Malaysian Journal of Microbiology, Vol 14(2) Special Issue 2018, pp. 187-194

Malaysian Journal of Microbiology
Published by Malaysian Society for Microbiology
(In SCOPUS since 2011)

Removal of COD, BOD and nutrients in swine manure wastewater using freshwater green microalgae

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ABSTRACT

Aims: The aim of this study was to investigate the effectiveness of freshwater green microalgae in remediating swine manure wastewater. Two different species of freshwater green microalgae (*Scenedesmus quadricauda* and *Stigeoclonium* sp.) were used in this study.
Methodology and results: Laboratory experiments were performed to compare the growth rate and nutrients (total phosphorus, ammonia nitrogen, nitrate nitrogen and nitrite nitrogen) uptake by these two species of microalgae in swine manure wastewater. Experimental work was carried out for 14 days at room temperature of 30±1 °C with about 1520 Lux of light intensity. The results showed that both microalgae grew well in swine manure wastewater. *S. quadricauda* performed better 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.
Conclusion, significance and impact of study: The results of this study indicate that these two species of microalgae have potential to be used in the remediation of swine manure wastewater.

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

INTRODUCTION

Swine manure wastewater, or wastewater produced in pig farming, is an agricultural wastewater which contains very high nitrogen, phosphorus and other organic matter (Godos *et al*., 2010). Eutrophication is one of the severe environmental problems which is caused by improper management of this kind of wastewater (Ji *et al*., 2013). The traditional method of swine manure management is the agriculture land disposal method, where the wastewater is re-used or disposed in agricultural land as natural fertilizer. However, recent intensive farming method has caused the natural capacity of the farm surrounding lands to overflow and unable to cope with swine wastewater (Godos *et al*., 2009). 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 (Godos *et al*., 2009). Therefore, the development of cost-effective and environmental-friendly methods for the treatment of swine manure is mandatory.

Microalgae are micrometer-sized single-cell aquatic organisms that exhibit photosynthetic metabolism and potential for wastewater treatment. The efficiency of their capacity to assimilate nutrients and organic matters had caused them to be utilized in tertiary treatment of wastewater (Larsdotter, 2006). A wide range of research work has been conducted on using microalgae to remediate industrial, municipal and agricultural wastewaters. 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 (Markou *et al*., 2012). A 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 (Ruiz-Marin *et al*., 2010). 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 (Kothari *et al*., 2012).

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 (Olguín *et al*., 2003). 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 (Godos *et al*., 2009; Wang *et al*., 2012). For example, 80% of nitrate was removal by green alga *Botryococcus braunii*.

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ISSN (print): 1823-8262, ISSN (online): 2231-7538

However, there seem to be a 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.

The aim of this study was to evaluate the performance of *S. quadricauda* and *Stigeoclonium* sp*.* in the remediation of swine manure wastewater. Batch experiments were conducted to analyse the growth rate of both green microalgae in swine manure wastewater. The efficiency of *S. quadricauda* and *Stigeoclonium* sp. in reducing COD, BOD, TP, NH_3-N, NO_3-N and NO_2-N in swine manure wastewater will also be determined.

MATERIALS AND METHODS

Microalgae strains and culture conditions

Both freshwater green microalgae strains of *Scenedesmus quadricauda* and *Stigeoclonium* sp. were obtained from the School of Biological Sciences, Universiti Sains Malaysia. The two strains of freshwater green microalgae were cultured in Bold's Basal medium (BBM) which consisted of 25 mg/L $NaNO_3$, 7.5 g/L $MgSO_4 \cdot 7H_2O$, 7.5 g/L K_2HPO_4, 2.5 g/L $CaCl_2 \cdot H_2O$, 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 $FeSO_4 \cdot 7H_2O$, 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 $MnCl_2 \cdot 4H_2O$, 1.59 g/L $CuSO_4 \cdot 5H_2O$, 0.71 g/L MoO_3, 0.49 g/L $Co(NO_3) \cdot 4H_2O$.

A 20% of each microalgae species was inoculated in a 250 mL Erlenmeyer flask which contained 100 mL of autoclaved BBM, and shaken at 130 rpm under room temperature of 30±1 °C with continuous light supply at about 1520 Lux (Philips TLD 18W/54-765 fluorescent lamp).

