PHYCOREMEDIATION OF SWINE MANURE WASTEWATER BY FRESHWATER GREEN MICROALGAE

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ABSTRACT

Microalgae are micrometer-sized single-cell aquatic organisms that exhibit photosynthetic metabolism and potential for wastewater treatment. The aim of this study was to determine the growth rate and nutrients removal using two different species of freshwater microalgae (Scenedesmus quadricauda and Stigeoclonium sp.*) in remediation of swine manure wastewater. The experimental works were carried out at temperature of 30±1 ^oC under 1524 Lux light intensity for 14 days. Scenedesmus quadricauda revealed its effectiveness in remediating swine manure wastewater, by reducing 83.99% of COD, 80.39% of BOD_5, 84.78% of total phosphorus (TP), 91.79% of ammonia nitrogen (NH_3-N), 89.79% of nitrate nitrogen (NO_3-N) and 87.14% of nitrite nitrogen (NO_2-N) compared to Stigeoclonium* sp. *which was only able to remove 79.26% of COD, 76.27% of BOD_5, 75.17% of TP, 86.42% of NH_3-N, 84.38% of NO_3-N and 82.38 NO_2-N. Both microalgae were able to grow well in swine manure wastewater. Therefore, they can be used for phycoremediation of swine manure wastewater.*

Keywords: Microalgae, phycoremediation, *Scenedesmus quadricauda*, *Stigeoclonium* sp., swine manure wastewater.

INTRODUCTION

Many environmental problems are caused by large amount of wastes produced from the intensive livestock industries worldwide. Swine manure is one of the most polluting agro-industrial wastewaters worldwide [1]. It contains high concentrations of nitrogen, phosphorus and organic matter. This can cause severe environmental problems such as eutrophication if it is not properly managed. Eutrophication has been identified as an important environmental and public health concern [2]. Therefore, the development of cost-effective and environmental-friendly methods for the treatment of swine manure is mandatory.

Agriculture land disposal methods have traditionally been used to solve swine manure management. However, the recent intensive farming method has caused the natural capacity of the farm surrounding lands to overflow and unable to cope with swine wastewaters [3]. Conventional biological treatments such as activated-sludge or sequential-batch processes have not been promoted and implemented in rural areas due to the high complexity and energy inputs associated with these technologies [3].

Microalgae can play a key role in the quest for sustainable wastewater treatment in the 21st century. Microalgae-based treatment can be used for tertiary treatment of wastewater due to their capacity to assimilate nutrients [4]. Recently there is a lot of on-going research on the treatment of industrial, municipal and agricultural wastewaters by microalgae system. It was found that when cultivating *Arthrospira platensis* in olive-oil mill wastewater the maximum removal of chemical oxygen demand (COD) was 73.18% while phenols, phosphorus and nitrates in some were completely removed [5]. The comparison of two species of microalgae growing as immobilized and free-cells to test their abilities to remove total nitrogen (TN) and total phosphate (TP) in batch cultures with urban wastewater also had been published [6]. In addition, a research work had reported that *Chlorella phyrenoidosa* could remove about 80-85% of TP and 60-80% of TN from dairy wastewater [7].

Treatment and disposal of swine manure are among the most important environmental problem to be solved in many countries. Treating animal waste involves anaerobic treatment followed by post-treatment in high-rate oxidation ponds is an effective and widely used methods [8]. Many treatments of swine wastewater by microalgae have been published, even in pilot-scale operation, as a means of providing environmental protection and recovery of nutrient [3, 9]. There seem to be dearth of information on the use of *Scenedemus quadricauda* and *Stigeoclonium* sp. in the treatment of swine manure wastewater. Therefore, this research study represents one of the few studies in this area.

This work evaluates the performance of *Scenedesmus quadricauda* and *Stigeoclonium* sp. in the phycoremediation of swine manure wastewater. Batch experiments were conducted to analyse the growth rate of green microalgae in swine manure wastewater. The study was designed to determine the efficiency of *Scenedemus quadricauda* and *Stigeoclonium* sp. in reducing COD, BOD, TP, NH_3-N, NO_3-N and NO_2-N in swine manure wastewater.

