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Research Article

A STUDY ON SWINE WASTEWATER TREATMENT AFTER BIOGAS DIGESTION USING *ACTINASTRUM* SP.

Duong Thi Giang Huong
Saigon University
Corresponding author: Duong Thi Giang Huong – Email: huongduong@sgu.edu.vn
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ABSTRACT

Currently, algae have been focused on research to apply in different fields. With the desire to contribute to the application of microbiological technology to reduce pollution in organically polluted wastewater per the situation of Vietnam, this study is conducted to apply Actinastrum sp. *to treat nitrogen and phosphorus in swine wastewater after biogas digesters. Empirical results suggest that the algae are capable of treating livestock wastewater, meeting the standards of national regulation QCVN 62-MT:2016/BTNMT with maximum COD and TN removal of 84.62% and 81.88%, respectively, when supplemented with an algal density of* 2.5×10^6 *cells/mL.*

Keywords: swine wastewater; post-biogas digestion; *Actinastrum* sp.

1. Introduction

The animal husbandry industry of the southern region in general, as well as Ho Chi Minh City in particular, has been developing rapidly, especially with the model of small and medium pig farms in Cu Chi, Hoc Mon, and District 12. However, the arising problem is that the wastewater treatment of pig farming in Ho Chi Minh City has not been given due attention. The capital for the swine wastewater treatment system is too high and does not bring direct benefits to investors, resulting in many inadequacies in the treatment process. Currently, most pig farms in Ho Chi Minh City are applying the treatment of animal waste by biogas tanks. However, the biogas digestion is suitable for small and medium-sized animal husbandry household, but is not capable enough to handle the discharge flow from large scale breeding farm. Thus, the high levels of organic and microbiological contaminants remain, and the quality of post-treatment wastewater does not meet the national technical regulation on the effluent of livestock (QCVN 62-MT:2016/BTNMT, column B) for most farms (Pham et al., 2012). In particular, nitrogen compounds are usually not thoroughly treated by conventional filtration (Nguyen et al., 2010; Nguyen et al., 2016; Vu et al., 2015) due to their high solubility. Therefore, reusing this water is a major challenge.

In nature, there are many aquatic species capable of treating wastewater very effectively, such as algae, water hyacinth, and some crustacean species. They can be applied as a stage in

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the wastewater treatment process with simple equipment and low operation cost and help achieve the permitted standard. In addition, many assessments show that microalgae consume nitrogen and phosphorus in wastewater as nutrients, so nitrogen- and phosphorus-rich biogas effluents are sufficient sources for these species. The fundamental role of algae in wastewater treatment is to eliminate nitrogen and phosphorus compounds in water; besides, algae also can kill some pathogens because of toxins released from their cells and changes in pH during the day due to the effect of their photosynthesis processes (Ansa et al., 2012). Moreover, lipid from microalgae is one of the raw materials for biodiesel production. Therefore, algae are often applied in wastewater treatment. However, most studies are conducted based on the treatment ability of S*pirulina* sp., *Scenedesmus* sp. and *Chlorella* sp. or the mixture of *Actinastrum* sp. with the mentioned microalgae (Gupta, & Bux, 2019). *Actinastrum* sp. has not been popularly solely studied to treat both municipal and agricultural wastewater treatment despite its capability to absorb nutrients well, widely applied, grow fast, and kill pathogens (Ansa et al., 2012).

For these reasons, this research was conducted to evaluate the *Actinastrum* sp. genus's ability to grow and remove total nitrogen, total phosphorus, COD at a reasonable cost, limit eutrophication, and diversify the agents used in swine wastewater treatment after biogas digestion.

2. Methodology

*2.1. **Actinastrum** **sp.** density*

The experiment was conducted with *Actinastrum* sp. isolated from the water samples collected from the pig farms in the surrounding area of Cu Chi pig farm at the Institute of Tropical Biology. The sampling procedure followed TCVN 6663-1:2011 (ISO 5667-2:2006) (VEA, 2011). After isolation, samples of pure algae were obtained and cultured in 100 mL, 250 mL, and 1000 mL erlenmeyer flask.