Swine manure wastewater

Swine manure wastewater was collected from a private swine farm which was located in Penang, Malaysia. The farm has about 400 pigs. Pre-treatment of the collected wastewater sample 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 °C for 2 days to allow settling of particulate solids and the supernatant was used for microalgae growth and phycoremediation studies. The characteristics of wastewater was analysed by determining its pH, COD, BOD_5, TP, NH_3-N, NO_3-N and NO_2-N (APHA, 1998).

Phycoremediation of swine manure wastewater

Both strains of green microalgae were acclimatized in different dilutions of swine manure wastewater for one week before being used in the remediation study. This step is performed in order to achieve good growth of both strains of microalgae in swine manure wastewater.

A 20% of each microalgae species with initial chlorophyll concentration of 0.5-1.0 mg/L respectively was transferred into the 100 mL of swine manure wastewater. The experimental work was carried out for 14-days (Abou-Shanab *et al*., 2013; Zhu *et al*., 2013). 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 rate of both strains of microalgae, *S. quadricauda* and *Stigeoclonium* sp. were based on the chlorophyll α content spectrophotometrically. A 20 mL of both microalgae samples were taken out from each flask every two days. The samples were filtered with 0.45 µm cellulose nitrate membrane filters. The filtrates were discarded while the pellets of microalgae which were retained on the membrane filters were dissolved using 10 mL of acetone (90%). The mixtures were stored at 4 °C in dark for 24 h. Absorbance of the chlorophyll extracts of each microalgae species was determined after 24 h at wavelength 647 nm and 664 nm using Shimadzu UV-Visible Spectrophotometer. The chlorophyll α content was determined using equation (1) which correlates the absorbance and chlorophyll α content in 90% of acetone extracts (Ritchie, 2006).

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

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

A 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 (APHA, 1998) method 5210B. The percentage reduction of each parameter was obtained by applying equation (2),

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

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 DISCUSSION

Characterization of swine manure wastewater

The characteristics of the raw swine manure wastewater were determined by analyzing the following parameters -

ISSN (print): 1823-8262, ISSN (online): 2231-7538

pH, COD, BOD_5, Salinity, TP, NH_3-N, NO_3-N and NO_2-N. All the results of the analysis are listed 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 (Gan *et a*l., 2014). 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 (Wang *et al*., 2012). *Stigeoclonium* sp. was found to adapt faster and grew better than *S. quadricauda* in swine manure wastewater. *Stigeoclonium* sp. grew extremely well from the 6^{th} day of experiment while the stable growth of *S. 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 (Kim *et al*., 2015).

Chemical oxygen demand (COD) reduction

The percentage of COD reduction of swine manure wastewater using *S. quadricauda* and *Stigeoclonium* sp*.* is shown in Figure 2. Both species of microalgae (*S. quadricauda* and *Stigeoclonium* sp.) showed high efficiency in COD reduction of swine manure wastewater. *S. 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 *S. 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 (Gan *et al*., 2014). 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 (Wang *et al*., 2012).

Biochemical Oxygen Demand (BOD_5) reduction

Figure 3 shows the percentage reduction of BOD_5 of swine manure wastewater by *S. quadricauda* and *Stigeoclonium* sp. with respect to the cultivation days. *S. 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 the control which was due to the existence of bacteria which also played a role in the degradation of organic compounds.

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 (Markou *et al*., 2014). *S. 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 (Ge and Champagne, 2016). The higher reduction efficiency of TP by both of *S. quadricauda* and *Stigeoclonium* sp*.* in swine manure wastewater indicated that the existence of bacteria also contributed to the assimilation of phosphorus (El-Sheekh *et al*., 2012). The uptake of total phosphorus by *S. quadricauda* is better than *Stigeoclonium* sp*.* This is because the bacteria in the culture sample which contained *S. quadricauda* has better adaptability and assimilation rate than the culture sample which contained *Stigeoclonium* sp*.* There were fluctuations in the control experiment. The decrease of percentage removal of TP on the 6^{th} (sixth) day is due to the increases in concentration of TP. This is probably due to the minimal 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 (Moore *et al*., 2013). 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 (Moore *et al*., 2013).