MATERIALS AND METHODS

Microalgae and Culture Conditions

Pure microalgal strains of *Scenedesmus quadricauda* and *Stigeoclonium* sp. were obtained from the School of Biological Sciences, Universiti Sains Malaysia and maintained in Bold's Basal medium (BBM) which consists of 25mg/L $NaNO_3$, 7.5 g/L $MgSO_4.7H_2O$, 7.5 g/L K_2HPO_4, 2.5 g/L $CaCl_2.H_2O$, 2.5 g/L NaCl, 11.42 g/L H_3BO_4, 50 g/L EDTA.Na_2, 31 g/L KOH, 4.98 g/L $FeSO_4.7H_2O$, 1.0 mL concentrated HCL and 1.0 mL trace elements solution. The trace elements solution contained 8.82 g/L $ZnSO_4$, 1.44 g/L $MnCl_2.4H_2O$, 1.59 g/L $CuSO_4.5H_2O$, 0.71 g/L MoO_3, 0.49 g/L $Co(NO_3).4H_2O$. 20 mL of each microalgae species were inoculated in 250 mL Erlenmeyer flask which contained 100 mL of autoclaved BBM and shaken at 130 rpm. All the cultures were done in triplicate and maintained at a temperature of 30±1 ºC with continuous light supply at 1524 Lux (Philips TLD 18W/54-765 fluorescent lamp).

Swine Manure Wastewater

Swine manure wastewater from a private farm with about 400 swine in Penang, Malaysia was collected. Pre-treatment was carried out by sedimentation and filtration with a filter cloth to remove large and non-soluble particulate solids. After filtration, the substrate was stored at 4 ºC for 2 days to allow settling of particulate solids and the supernatant was used for microalgae growth and phycoremediation studies. The wastewater was characterized by determining the pH, COD, BOD_5, TP, NH_3-N, NO_3-N and NO_2-N [10].

Adaptation of Microalgae in Diluted Swine Manure Wastewater

Adaptation process of microalgae in swine manure wastewater was carried out in order to achieve good growth of microalgae, without being easily eliminated due to competition with bacteria and other possibly existing algae species. *Scenedesmus quadricauda* and *Stigeoclonium* sp. was acclimatized in the diluted swine manure wastewater. Chlorophyll α content of the microalgae was obtained every day for one week to determine the ability of microalgae to grow in the diluted swine manure wastewater.

Phycoremedation of Swine Manure Wastewater

A 250 mL of Erlenmeyer flask which contained 100 mL of swine manure wastewater were prepared for cultivation of microalgae. 20 mL of each microalgae species with chlorophyll concentration of 0.5-1.0 mg/L each was transferred into the 100 mL of swine manure wastewater. The experimental work was carried out for 14-days [11, 12]. The reduction of COD, BOD_5, TP, NH_3-N_2, NO_3-N and NO_2-N was monitored during the cultivation period. Sampling was carried out at an interval of 2 days during the cultivation period. All the experiments were carried out in triplicates.

Analytical Methods

The growth of *Scenedesmus quadricauda* and *Stigeoclonium* sp. was determined by determining the chlorophyll α content spectrophotometrically. 20 mL of microalgae were extracted in 10 mL of acetone (90%) and stored at 4 ºC in dark for 24 hours after filtered with 0.45 µm membrane filter. Absorbance of the chlorophyll extracts of each microalgae species were determined after 24 hours at wavelength 647 nm and 664 nm using Shimadzu UV-Visible Spectrophotometer. The chlorophyll α content was determined by using equation (1) which correlates the absorbance and chlorophyll α content in 90% of acetone extracts [13].

$$chl \propto (mg/L) = -1.7858A_{647} + 11.8668A_{664} \tag{1}$$

where A_{647} is the absorbance at 647 nm and A_{664} is the absorbance at 664 nm.

20 mL of the sample was collected every 2 days for growth rate and parameters (NH_3-N, NO_3-N, NO_2-N, TP, COD, and BOD_5) reduction analysis. The samples were first filtered by using 0.45 µm cellulose nitrate membrane filters. After that, the supernatants were used for parameters reduction analysis, while the membrane filters with chlorophyll residues were used for growth analysis. The filtrates were analyzed for COD, NH_3 -N, NO_3 -N, NO_2 -N and TP following HACH DR 2800 Spectrophotometer Procedures Manual (HACH, 2007). BOD was determined every four days according to [10] method 5210B. The percentage reduction of each parameter was obtained by applying equation (2),

$$percentage\ of\ reduction\ (\%) = \frac{C_i - C_o}{C_i} \times 100\% \tag{2}$$

where C_i is the initial concentration of the parameter at initial time t_o and C_o is the final concentration at time t.

RESULTS AND DISCUSSIONS

Characterization of Swine Manure Wastewater

The characteristics of raw swine manure wastewater was determined by analyzing the following parameters - pH, COD, BOD_5, Salinity, TP, NH_3-N, NO_3-N and NO_2-N. All the analysis results are shown in Table 1.

