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Cultivation of *Spirulina platensis* using Anaerobically Swine Wastewater Treatment Effluent

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ABSTRACT

In this study, *Spirulina platensis* was cultivated by using effluent from anaerobically treated swine wastewater at concentration of 0, 10, 20, 40, 60, 80 and 100% dilution. Results showed that the most suitable concentration for maximum growth of *S. platensis* was 10% dilution with $NaHCO_3$ and $NaNO_3$ at 8.0 g L^{-1} and 1.5 g L^{-1}, respectively. From a batch culture with 10% dilute concentration and added nutrients, highest wastewater treatment efficiency occurred after 12 days retention time when maximum removal efficiency was measured at 23, 45, 49, 92 and 67% for COD, BOD, NO_3^-, NH^+_4 and PO_4^{3-}, respectively. Maximum growth of *S. platensis* was measured by 17.8×10^4 cells mL^{-1} and value of OD_{560} at 1.09 with 55.88 % protein content by dry weight. This suggested that *S. platensis* could be applied for use as a food nutrient in the farm that could decrease farm cost.

Key Words: *Spirulina platensis*; Swine wastewater; Treatment efficiency; Nutrient

INTRODUCTION

Swine waste generated from pig-domesticating industry is considered a primary source for fresh water pollution. Although many treatment systems have been investigated, the effluents are still high in of inorganic compounds, which cause deterioration of water resources. These compounds are also important nutrient sources for aquatic plants and specially algae (Finlayson *et al*., 1987; Tiow-Suan & Anthony, 1988; Sevrin-Reyssac, 1998). Algae use solar energy, while absorbing nutrients from wastewater to fix carbon substances to produce plant biomass that would constitute a valuable source of compounds for animal feeding supplement and that can be at the same time, as a decaying organic pollutant that decreases water pollution.

In recent years, algae have been applied to cultivate in many kinds of wastewater to improve water quality such as *Phormidium*, *Spirulina*, *Chlorella* and *Scendesmus* (Gantar *et al*., 1991; Anaga & Abu, 1996; Blier *et al*., 1996; Olguin *et al*., 1997; Phang *et al*., 2000; Travieso *et al*., 2006; Jongkon *et al*., 2008; Ungsethaphand *et al*., 2009). The production of algae yields a biomass that should be valuable as an animal feed supplement.

This study involved the selection of *Spirulina platensis*, which was cultivated in effluent from anaerobically treated swine wastewater. *S. platensis* is a plank tonic cyanobactera that grows fast as a valuable product (Ciferri & Tiboni, 1985; Cohen *et al*., 1987; Henrikson, 1989). Moreover, the farmers are able to use it in their swine farms as source of important protein substances thus helping to economize in terms of buying feed supplements, which are usually expensive while at the same time, increasing the quality of wastewater.

MATERIALS AND METHODS

Experiment I: Suitable dilutions from anaerobically treated swine wastewater for the growth of *S. platensis*: Effluent from anaerobically treated swine wastewater was first sanitized using sodium metabisulfite ($Na_2S_2O_5$) at 0.2 g L^{-1} for a day and diluted with distilled water to produce dilution percentages of 0, 10, 20, 40, 60, 80 and 100 in a 20-L container with a size of 5 L. *S. platensis* stock solution (OD_{560} at 1.0) with 10% dilution was added. The experiment was replicated three times in a Completely Randomized Design. The cultures were maintained for 14 days with sufficient aeration. The cellular concentration growths were determined by cells number. Such effluent had values of 1200-1800 mg L^{-1} COD, 760-1600 mg L^{-1} BOD, 40-90 mg L^{-1} NO_3^-, 73-210 mg L^{-1} NH_4^+, 260-675 mg L^{-1} PO_4^{3-}, 27-32ºC temperature and pH of 7.5-8.9.

