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Kinetics of Removing Nitrogenous and Phosphorus Compounds from Swine Waste by Growth of Microalga, *Spirulina platensis*

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Abstract *Spirulina platensis* was grown in swine waste to reduce inorganic compounds and simultaneously produce feed resources. *Spirulina platensis* prefers nitrogenous compounds in the order: NH_4^+-N>NO_3^--N>simple-N such as urea and simple amino acids. It even consumes NH_4^+-N first when urea or nitrate are present. Therefore, the content of residual NH_4^+-N in *Spirulina platensis* cultures can be determined by the relative extent of the following processes: (i) algal uptake and assimilation; (ii) ammonia stripping; and (iii) decomposition of urea to NH_4^+-N by urease-positive bacteria. The removal rates of total nitrogen and total phosphorus were estimated as an indicator of the treatment efficiency. It was found that *Spirulina platensis* was able to reduce 70–93% of PO_4^{3-}-P, 67–93% of inorganic nitrogen, 80–90% of COD, and 37-56% of organic nitrogen in various concentrations of swine waste over 12 days of batch cultivation. The removal of inorganic compounds from swine waste was mainly used for cell growth, however, the organic nitrogen removal was not related to cell growth. A maximum cell density of 1.52 dry-g/l was maintained with a dilution rate of 0.21/day in continuous cultivation by adding 30% swine waste. The nitrogen and phosphorus removal rates were correlated to the dilution rates. Based on the amino acid profile, the quality of the proteins in the *Spirulina platensis* grown in the waste was the same as that in a clean culture.

Key words: Swine waste, *Spirulina platensis*, total nitrogen and phosphate, chemical oxygen demand, removal rates

The biological treatment of wastewater is an active research field and growing in development in many industrialized or developing countries [7, 8, 25]. The need for efficient processes comes from the heavy polluting effect of urban effluents rich in ammonium and phosphate ions that cause eutrophication [19]. Since physico-chemical techniques are costly and not so efficient and lead to secondary pollution problems, biological treatments are more appealing. Among these, solar biotechnology through microalgal culture appears to be feasible and interesting because of the generation of a useful biomass that can be exploited for various purposes [10, 18, 22]. Microalgae have received much attention in recent years as an alternative biosystem for wastewater treatment, especially in tropical and subtropical regions [6]. Algal systems have traditionally been employed as a tertiary treatment process and have recently been proposed as a potential secondary treatment system [13]. Algal systems are also attractive due to their potential biomass production as a source of fine chemicals and animal feed [4, 20, 21, 23]. *Spirulina platensis* grown on wastewater can be used as a dietary supplement for animals as well as other purposes (energy source, fuel, fertilizer, fine chemicals, etc.) [12, 15].

Swine waste contains many carbonaceous and nitrogenous substances as pollutants. A primary biological treatment has been designed for the maximum biodegradation of organic matter [3]. Ammonia nitrogen is present in swine waste at very high concentrations and can be transformed in a secondary treatment [2, 24, 26]. The removal of nitrogen from swine waste is important because it promotes the eutrophication of the receiving water course [1, 14].

The main purpose of tertiary treatment is to remove the inorganic nutrients from the secondary treatment effluent [17]. There are two kinds of tertiary treatment; physico-chemical treatment and an advanced biological treatment. An algal culture and nitrification-denitrification are both biological treatments [11].

In this study, *Spirulina platensis* was directly grown in swine waste. The cultivation of microalgae is a biological

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process for removing nitrogenous compounds from wastewater [16] where the microalgae efficiently take up the nitrogenous compounds, and phosphorus can also be removed simultaneously. The aim of this work was to investigate the possibility of growing *Spirulina platensis* to treat swine waste by removing its highly concentrated phosphorus and nitrogen, and produce a biomass with good nutritional characteristics suitable for animal consumption.

