swine farm wastewater. It was evident that the decrease in odor accompanied the decline in NH_4^+-N concentration, regardless of whether *S. platensis* was added or aeration alone was applied. Ammonia may have escaped into the atmosphere. The experiment involved using covered tanks, although the covers were not completely sealed, and ammonia could still escape. The control group, not receiving aeration, showed almost no change in ammonia-nitrogen levels.

The average NH_4^+-N concentration of the initial wastewater samples was approximately 82.1 mg/L. At the seventh day, the second experimental group had an average NH_4^+-N concentration of 6.6 mg/L. The first experimental group had an average NH_4^+-N concentration of 52.8 mg/L, and the control group had an average PO_4^{3-} of 79.7 mg/L.

Nitrate (NO_3^-) Concentration

The second experimental group showed 54.0 % removal of NO_3^- by the seventh day. The control group also showed an

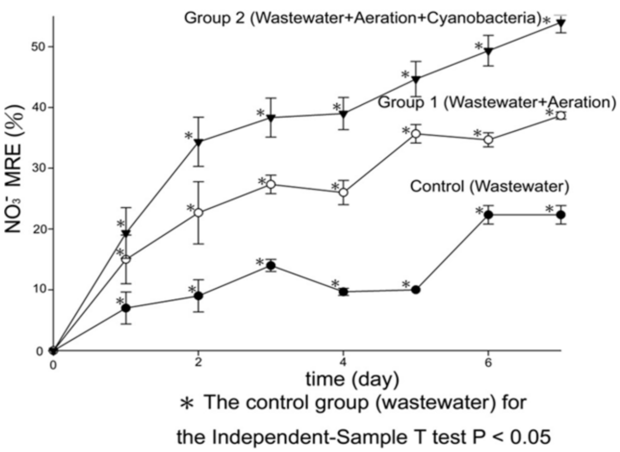

Fig. 6: *S. platensis* NO_3^- removal efficiency (MRE) that grew in 20 % concentration unsterilized raw wastewater

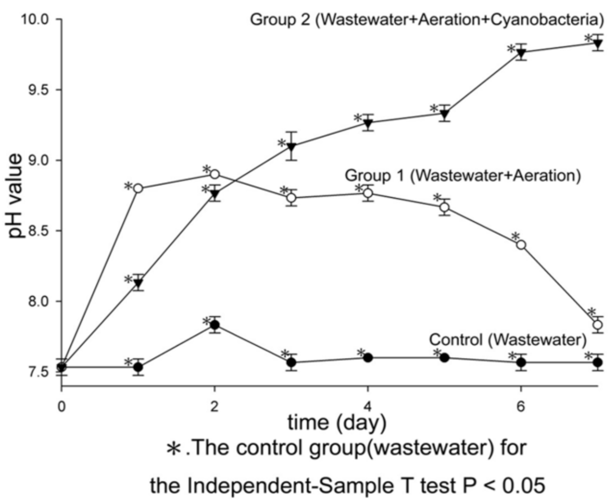

Fig. 7: The pH values changed in the control and experimental tanks

NO_3^- reduction, albeit at a reduced efficiency (Fig. 6). As expected, the observed NO_3^- removal rate for the aeration group was between those of the control and Experimental Group 2. The average NO_3^- concentration of the initial wastewater samples was approximately 20.3 mg/L. At the seventh day, the second experimental group had an average nitrate concentration of 9.3 mg/L. The first experimental group had an average NO_3^- concentration of 12.6 mg/L, and the control group had average NO_3^- of 15.7 mg/L.

Changes of pH values: The average pH value of the initial wastewater samples was ~7.53. In the second experimental tank, the pH values showed increased from 7.53 to 9.83 during the experimental period. In the first experimental tanks, the pH values rose to 8.90 at the second day, then gradually dropped to 7.83 at the seventh day. The pH values in the control tanks, rose to 7.83 at the second day, then gradually lowered to 7.57 at the seventh day (Fig. 7).

Discussion

Previous studies described the addition of sodium bicarbonate and urea (Gantar *et al.*, 1991; Lincoln *et al.*, 1996; Ratana *et al.*, 2010; Mezzomo *et al.*, 2011) to adjust pH and supplement the available nitrogen. However, this research was intended to use *S. platensis* to consume nutrients in the wastewater and bring the wastewater to a quality that could be safely discharged into drainage, and thus, the experiment did not involve the addition of any supplements. The product yield was not our first priority. However, *S. platensis* productivity reached 1.2 g/L (dry weight) by the sixth day of growth.

In discharging wastewater, diluted wastewater increases the amount of water; however, the wastewater concentration cannot be significantly greater because this would result in increased turbidity, preventing light from penetrating the water and inhibiting the cyanobacterial growth. Additionally, the high concentrations of ammonia and other ingredients may inhibit *S. platensis* growth (Cañizares-Villanueva *et al.*, 1995). It is also possible that increased wastewater concentrations may result in autochthonous algae outcompeting *S. platensis* (Gantar *et al.*, 1991).

Previous studies have also shown that phosphorus may be removed from wastewater by direct cellular absorption under aerobic conditions, or by reaction with ammonia and sediment under anoxic conditions (Maekawa *et al.*, 1995; González *et al.,* 1997; Su *et al.*, 1997). This study showed that cyanobacteria could increase the phosphorus removal rate by approximately 20% after four days of treatment. This study also showed that the anthropic acclimated *S. platensis* could significantly increase NO_3^-, NH_4^+-N, SS, and COD removal rates, as well as increase the pH of the wastewater.

The *S. platensis* cells clumped together in the first three days of culture in the 20% wastewater, which might have been caused by either the filtering collection of the cells or the wastewater hypertonic osmotic pressure. Lincoln *et al.* (1996) showed that at least half of *S. platensis* biomass is edible protein that contains γ-linolenic acid, palmitic acid, vitamins (biotin, B6, B12, folic acid, panthothenic acid, riboflavin, B1, and nicotinic acid), essential amino acids (methionine, leucine, isoleucine, phenylalanine, threonine, and lysine), and phycocyanin (Cañizares-Villanueva *et al.*, 1995; Ratana *et al.*, 2010), which may be extracted for healthy food.

The Lai, Yung-Mei swine farm currently uses underground water to dilute wastewater, and the company has constructed a 50-ton bioreactor and a 100-ton bioreactor, both of which contain an aeration system, but without any night illumination. The farm owner has determined that the *S. platensis* production current market price for fodder is sufficient to cover the variable and installation costs. In the summer, the *S. platensis* production from both tanks is approximately 75 kg each week. In the winter, half a month is required to produce nearly the same amount. The *S. platensis* market price for animal feed is approximately 20 USD/kg. The company estimated that the annual *S. platensis* production value is approximately 36,000 USD. The construction costs of the tanks were about 30,000 USD.

In conclusion, artificially selected *S. platensis* can effectively treat swine farm wastewater to meet Taiwan's EPA requirements for COD and SS effluent standards for release into waterways and significantly increase the removal rates of NO_3^-, NH_4^+-N, and PO_4^{3-} from pig farm wastewater. (In Taiwan, no phosphate, ammonia-nitrogen, or nitrate effluent standards have yet been established.

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