Table 1. Characteristics of Swine Manure Wastewater

Parameters	**Swine manure wastewater**
pH	8.4
COD (mg/L)	1474
BOD_5 (mg/L)	510
TDS (mg/L)	2630
Salinity (o/oo)	2.8

TP (mg/L)	149
NH_3-N (mg/L)	186
NO_3-N (mg/L)	160
NO_2-N (mg/L)	140

Microalgae Growth in Swine Manure Wastewater

The growth of *Scenedesmus quadricauda* and *Stigeoclonium* sp. in swine manure wastewater was determined by the variation of chlorophyll α content with respect to cultivation periods as shown in Figure 1. A short period of lag phase was observed for both microalgae in the swine manure wastewater although the adaptation process had been carried out in the preliminary study. The occurrence of lag phase is due to the hindrance by bacteria which existed in the swine manure wastewater. Some previous studies showed the existence of bacteria which led to competition with microalgae for inorganic nutrients [14]. The deep brown colour of raw swine wastewater also contributed to the lag phase as suspended compounds or impurities in swine manure wastewater limited the penetration of light into swine manure wastewater and hence impeded microalgae photosynthesis [9]. *Stigeoclonium* sp. was found to adapt faster and grew better than *Scenedesmus quadricauda* in swine manure wastewater. *Stigeoclonium* sp. started to grow tremendously from 6^{th} day of experiment while the stable growth of *Scenedesmus quadricauda* started from the 8^{th} day. *Stigeoclonium* sp. are known to have high tolerance toward a wide range of water conditions, including water bodies with significant amount of heavy metals and organic compounds [15].

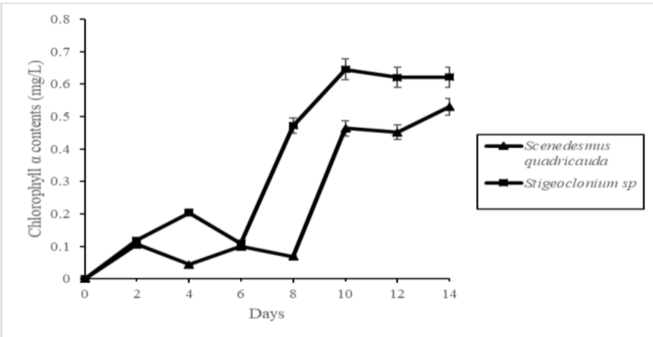

Figure 1. Growth rate of *Scenedesmus quadricauda* and *Stigeoclonium* sp. in swine manure wastewater

Chemical Oxygen Demand (COD) Reduction

The percentage of COD reduction of swine manure wastewater using *Scenedesmus quadricauda* and *Stigeoclonium* sp. is shown in Figure 2. Both species of microalgae (*Scenedesmus quadricauda* and *Stigeoclonium* sp.) showed high efficiency in COD reduction of swine manure wastewater. *Scenedesmus quadricauda* and *Stigeoclonium* sp. reduced 83.99% and 79.26% of COD of swine manure wastewater respectively for 14 days. The percentage of COD reduction remarkably increased after 2 days of cultivation for both *Scenedesmus quadricauda* and *Stigeoclonium* sp. The sharp increase in COD reduction is due to the rapid assimilation by microalgae and some residual bacteria. Bacteria degraded some of the long-chained organic compound into smaller digestible molecules for microalgae uptake and at the same time utilized part of the organic compounds [14]. It is also believed that under moderate light condition, microalgae developed mixotrophic growth by utilizing the organic components of complex nutrients in swine manure wastewater, resulting in rapid assimilation of organic carbon [9].

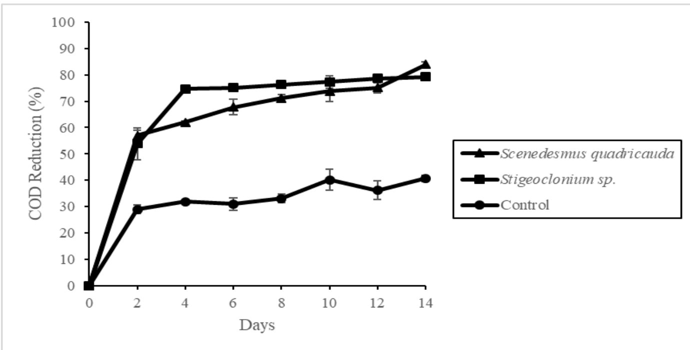

Figure 2. Percentage removal of chemical oxygen demand (COD) from swine manure wastewater by *Scenedesmus quadricauda* and *Stigeoclonium* sp.