MATERIALS AND METHODS

Microorganism and Culture Conditions

The strain of *Spirulina platensis* (LB 1926) was obtained from the Collection of Algae at the University of Texas (Austin, U.S.A.). The composition of the basal culture medium was 10 ml of A solution (Na_2CO_3, 0.042; $NaHCO_3$, 0.042; $MgSO_4 \cdot 7H_2O$, 24.4; KCl, 6.0; $NaNO_3$, 10.0; $CaCl_2 \cdot 2H_2O$, 3.0; KH_2PO_4, 5.0; Tris buffer, 10.0 in g/100 ml); Vitamin B_{12}, 1.0 μg; 10 ml of PI solution (H_3BO_3, 3.4 g/l; $CoCl_2 \cdot 6H_2O$, 1.21 mg/l; $MnCl_2 \cdot 4H_2O$, 0.43 mg/l; $ZnCl_2$, 31.5 mg/l; H_2SO_4, 1 mg/l; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 31.2 mg/l); 3 ml of chelate iron solution ($Na_2 \cdot EDTA$, 10.0 g in 500 ml of hot distilled water; $FeCl_3 \cdot 6H_2O$, 0.81 g in 500 ml 0.1 N HCl); NaCl, 36.0 g/l; distilled water, 926 ml. All of the chemicals were purchased from Sigma Co. (St. Louis, U.S.A.). The pH of the medium was adjusted to 8.08 by 10% HCl. The cultures were agitated at 120 rpm in a shaking incubator under continuous illumination with 20 W cool white fluorescent lamps at 12 W/m^2 and 32°C.

Preparation of Medium Containing Swine Waste

The swine waste was obtained from a swine farm located near Chunchon area, immediately transported to the laboratory, centrifuged, and then stored in a refrigerator at 4°C until use. The swine waste was centrifuged at 3,000 ×g for 5 min, and the supernatant was used for the cultivation of *Spirulina platensis*. The supernatant was diluted to 5%, 10%, 20%, 30%, and 60% by adding the above basal medium, of which the dilution percentage was defined as dilution ratio in Table 2. As shown in Table 1, most of the nitrogen in the swine waste was present in organic forms, mainly, as NH_4^+-N, and most of the phosphorus was as orthophosphate.

Table 1. Chemical composition of swine waste.

Component	Concentration (mg/l)
NH_4^+-N	1,200* - 4,100
NO_3^--N	50- 150*
Kjeldahl-N	1,500* - 4,400
Orthophosphate	80*- 270
Total phosphate	220*- 340
COD	15,200- 29,900*

*Value of swine waste used in this work.

Table 2. Growth parameters from batch cultivation of *Spirulina platensis* in various concentrations of swine waste.

Dilution ratio† (%)	Specific growth rate μ (1/day)	Max. cell density X (g-dry wt./l)	Max. biomass productivity P (g-dry wt /l/day)
0*	0.31	1.18	0.37
5	0.30	1.20	0.36
10	0.27	0.91	0.25
20	0.24	0.68	0.16
30	0.22	0.58	0.13
60	0.20	0.53	0.11

*Base culture, no added swine waste.
†The percentage of diluting the wastes with fresh culture medium.

Batch and Continuous Cultivations

The batch cultivation was conducted in a 1-l shake flask at 32°C, 120 rpm, for 12 days by adding various concentrations of swine waste. The initial cell density was 0.24 (g/l) in each flask. At 1 to 2 days intervals, 20 ml of the culture broth were collected from each flask. Continuous cultivation was performed using a 14-l bioreactor (working volume was 12 l), as shown in Fig. 1. The culture homogeneity was ensured by agitating with an impeller at 120 rpm. The feed medium was pumped into the reactor using a peristaltic pump. The samples were centrifuged at 3,000 ×g for 5 min and the supernatant was analyzed for residual inorganic nitrogen and phosphorus concentrations. The collected algae were dried at 100°C-110°C for 6 h, and then the dry weight was measured. To minimize the analytical errors, the pellet was washed with distilled water to remove any salts.

Fig. 1. A photograph of the photobioreactor used for growing *Spirulina platensis*.

Kinetics of Cell Growth and N, P Removal

The specific growth rate of algae in a batch culture can be calculated as follows:

$$\mu=\ln(X_f/X_i)/(t_f-t_i) \quad (1)$$

where X_i=initial biomass concentration (g/l)
X_f=biomass concentration after time interval, t (day)
μ=specific growth rate (1/day)
t_f=final cultivation time
t_i=initial cultivation time

For evaluating the feasibility of swine wastes treatment by *Spilurina platensis*, first-order reaction kinetics were employed as:

$$\ln(C_t/C_0)=-kt \quad (2)$$

where C_t=the waste concentration after t days (mg/l)
C_0=initial waste concentration (mg/l)
k=removal rate constant (1/day)
t=time (day)

The rate of adding a medium into a vessel in continuous cultivation is related to the volume of the vessel by the term dilution, D(1/day), and can be defined as:

$$D=F/V \quad (3)$$

where F is the flow rate (l/day) and V is the working volume (l).