The algae-culturing tanks were made of glass and have a volume of 8 liters with a depth of 40-50 cm to create an appropriate retention time, reduce the volume lost due to sediment and ensure temperature to be less affected by solar radiation (Le, & Nguyen, 2015).

Algae culture media include Combo, Z8 and TT3. The **improved Combo** medium (pH = 7.8) contains 10mL/L of 85.01 g/L $NaNO_3$, 36.76 g/L $CaCl_2.2H_2O$, 36.97 g/L $MgSO_4.7H_2O$, 12.6 g/L $NaHCO_3$, 28.42 g/L $Na_2SiO_3.9H_2O$, 8.71 g/L K_2HPO_4, 24 g/L H_3BO_3, 7.45 g/L KCl, and micronutrient as well as 1 mL/L vitamin B12, vitamin H, vitamin B1, and 1cc CR1 Soil. **TT3** contains 70 mg/L KNO_3, 10mg/L KH_2PO_4, 5 mg/L EDTA, 7 mg/L urea, 7 mg/L citric acid, 5 mg/L $NaSiO_3$, and 2 mg/L $FeCl_3$.

The algae were isolated according to the colony culture method in Combo medium. Then, they were transferred to test tubes containing Combo medium (V = 5 mL/test tube) and illuminated by neon light for about 2-3 weeks. Then, the algae are inoculated into 100 mL erlenmeyer flasks containing TT3 medium, aerated, illuminated by neon light and maintained at 26^{o}C for 24 hours. *Actinastrum* sp. algae are transplanted repeatedly until a sufficient amount for the wastewater tanks were obtained. The growth of algae is monitored through pH, OD (optical density) and Chlorophyll-a content (three times per day).

The density of the added algae solution was determined by counting the number of cells with the Thoma cell counting chamber (10× objective lens), while the chlorophyll-a content was extracted using acetone and then determined by the photometric method.

2.2. Nitrogen removal using Actinastrum sp.

The experiment is arranged completely randomly with five treatments and three replicates. Using six 5-liter glass bottles with lids, each contains three liters of livestock wastewater and different microbial supplement of 0 (Control), 1×10^6 (NT1), $1,5\times10^6$ (NT2), 2×10^6 (NT3), $2,5\times10^6$ (NT4), and 3×10^6 (NT5) cells/mL, respectively. The control experiment had the same effluent environment but was not supplemented with *Actinastrum* sp. The bottles were ensured optimal sunlight for bacterial photosynthesis and aerated for 8-9 hours/day.

The experiment aimed at determining the number of algae needed to supplement to achieve the optimal nitrogen treatment efficiency through the analysis results of $N\text{-}NH_4^+$ and TN parameters.

The ability of microalgae to adapt to environmental conditions is monitored through measuring OD and chlorophyll-a content. The performance of treating pollutants is assessed based on the parameters of total nitrogen, total phosphorus, COD, $N\text{-}NH_4^+$, and $P\text{-}PO_4^{3-}$ to determine the treatment capacity of *Actinastrum* sp. algae.

The samples are analyzed at the laboratory of the Environmental Sciences Department, Saigon University. Collected data were then processed with Microsoft Excel 2016.

3. Results and discussion

3.1. Actinastrum gracile growth

3.1.1. In TT3 medium

Isolation experiments obtained genus *Actinastrum* sp. *Actinastrum gracile* (*A. gracile*) reaches a maximum density of 24.41×10^6 cells/mL on the day 16th when cultured in the TT3 medium with OD (Abs) at 686 nm of 1.649 Abs. The correlation between cell density and biomass determined by optical density measurement (OD) method is shown in Figure 1 with $R^2 = 0.9883$.