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

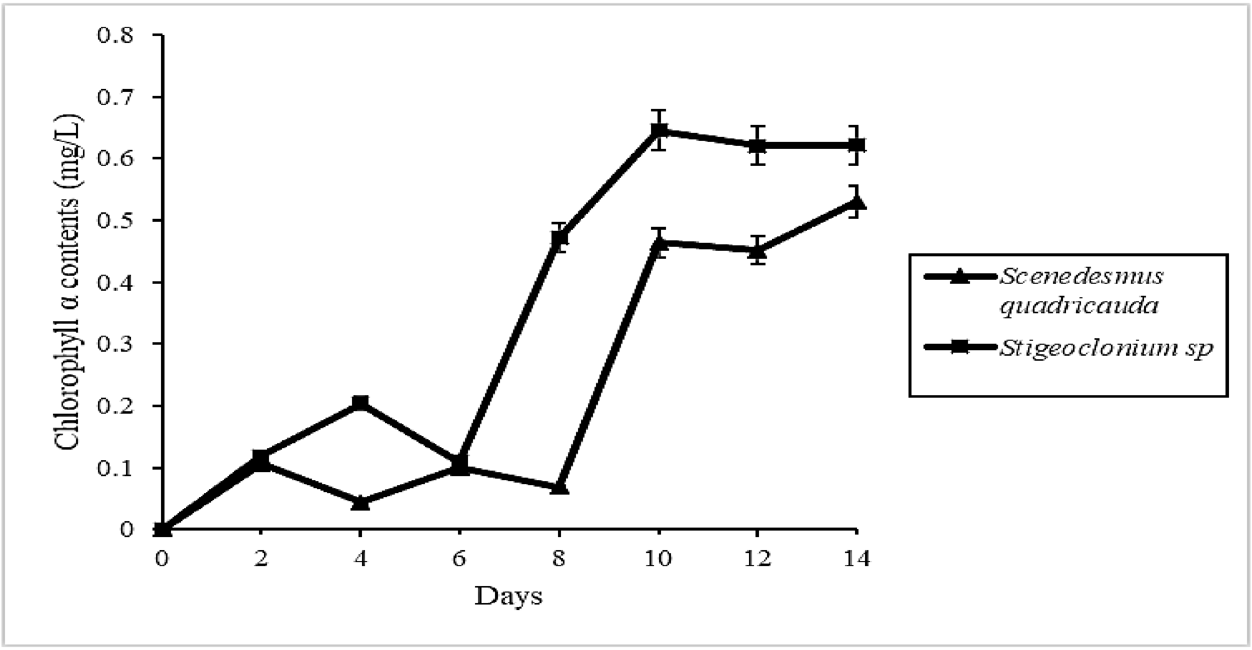

Figure 1: Growth rate of *S. quadricauda* and *Stigeoclonium* sp. in swine manure wastewater.