Biochemical Oxygen Demand (BOD_5) Reduction

Figure 3 shows the percentage reduction of BOD_5 of swine manure wastewater by *Scenedesmus quadricauda* and *Stigeoclonium* sp. with respect to the cultivation days. *Scenedesmus quadricauda* reduced 80.39% while *Stigeoclonium* sp. reduced 76.27% of BOD_5 from swine manure wastewater. The higher reduction of BOD_5 by both microalgae was probably due to nutrient emission during bacterial metabolism that increased the organic content in swine manure wastewater. Some fluctuation occurred in control is due to the existence of bacteria which also played a role in the degradation of organic compounds.

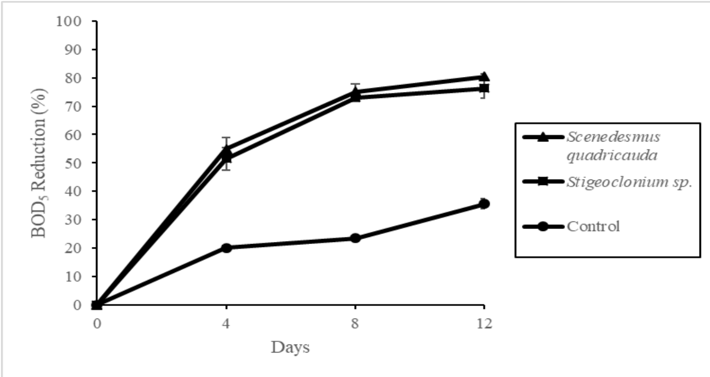

Figure 3. Percentage removal of biochemical oxygen demand (BOD_5) from swine manure wastewater by *Scenedesmus quadricauda* and *Stigeoclonium* sp.

Swine Manure Wastewater Nutrient Uptake by Microalgae
Reduction of Total Phosphorus (TP)

As shown in Figure 4, the phosphorus reduction observed within 14 days of cultivation exhibited similar trends to both microalgae. The increasing TP uptake in the first few days was due to the metabolism of both microalgae. Phosphorus plays a significant role in microalgae metabolism and cell growth [16]. *Scenedesmus quadricauda* reduced TP higher than *Stigeoclonium* sp. which is 84.78% and 75.17% respectively. Phosphorus was removed from algal culture through a combination of assimilation and chemical precipitation process [17]. The higher reduction efficiency of TP by both of *Scenedesmus quadricauda* and *Stigeoclonium* sp. in swine manure wastewater indicated that the existence of bacteria also contributed for the assimilation of phosphorus[18]. The uptake of total phosphorus by *Scenedesmus quadricauda* is better than *Stigeoclonium* sp. This is because the bacteria in the culture sample which contained *Scenedesmus quadricauda* has better adaptability and assimilation rate than the culture sample which contained *Stigeoclonium* sp. There were fluctuations in the control experiment. The decreases of percentage removal of TP in the 6^{th} (sixth) day is due to the increases in concentration of TP. This is properly because of the minimum supply of oxygen in wastewater. The lack of oxygen can trigger the release of phosphorus from sediments. Unlike green microalgae, there are only a few specific autotrophic bacteria species that can utilize carbon dioxide for photosynthesis and production of oxygen [19]. The oxygen used in decomposition of organic compounds was not compensated and hence created a minimum oxygen condition which triggered the release of phosphorus from the sediments [19].

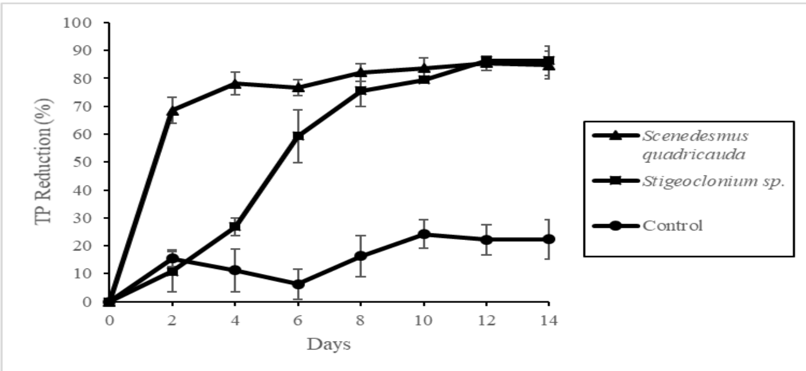

Figure 4. Percentage removal of total phosphorus (TP) from swine manure wastewater using *Scenedesmus quadricauda* and *Stigeoclonium* sp.