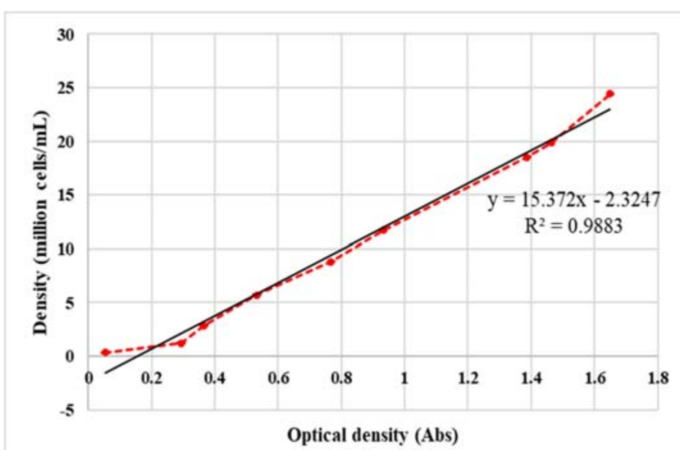

Figure 1. *Correlation between algae density and optical density of A. gracile*

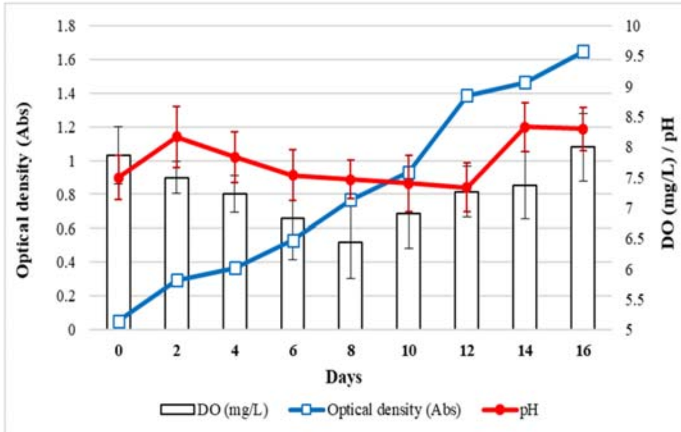

Figure 2. *A. gracile growth rate in TT3 medium after 16 days*

Biomass accumulation of *A. gracile* after 16 days shows that they have very good growth ability in the TT3 environment. pH value is relatively stable, fluctuating between 7 and 8.5, while DO tends to decrease in the first 8 days (6.4 mg O_2/L) due to the algae's consumption for growth and then increases up to 8.0 mg O_2/L on the 16th day for algae photosynthesis.

3.1.2. In swine wastewater

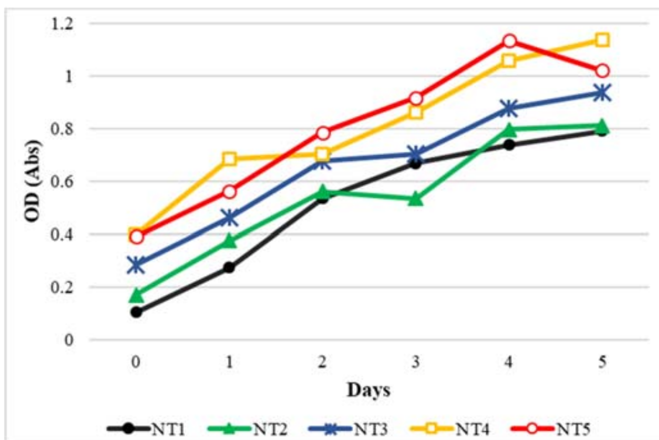

Figure 3. *A. gracile growth capability in swine wastewater at a different initial density*

Actinastrum gracile is added to wastewater with a density of 50-60 million cells/mL. The additional algae solution is naturally deposited or centrifuged 6,000 cycles/minute for about 10 minutes to extract the algae solution with a density of 50-60 million cells/mL to avoid diluting the concentration of pollutants in the previous wastewater when supplemented.

As a result, after being added into the wastewater, the *A. gracile*'s growth rate is quite rapid in the first three days, turning the water to dark green color. In addition, the wastewater no longer smells of stubs and oil streaks float to the surface; meanwhile, the control tanks are still foul-smelling. The growth time of *Actinastrum gracile* was found to be short compared to that when cultured in the TT3 environment. Algae densities in most experimental tanks increase gradually on days 4-5, except for the appearance of dead yellowish *A.gracile* cells in NT5 tanks due to the huge amount of added algae to the wastewater that leads to the lack of nutrients for all the algae to grow.