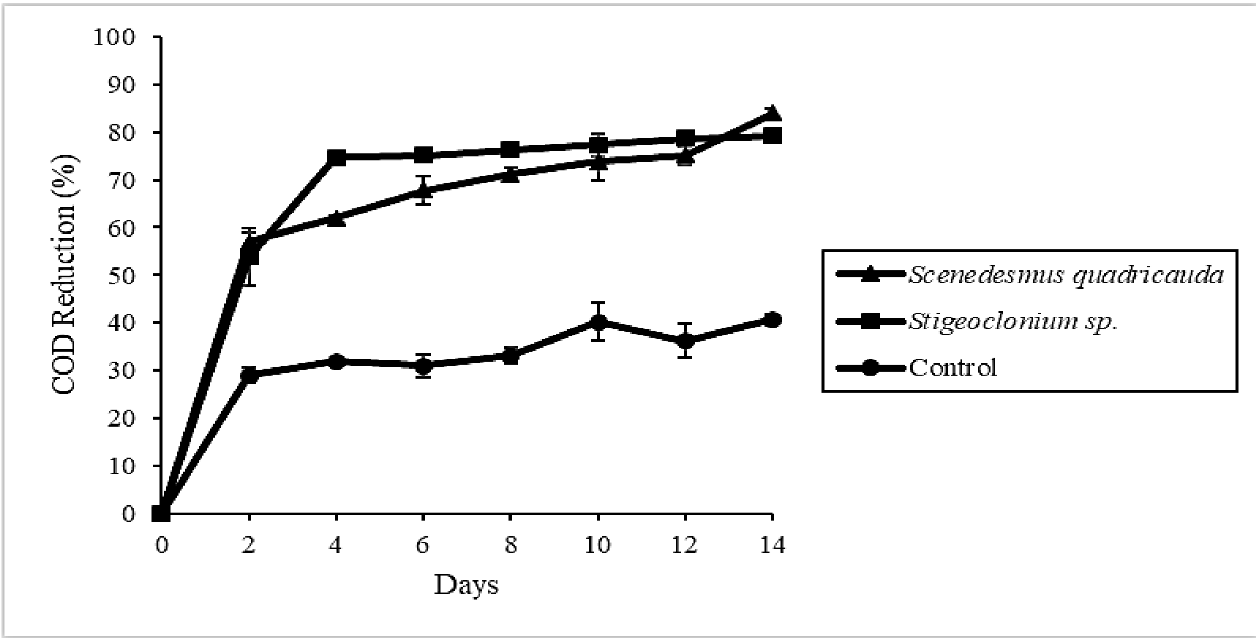

Figure 2: Percentage removal of chemical oxygen demand (COD) from swine manure wastewater by *S. quadricauda* and *Stigeoclonium* sp.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

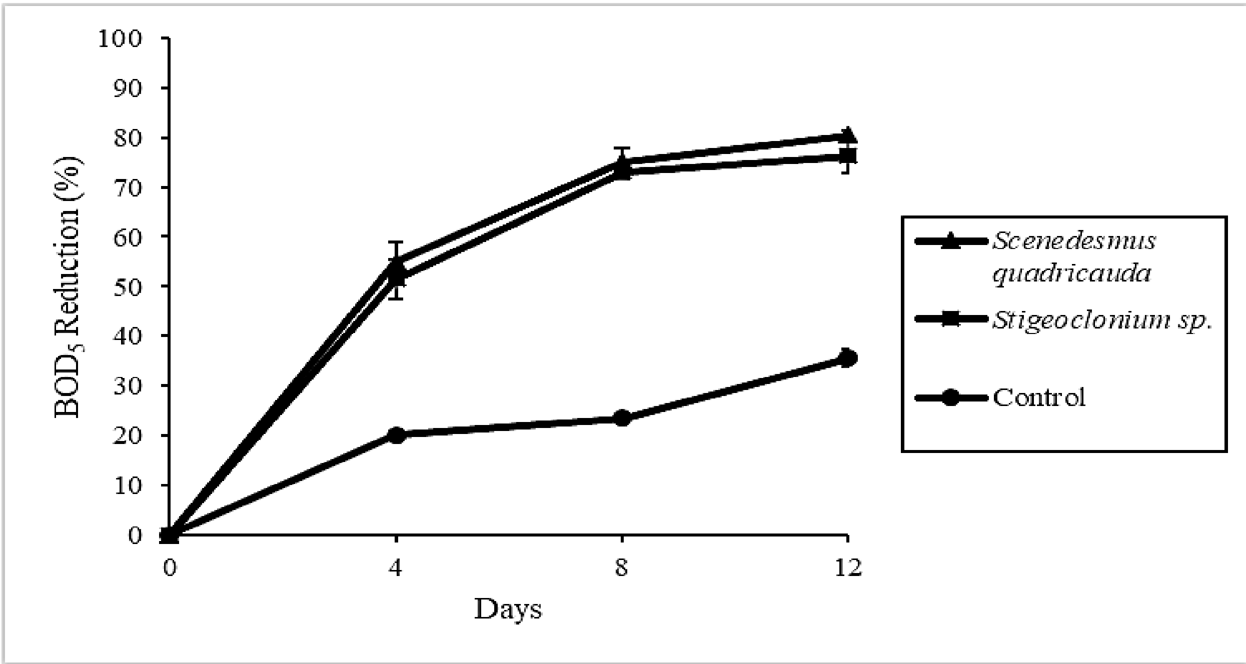

Figure 3: Percentage removal of biochemical oxygen demand (BOD_5) from swine manure wastewater by *S. quadricauda* and *Stigeoclonium* sp.