Experiment II: Feed level suitable for growth of *S. platensis* in anaerobically treated swine wastewater effluent: *S. platensis* was maintained with the suitable swine waste concentration diluting base on a previous study in addition to the 4 types of feeds resulting to the following dilution levels:

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1) $NaHCO_3$ at 4, 6, 8 and 10 g L^{-1}
2) $NaNO_3$ at 0.5, 1, 1.5 and 2 g L^{-1}
3) K_2HPO_4 at 0.1, 0.3, 0.5 and 0.7 g L^{-1} and
4) N-P-K fertilizer (16-16-16) at 0.2, 0.4, 0.6 and 0.8 g L^{-1}.

The treatment was analyzed for the statistical significant difference by using the Analysis of Variance (ANOVA) and within Fisher's least significant difference (LSD) the 95%, confidence level.

Experiment III: Suitable condition and production of *S. platensis* in effluent from anaerobically treated swine wastewater: The batch culture of *S. platensis* was conducted using the suitable dilution from Exp. I and feed level from Exp. II in a 100 L cement pond with one meter diameter. The control used only the suitable dilution from Exp. I, no feed level. In a period of 16 days, the effluent was analyzed every 2 days. The cellular concentration growths were determined by optical density measurement at 560 nm and numbers of cell. The algae biomass was harvested and analyzed for the total protein. The treatment efficiency was assessed by determining the percentage reduction in COD, BOD, NH^+_4, NO^-_3 and PO_4^{3-}.

Table I: The highest growth of *S. platensis* by measuring OD_{560} and cells number on four types of nutrients composition

Nutrient Elements ($g\ L^{-1}$)	Cellular Concentration	
	OD_{560}	Cell number ($x10^4$) cells mL^{-1}
$NaNO_3$		
0	0.4235 ± 0.01^a	9.1386 ± 0.57^a
0.5	0.4879 ± 0.002^b	10.9571 ± 0.48^{abc}
1	0.4645 ± 0.03^b	11.5292 ± 0.85^{bc}
1.5	0.5298 ± 0.03^c	12.7158 ± 0.89^c
2	0.4830 ± 0.01^b	10.1883 ± 0.70^{ab}
$NaHCO_3$		
0	0.4830 ± 0.04^b	13.7441 ± 0.84^{ab}
4	0.4318 ± 0.01^a	13.1437 ± 0.97^a
6	0.4926 ± 0.06^b	12.0075 ± 0.52^a
8	0.5713 ± 0.11^c	16.0900 ± 1.22^b
10	0.4606 ± 0.02^{ab}	14.3142 ± 0.54^{ab}
NPK		
0	0.4393 ± 0.06^c	9.8850 ± 1.42^c
0.2	0.3663 ± 0.04^a	9.7758 ± 1.04^b
0.4	0.3426 ± 0.01^b	8.3033 ± 0.50^b
0.6	0.3638 ± 0.01^b	9.0600 ± 0.26^b
0.8	0.2592 ± 0.03^b	5.2908 ± 0.70^a
K_2HPO_4		
0	0.4393 ± 0.04^c	9.8850 ± 0.79^c
1	0.4608 ± 0.08^c	10.3242 ± 0.78^c
3	0.3147 ± 0.01^{ab}	8.3440 ± 0.44^a
5	0.3529 ± 0.03^b	10.0175 ± 0.36^c
7	0.2828 ± 0.01^a	$8.1400\pm0.1.59^a$

Note: Values followed by different letters in a column were significantly different (P‹0.05)

RESULTS AND DISCUSSION

Suitable dilutions from anaerobically treated swine wastewater for the growth of *S. platensis*: From this study, cellular concentration growth of *S. platensis* is illustrated in Fig. 1. It was found that *S. platensis* was able to grow in wastewater from anaerobically treated swine wastewater only with low dilution, because the algae was not able to adapt itself due to high substances, with high color and turbidity, which affected the rate of photosynthesis in algae. At 10% dilution, the algae showed the highest growth and could be survivable all throughout the experimental period with highest cells number observed during day 8 of the study period, which was 1.2×10^5 cells mL^{-1}. This was followed by 20% dilution, which produced 6.1×10^4 cells m L^{-1} during day 6. However, results showed that algal growth was at a short period only because the feed was not sufficient to the growth and there was, therefore, a need to add some important nutrients in order to improve the growth of algae.