3.2. Capability of A. gracile in treating swine wastewater

3.2.1. Ammonium and total nitrogen

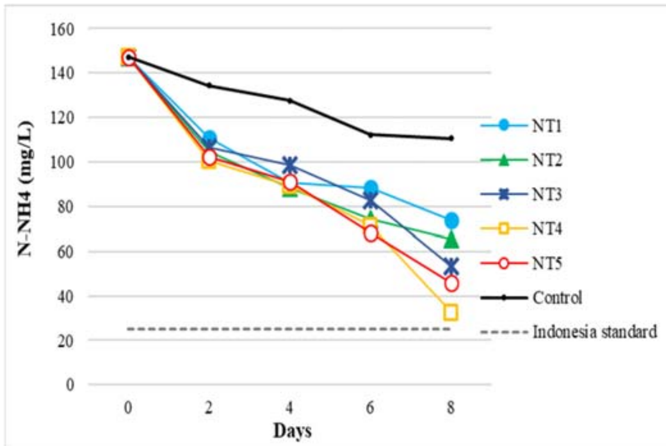

Figure 4. *Ammonium removal by A.gracile*

After the addition of *A. gracile* at different density values, the concentration of N-NH_4^+ in the swine wastewater decreases rapidly within two days. Observation shows that ammonium removal efficiencies of the algae-supplemented experiments fluctuate between 24.89% (NT1) and 31.67% (NT4), while that of the control tanks barely signifies (8.82%). On the later of the first two days, these efficiencies begin to differentiate from one another. Specifically, on day 6, the initial 1×10^6 cells/mL *A.gracile* consume a total of 40.05% of N-NH_4^+ whilst 3×10^6 cells/mL of the algae use 53.62%. This corresponds with the fact that the greater the cell density, the higher the need for N-NH_4^+ uptake as the remaining N-NH_4^+ concentration in NT5 is 68.33 ± 7.02 mg/L. However, N-NH_4^+ removal in NT5 experiences a downfall because there appear to be dead algae cells due to phosphorus deficiency on day 8, while NT4 proves to be more efficient as its removal efficiency reaches 77.83% and the N-NH_4^+ concentration after treatment is 32.67 ± 5.51 mg/L. Although the remaining content of N-NH_4^+ is a little above the Indonesia standard (25 mg/L), *A.gracile* proves its ability to treat N-NH_4^+ in livestock wastewater when compared to the control experiment (a total of 23.76% of N-NH_4^+ removed).

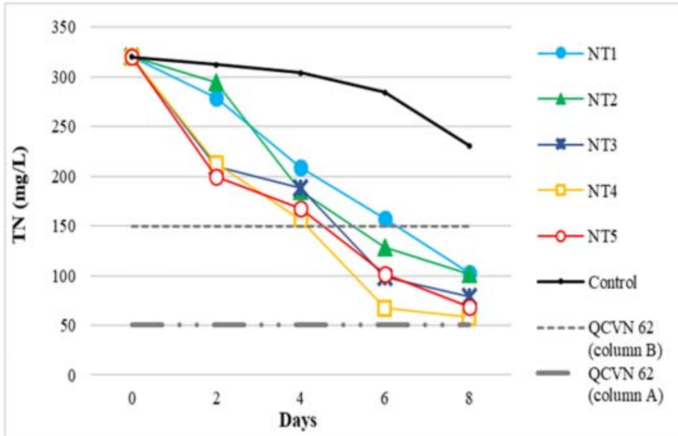

Figure 5. *TN removal by A.gracile*

Figure 5 illustrates the total nitrogen (TN) content decreases with time, rapidly with the supplement of *A.gracile* and slowly without. TN concentration of the raw wastewater is 320 ± 5.57 mg/L, with $N\text{-}NH_4^+$ account for 45.9% total amount. As $N\text{-}NH_4^+$ content drops distinctly after two days of treatment, TN content also declines to 200.0 ± 8.89 mg/L for NT5 (37.5%). After eight days of treatment, the remaining of TN is 58.0 ± 6.56 mg/L for NT4 (the initial algae density of 2.5×10^6 cells/mL) and 68.33 ± 8.74 mg/L for NT5 (3×10^6 cells/mL). When compared with the control experiment with the efficiency of 27.92%, it is suggested that the presence of *A.gracile* promotes TN removal in livestock wastewater, meeting the effluent standard of 150 mg/L (QCVN 62-MT:2016/BTNMT, Column B).