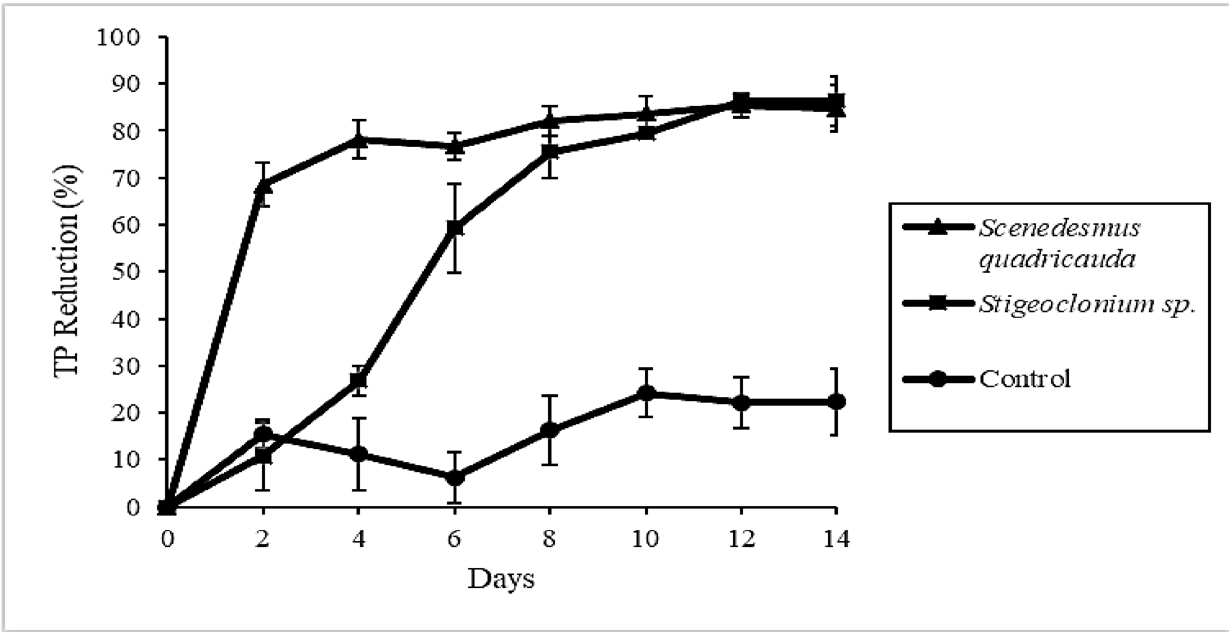

Figure 4: Percentage removal of total phosphorus (TP) from swine manure wastewater using *S. quadricauda* and *Stigeoclonium* sp.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