Feed level suitable for growth of *S. platensis* in anaerobically treated swine wastewater effluent: Four types of nutrients were added to serve as a source of nutrients, with $NaNO_3$ (1.5 g L^{-1}) and $NaHCO_3$ (8.0 g L^{-1}), the algae had a greater increase in number of cells as compared to other treatments, while the number of cells and OD increased gradually throughout the study period with significant ($P<0.05$) different from the control (Table I). This might be caused by $NaNO_3$ and $NaHCO_3$ serving as good source of nitrogen, particularly $NaHCO_3$, which is significantly needed by algae during its growth (Huertas *et al*., 2000; Abu *et al*., 2007). When the alga is fed with $NaHCO_3$ and as a source of carbon, the emitted carbon dioxide gas is used in photosynthesis. The suitable amount of $NaHCO_3$ for *S. platensis* should range from 4.5-8.5 g L^{-1} and the resulting NaOH from the process could help in increasing the pH value into 9 (Venkataraman, 1983; Materassi *et al*.,1984; Binaghi *et al*., 2003).

When K_2HPO_4 was added to the dilution as a nutrient source, it was found that adding 0.1 g L^{-1} caused algae to have the best growth but not significantly ($P<0.05$) different with the control based on the OD value and amount of cells. With N-P-K fertilizer added situation, the results showed that the fertilizer was not effected the growth of *S. platensis*. The control was highest in cellular concentration.

However, in the study the cell concentration was low, because of changing in environment when transferred from the commercial medium to wastewater. The algae were not able to adapt and died too early. With inoculums of 10% *S. platensis* solution at the beginning could be not enough for the start up so it caused algae died by photo-oxidation (Abeliovich & Shilo, 1972; Benchokroun *et al*., 2003; Baroli *et al*., 2004). For these reasons in the next experiment, 20% *S. platensis* solution was used as starter. However, if algae concentration is too high at the beginning, it causes low in photosynthesis, because of shading and increase in investment (Vonshak *et al*., 1982; Gitelson *et al*., 1996).

Production of *S. platensis* in effluent from anaerobically treated swine wastewater: The batch culture of *S. platensis* was conducted using effluent at 10% dilution and initial 20% *S. platensis* stock solution with $NaNO_3$ at 1.5 g L^{-1} and $NaHCO_3$ 8 g L^{-1} added. The controls had no nutrient added.

Fig. 1: Cellular concentration of *S. platensis* on anaerobically treated swine wastewater effluent from difference dilutions

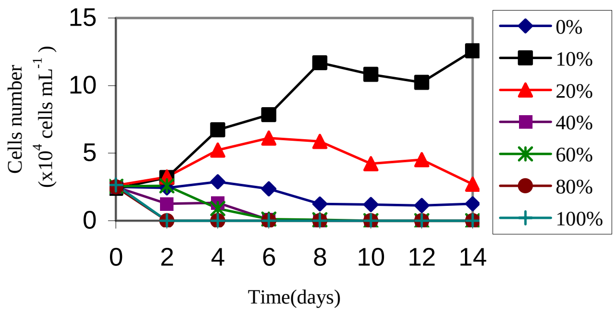


Fig. 2: Growth of *S. platensis* cultivated under batch culture in 10% dilution from anaerobically swine wastewater treatment