The net change in the cell concentration over a time period can be expressed as:

$$dX/dt=\mu X-DX \quad (4)$$

Under steady-state conditions the cell concentration remains constant, thus when dX/dt=0,

$$\mu=D \quad (5)$$

Analytical Techniques

The total Kjeldahl nitrogen (TKN, equal to NH_4^+-N plus organic N) was quantified by a Tecator Kjeltic Auto 1030 Analyzer (Albas co., New Brunswick, U.S.A.). The ammonium concentration was analyzed by the indophenol-blue method while the nitrate concentration was analyzed by Ion Chromatography (Waters, Chicago, U.S.A.). The total phosphorus was measured by the method in AOAC [9]. The chemical oxygen demand (COD) was estimated by the titrimetric method [9]. The chlorophyll *a* was extracted by sonication in 80% acetone and its concentration was determined from its absorbance at 663 nm and an extinction coefficient of 0.82 [5].

RESULTS AND DISCUSSION

The cell densities during the batch growth of *Spirulina platensis* in swine waste are shown in Fig. 2. Higher concentrations of swine waste slowed the cell growth due to high concentrations of ammonium. Even though organic nitrogen species in the manure are the most favorable nitrogen source for the growth of *Spirulina platensis*, it becomes toxic to the cells when present at a high concentration. There are many other toxic wastes in crude animal wastes and very high concentrations of nitrogen and other nutrients will also inhibit the growth of algae. The best growth of *Spirulina platensis* was obtained with 5% swine waste, giving a final cell density of 1.2 g/l. Table 2 shows the growth parameters derived from the data in Fig. 2. The

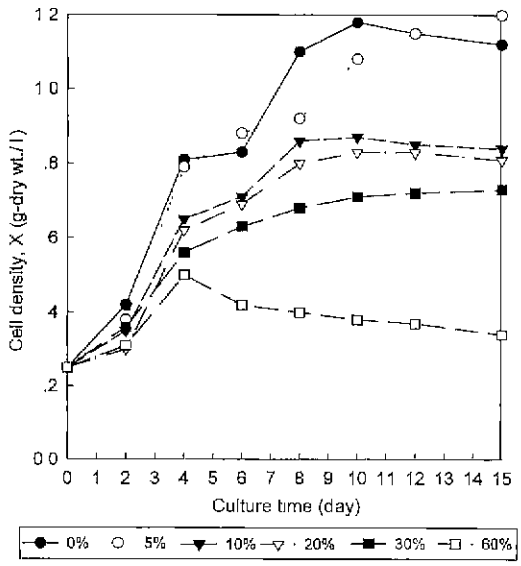

Fig. 2. Comparison of cell growth for several concentrations of swine waste in batch cultivation of *Spirulina platensis*.

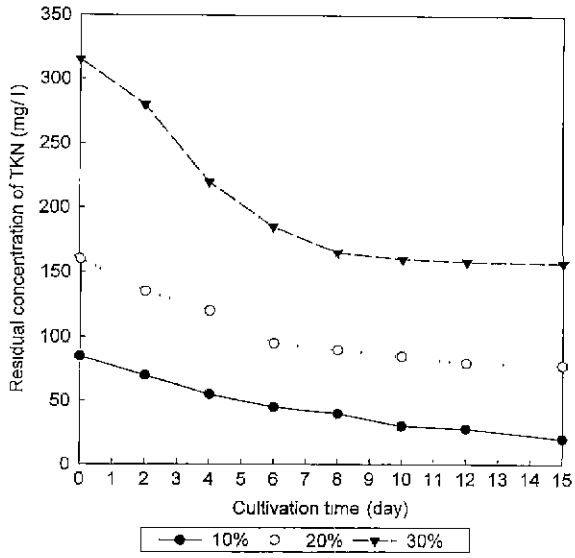

Fig. 3. Reduction of total nitrogen (TKN) during batch growth of *Spirulina platensis* for different initial concentrations of swine waste.

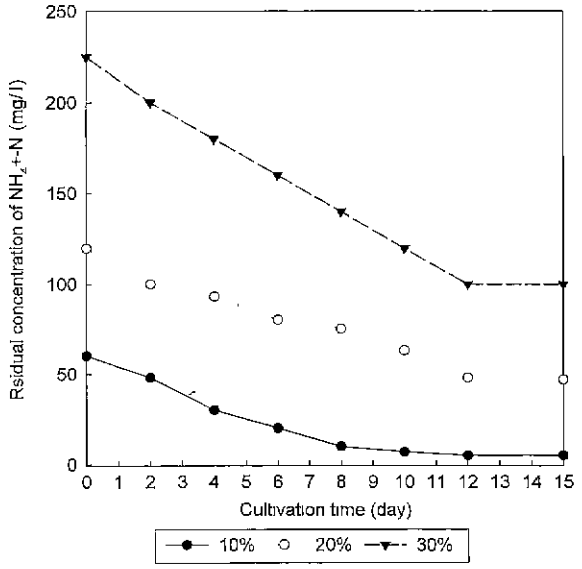

Fig. 4. Reduction of NH_4^+-N during batch growth of *Spirulina platensis* for different initial concentrations of swine waste.

maximum specific growth rates of the base culture and 5% swine waste culture were 0.31 and 0.30 l/day, respectively.