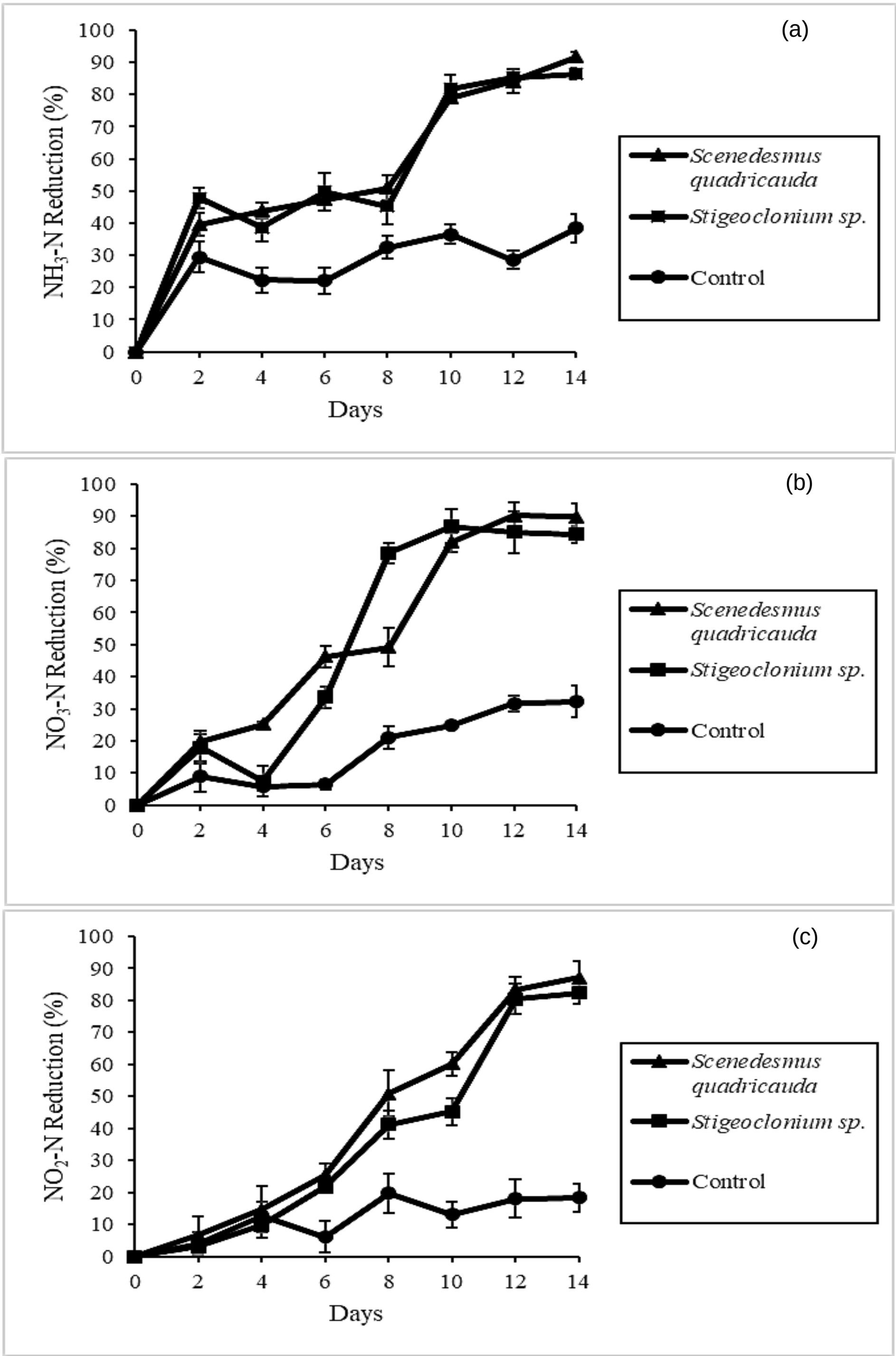

Figure 5: Percentage removal of (a) ammonia nitrogen, (b) nitrate nitrogen and (c) nitrite nitrogen from swine manure wastewater by *S. quadricauda* and *Stigeoclonium* sp.

ISSN (print): 1823-8262, ISSN (online): 2231-7538

Reduction of ammonia nitrogen, nitrate nitrogen, and nitrite nitrogen

From Figure 5 (a-c), it can be seen that both microalgae performed well in removal of ammonia nitrogen, nitrate nitrogen and nitrite nitrogen. *S. 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 (Hii *et al*., 2011). 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 exists 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 (Becker, 1994). 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 its presence 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 (Läuchli and Bieleski, 1983).

CONCLUSION

Both freshwater green microalgae, *S. quadricauda* and *Stigeoclonium* sp. have shown the ability to grow well in swine manure wastewater. Both microalgae are effective in the removal of COD, BOD_5 and nutrients from swine manure wastewater. Conversely, *S. quadricauda* performed better in phycoremediation of swine manure wastewater compared to *Stigeoclonium* sp. The positive results for both strains of microalgae in phycoremediation of swine manure wastewater are important to introduce both microalgae as a promising agent for treating swine manure wastewater to prevent environmental pollution.

ACKNOWLEDGEMENT

The authors gratefully acknowledge financial support from Universiti Sains Malaysia in the form of Research University Grant No. 1001/PTEKIND/811291, which have resulted in this paper.

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