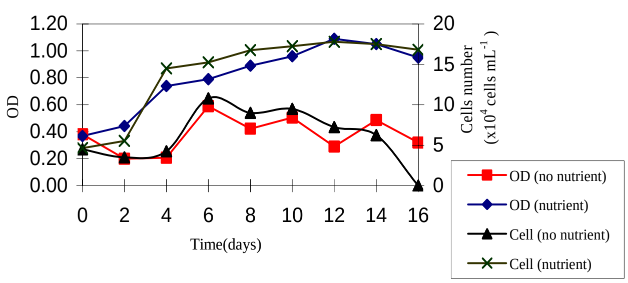
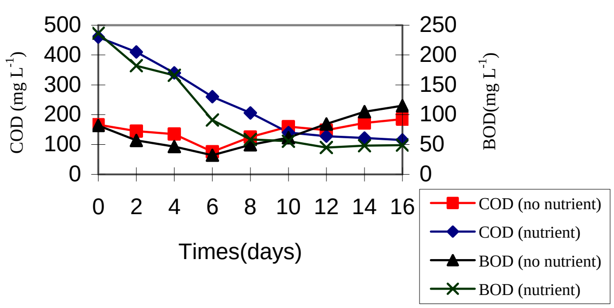


Fig. 3: COD and BOD content of effluent from *S. platensis* batch culture system



Results showed that treatments applied with nutrient supplements had better growth than those fed with no nutrient supplements in control and at day 12, had the highest number of cells at 17.8×10^4 cells mL^{-1} and OD_{560} value of 1.09. In the control experiment with no nutrient supplement, algae adjusted themselves much slower as indicated by the highest number of cells in day 6 (10.8×10^4 cells mL^{-1}), OD value of 0.59. At the end of the culture period (day 16), mortality was 100% (Fig. 2).

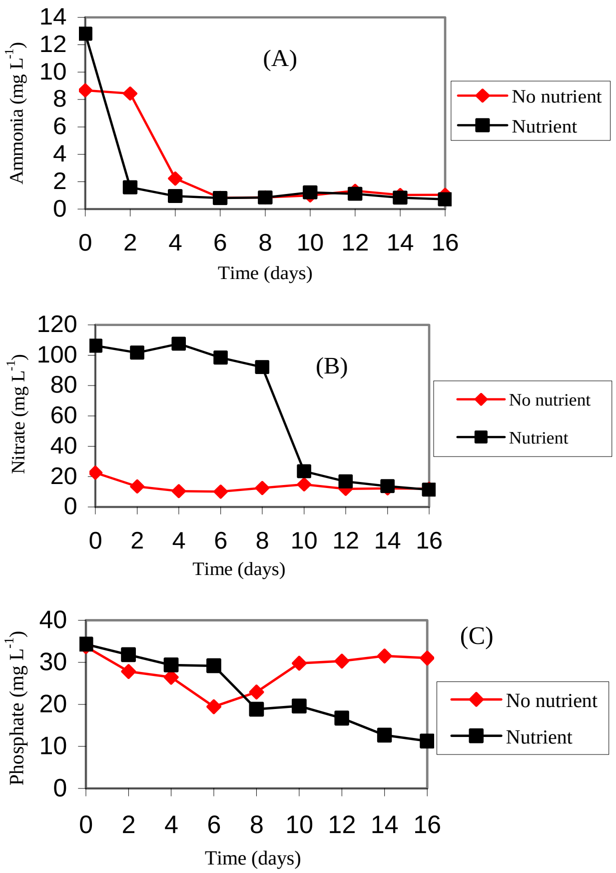
With regards to the efficiency of wastewater treatment, results showed that the use of nutrient supplements increased COD, BOD from 166 and 82 mg L^{-1} in control with no nutrient supplement to 460 and 236.5 mg L^{-1}, respectively. With nutrient supplement treatment indicated the highest growth and number of cells were observed at day 12 of the study with lowest COD and BOD values at 128 and 45 mg L^{-1}, respectively. The removal efficiency was measured as 23% and 45%, respectively compared with initial wastewater (no nutrient supplement) (Fig. 3). Afterwards, COD and BOD values were quite stable until the end of the study. As the control using no nutrient supplement, it was found that the algae had longer adjustment period than those fed nutrient supplements as indicated by quite consistent COD and BOD, which had the lowest values at day 6 with COD at 76 mg L^{-1} and BOD at 32 mg L^{-1}. The removal efficiency was measured 54% and 61%, respectively. Afterwards, COD and BOD values slightly increased continuously, because of the mortality of algae by starving. After dying, algae were decomposed and released nutrient into water (Chuntapa *et al.*, 2003). Resultantly, COD and BOD values slightly increased continuously at the end of the study.