The amounts of residual TKN and NH_4^+-N in the swine waste decreased, depending on the cultivation time, as shown in Figs. 3 and 4. The culture exhibited a pattern of an exponential drop which complemented the changes in the cell density shown in Fig. 2. The effect of NH_3 stripping due to alkalinization of the swine waste may also have contributed to the initial NH_4^+-N loss. As shown in Table 3, over 90% of NH_4^+-N was removed from the 10% swine waste culture by the end of the experiment. The 10% swine waste culture exhibited a better removal performance than the 20% or 30% swine waste cultures. A significant reduction of nitrate in the swine waste was only found after the depletion of ammonium and nitrite. This is because ammonium and nitrite inhibited the nitrate uptake by *Spirulina platensis*.

Table 4 shows the result of the effect of different initial swine waste concentrations in batch cultivations on the total-P and PO_4^{3-}-P removal. The total-P and PO_4^{3-}-P

Table 3. Removal efficiencies of TKN and NH_4^+-N by growth of *Spirulina platensis* in different concentrations of swine waste.

Cultivation time (day)	Culture reduction (%)					
	TKN			NH_4^+-N		
	10%	20%	30%	10%	20%	30%
2	25.3	19.8	16.2	23.3	15.3	18.4
4	43.7	51.5	37.1	51.7	25.4	30.0
6	55.2	48.1	47.0	70.0	41.5	36.3
8	71.3	52.5	54.2	81.7	53.4	44.1
10	77.0	63.1	60.2	88.3	62.7	55.1
12	86.2	68 5	64.1	93.3	72.9	67.3

Table 4. Removal efficiencies of total-P(T-P) and PO_4^{3-}-P by growth of *Spirulina platensis* in various concentrations of swine waste.

Cultivation time (day)	Culture reduction (%)					
	T-P			PO_4^{3-}-P		
	5%	10%	20%	5%	10%	20%
2	33.3	20.1	8.9	33.0	20.0	5.9
4	51.5	36.0	28.9	53.3	33.3	25.7
6	63.6	44.3	37.8	62.7	46.7	48.0
8	66.7	56.0	46.7	73.3	60.1	57.9
10	74.5	68.7	50.0	86.7	73.3	62.4
12	81.8	72.0	51.1	93.3	82.2	67.8

concentrations during the batch cultures are shown in Figs. 5 and 6. The trend of change in the residual PO_4^{3-}-P content in each culture was similar to that of NH_4^+-N. The best removal was achieved with 5% swine waste culture (93% reduction), followed by 10% swine waste culture (82%), with only 67% PO_4^{3-}-P removed in the 30% swine waste culture.

Figure 7 illustrates the pattern of COD removal in the 10%, 20%, and 30% swine waste cultures. The maximal COD removal was estimated as 80% after 12-day cultivation. There were no significant differences in the COD removal between the cultures. Related to an increase in the cell dry weight, the total COD reached 1,600 mg/l in the culture medium after 12-day cultivation in the 30% swine waste medium. However, an initial drop in the total COD was found during the first 4 days, which suggested an uptake of

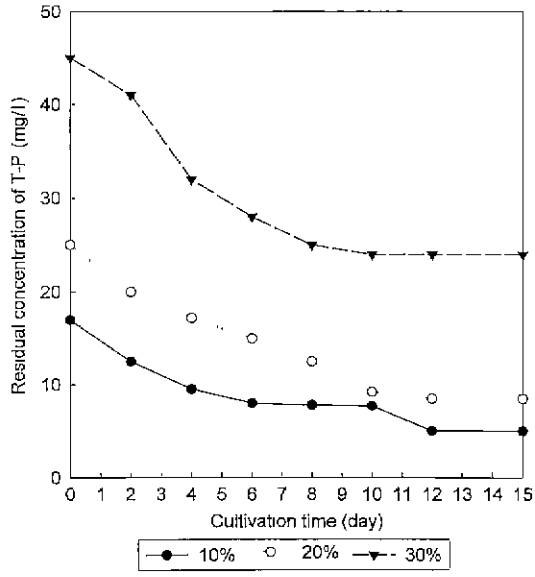

Fig. 5. Reduction of total phosphorus during batch growth of *Spirulina platensis* for different initial concentrations of swine waste.