3.2.2. Phosphate and total phosphorus

Different from the previous parameters, the removal of phosphate and total phosphorus occurs slowlier, reaching maximum efficiencies of 18.87% (NT5) and 16.30% (NT5) respectively (Figure 6 and Figure 7).

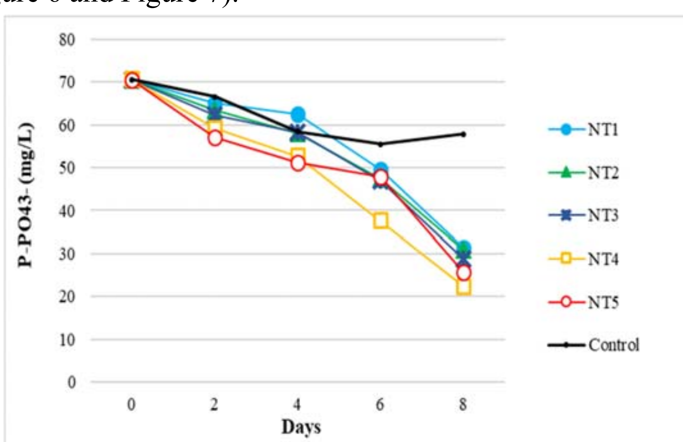

Figure 6. *Phosphate removal by A.gracile*

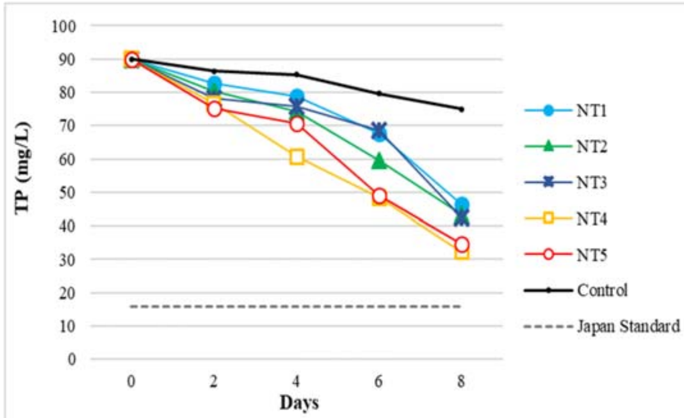

Figure 7. *TP removal by A.gracile*

Not until day 6 that there is a considerable reduction of P-PO_4^{3-} (29.72% - 46.70 %) and TP (24.81% - 46.30%). The P-PO_4^{3-} content of the raw wastewater is 70.67 ± 4.51 mg/L and then decreases slightly after 2 – 4 days might be for the metabolism by *A.gracile* that changes phosphorus in organic compounds to soluble forms (single, ortho) takes place alongside with the consumption of phosphorus. It is also noticed that the biomass of *A.gracile* increased very rapidly during the first four days as shown in Figure 3. Similar trends of P-PO_4^{3-} concentration during eight days of treatment are also observed in TP. TP removal efficiency reaches a maximum value of 64.07% on day 8 with the corresponding total phosphorus value of 32.33 ± 3.02 mg/L (NT4) for, whereas in the control tank, total TP removal only achieves 16.67% after a period of eight days of treatment.