When considering ammonia (Fig. 4A), the initial concentration was 12.8 mg L^{-1} in treatment using nutrient supplement as compared to 8.67 mg L^{-1} in treatments with no nutrient supplement. Results also showed that ammonia decreased very rapidly during the first two days of experiment in treatments using nutrient supplements. Ammonia that quickly decreased indicates the period of time needed for algae to adapt to the new environment. In this lag phase, algae used ammonium nitrate as a source of nitrogen at first (Becker, 1994; Costa *et al.*, 2001). In addition, in an aerobic condition, ammonia was also oxidized into nitrate. At the last stage of the experiment, it was found that ammonia reached the lowest amount (0.71 mg L^{-1}) in treatments using nutrient supplements thus showing a 92% efficiency of reducing ammonia when compared with initial wastewater (no nutrient supplement). The treatments not applied with nutrient supplements had lowest amount of ammonia at 0.83 mg L^{-1} at an efficiency of 75% in reducing ammonium N.

In terms of nitrogen (Fig. 4B), it was found that treatments applied with nutrient supplements, the initial value was 106.23 mg L^{-1} as compared to treatments with no nutrient supplement (22.8 mg L^{-1}). This study showed that treatments applied with nutrient supplements, nitrate had a high value and was quite consistent from the start of the experiment until day 8. This was because ammonia was oxidized to nitrate until ammonia in the wastewater began to disappear and algae started to use nitrogen in the form of nitrate such that the amount of nitrate decreased rapidly after day 8. At the final stage of the experiment, nitrate was measured as 11.36 mg L^{-1} with an efficiency rate of reducing nitrogen at 49% as compared to treatments applied with no nutrient supplements, which had only a slight decrease and the lowest value at day 6 (10.11 mg L^{-1}) with an efficiency value of reducing nitrogen at 54%.

In term of phosphate (Fig. 4C), treatments applied with nutrient supplements were found to have initial value of 34.29 mg L^{-1}, while treatments with no nutrient supplements had value of 33.72 mg L^{-1}. At the end of the

Fig. 4: Nutrient content of effluent from *S. platensis* batch culture system
(A) Ammonia; (B) Nitrate; (C) Phosphate



experiment, treatments with nutrient supplements had the lowest amount (11.28 mg L^{-1}), which was equivalent to an efficient phosphate reduction of 67% as compared to treatments with no nutrient supplements (19.45 mg L^{-1} & 42%, respectively).

S. platensis were cultivated for higher cellular concentration. The results showed that protein content of *S. platensis* cultured in a swine farm wastewater effluent at 10% dilutions with added nutrients contained 56% protein, while those treatments with no added nutrients contained 50% protein. In general, *S. platensis* contained a range of 55-72% protein (Hill, 1980; Nakamura, 1982; Venkataraman, 1983; Colla *et al*., 2005) depending mainly on the quality of nutrients added. In addition, cyanobacteria, *S. platensis* could then be fed as animal diet supplement that may lead to improved animal health (Belay *et al*., 1996).

CONCLUSION

In a batch culture, the most suitable conditions for culture of *S. platensis* in anaerobically treated swine wastewater should be at 10% dilution, initial 20% *S. platensis* stock solution with added nutrients consisting of 8 g L^{-1} $NaHCO_3$ and 1.5 g L^{-1} $NaNO_3$, suitable HRT at day 12 contain 17.8×10^4 cells mL^{-1} and an OD value of 1.09 with 56% protein contain. *S. platensis* algae can be cultured in small animal farms as an important source of protein in the farm and which entails a very low cost.

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