Reduction of Ammonia Nitrogen, Nitrate Nitrogen, and Nitrite Nitrogen

From Figure 5 (a), 5(b) and 5(c), it can be seen that both microalgae performed well in removal of ammonia nitrogen, nitrate nitrogen and nitrite nitrogen. *Scenedesmus quadricauda* removed 91.79% of NH_3-N, 89.79% of NO_3-N and 87.14% of NO_2-N while *Stigeoclonium* sp. removed 86.42% of NH_3-N, 84.38% of NO_3-N and 82.38% of NO_2-N within 14 days cultivation periods. Ammonia nitrogen is the dominant form of nitrogen sources among the three nitrogen species, followed by nitrate and lastly nitrite. Ammonia is a preferable nitrogen source for microalgae uptake, as ammonia can be directly utilized without the need for enzymatic reaction [20]. Nitrate is however needed to be converted to ammonia for microalgal assimilation. There are two enzymatic steps involved to convert nitrate into ammonia. Firstly, nitrate is reduced to nitrite by an enzyme called nitrate reductase which existed in microalgae. The process consumes a considerable amount of energy and electrons, thus the assimilation of nitrate is not preferred by microalgae. Furthermore, since ammonia is the end product in reduction of nitrate and can be directly utilized by microalgae, it inhibits and limits the nitrate uptake [21]. Therefore, a higher reduction rate in ammonia than nitrate should be expected. Nitrite is the least important nitrogen source compared to ammonia and nitrate due to its low stability and only present in lower concentrations. Nitrite reduction is more likely related to photosynthetic electron transport rather than assimilation and enzymatic reduction. Uptake rate of nitrite can be higher than that of nitrate in the presence of light [22].

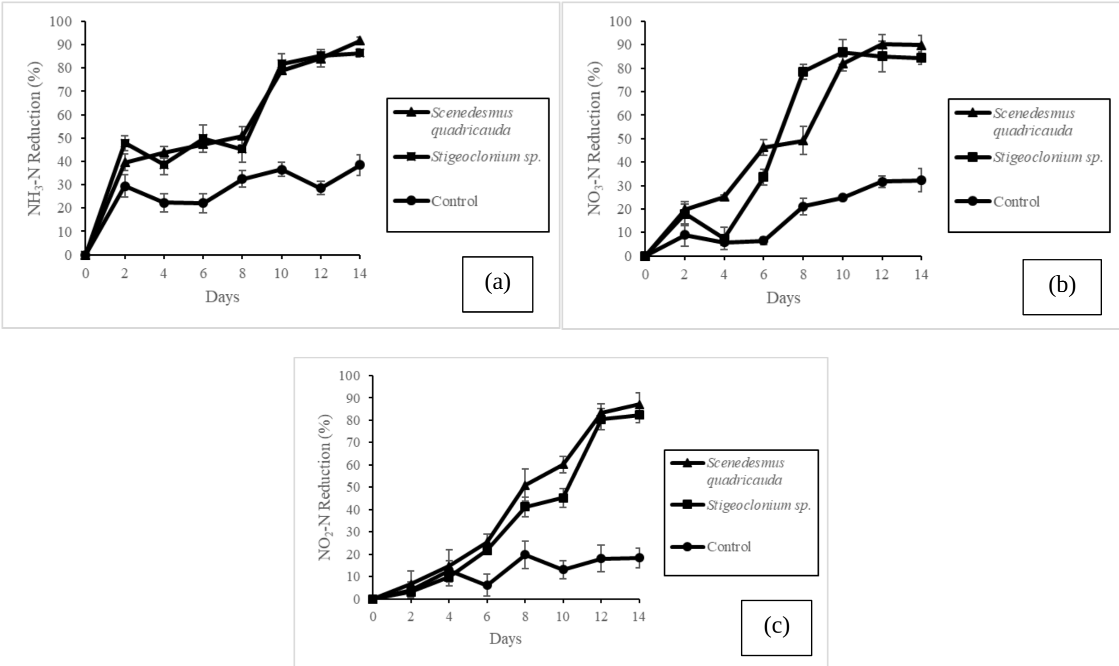

Figure 5. Percentage removal of (a) ammonia nitrogen, (b) nitrate nitrogen and (c) nitrite nitrogen from swine manure wastewater by *Scenedesmus quadricauda* and *Stigeoclonium* sp.

CONCLUSION

Scencedesmus quadricauda and *Stigeoclonium* sp. have shown the ability to grow in swine manure wastewater. Both microalgae are effective in the removal of COD, BOD_5 and nutrients from swine manure wastewater. Conversely, *Scencedesmus quadricauda* performed better in phycoremediation of swine manure wastewater compared to *Stigeoclonium* sp.

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