3.2.3. Organic matter COD

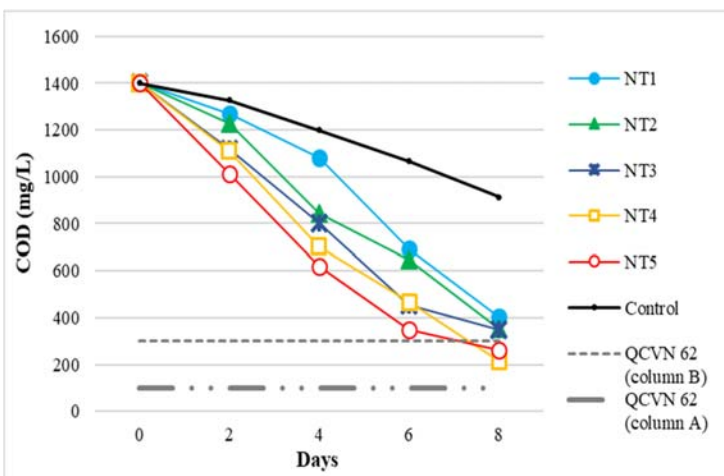

Figure 8. *COD removal by A.gracile*

Figure 8 suggests significant COD removal after two days with the initial concentration of 1,400.0 ± 9.17 mg/L. Microalgae species can synthesize organic matter themselves and also use nutrients in the water environment. COD removal efficiencies of all the 5 algae-supplemented experiments after eight days are above 71%. As the maximum results of 81.19% and 84.62% belong to NT5 and NT4, respectively, the remaining COD contents of these two experiments meet the QCVN 62-MT:2016/BTNMT standard, column B (300mg/L). In short, it is recommended that *A.gracile* can consume organic matter and, thus, reduce the COD content in raw livestock wastewater.

4. Conclusion

The nitrogen concentration in wastewater of *A. gracile* after eight days of treatment decreases from 320 $\pm$ 5.57 mg/L to about 58.0 $\pm$ 6.56 – 102.33 $\pm$ 5.51 mg/L, below permitted value in QCVN 62-MT:2016/BTNMT, column B (150 mg/L) in five tanks supplemented with different *A. gracile* density. Nitrogen removal efficiency is the highest at the density of 2.5×10^6 cells/mL and 3×10^6 cells/mL at 81.88% and 78.65% respectively.

Phosphorus removal efficiency after eight days of treatment in the tanks supplemented with *A. gracile* fluctuates between 48.52% and 64.07% with an initial concentration of 90.0 $\pm$ 10.15 mg/L.

The COD removal efficiency of *A. gracile* after eight days of treatment reaches a maximum efficiency of 84.62% with the remaining COD concentration of 215.33 $\pm$ 7.02mg/L.

However, the research needs further investigation on the adaptation of *A. gracile* in actual swine wastewater (at least eight days) for comparison of its treatment ability between the experiments. TSS and pH (before and after the experiment) should also be included for a full evaluation of *A.gracile* capability to treat the wastewater, meeting the standard of QCVN 62-MT:2016/BTNMT.

❖ ***Conflict of Interest:*** *Author have no conflict of interest to declare.*

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KHẢO SÁT KHẢ NĂNG XỬ LÍ NƯỚC THẢI CHĂN NUÔI HEO SAU HẦM BIOGAS BẰNG TẢO *ACTINASTRUM* SP.

Dương Thị Giáng Hương

Trường Đại học Sài Gòn

Tác giả liên hệ: Dương Thị Giáng Hương – Email: huongduong@sgu.edu.vn

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TÓM TẮT

Hiện nay tảo đã và đang được chú trọng nghiên cứu để ứng dụng vào trong các lĩnh vực khác nhau. Với mong muốn góp phần vào ứng dụng công nghệ vi sinh giảm thiểu ô nhiễm trong các nguồn nước thải ô nhiễm hữu cơ... phù hợp với tình hình Việt Nam, nghiên cứu này tiến hành ứng dụng tảo Actinastrum sp. *nhằm xử lí nitơ, photpho trong nước thải chăn nuôi heo sau hầm biogas. Kết quả thực nghiệm cho thấy chi tảo này có khả năng xử lí tốt nước thải chăn nuôi, đáp ứng các tiêu chuẩn theo Quy định Quốc gia QCVN 62-MT:2016/BTNMT với hiệu suất loại bỏ COD và TN tối đa lần lượt là 84,62% và 81,88%, khi được bổ sung mật độ tảo 2,5×10^6 tế bào/mL.*

Từ khóa: nước thải chăn nuôi heo; sau hầm bioga; tảo *Actinastrum* sp.