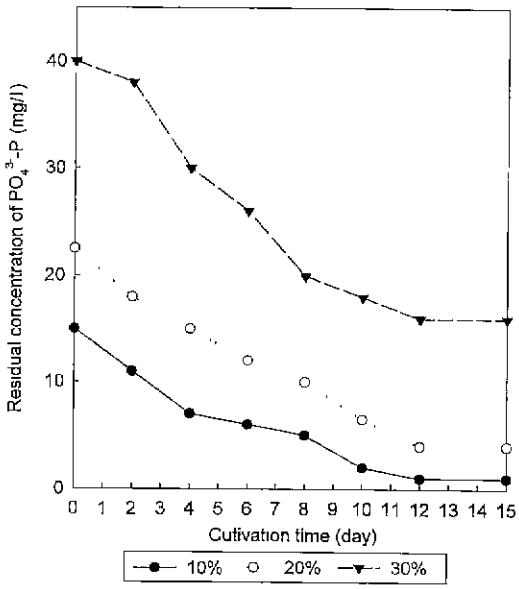

Fig. 6. Reduction of total PO_4^{3-}-P during batch growth of *Spirulina platensis* for different initial concentrations of swine waste.

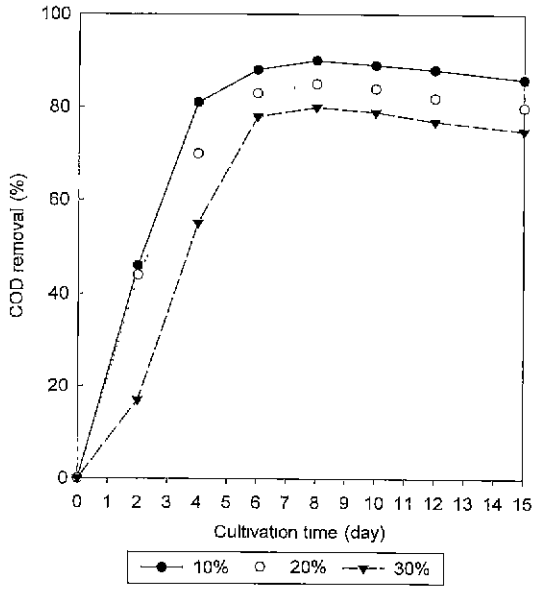

Fig. 7. Chemical oxygen demand (COD) profile in swine waste by cultivating *Spirulina platensis*.

dissolved organics by *Spirulina platensis* in addition to photosynthesis.

For this case, measurement of dry weight alone does not exactly reflect the growth of *Spirulina platensis*, because the medium was mixed with the wastes, bacteria, and other dry matters in the wastes might be added to the biomass. Since only algae contain chlorophyll, the measurement of this pigment concentration can be a reliable criterion to reflect the algal biomass concentration. Figure 8 shows that change in the chlorophyll content in the continuous

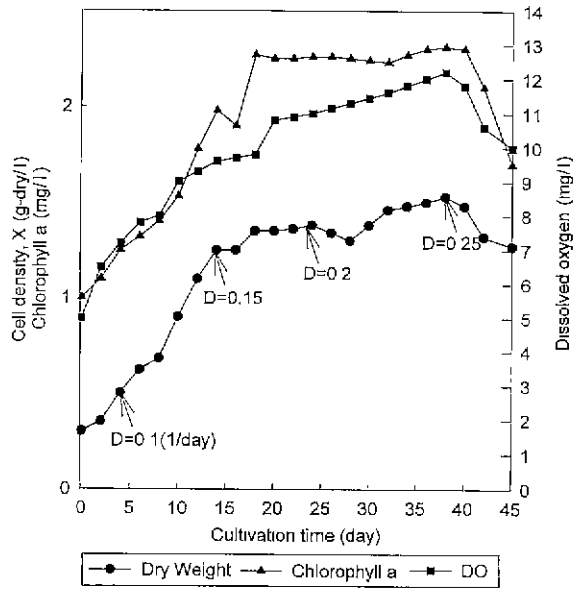

Fig. 8. Changes of cell density, chlorophyll *a* and dissolved oxygen when adding 30% swine waste for continuous cultivation of *Spirulina platensis*. Arrows indicate points of changing dilution rates.

Table 5. Growth parameters from continuous cultivation of *Spirulina platensis* in different flow rates of swine waste.

Dilution rate (1/day)	Specific growth rate (1/day)	Max. cell density (g/l)	Max. biomass productivity P (g/l/day)
0.10	0.20	1.12	0.22
0.15	0.17	1.29	0.22
0.20	0.23	1.52	0.35

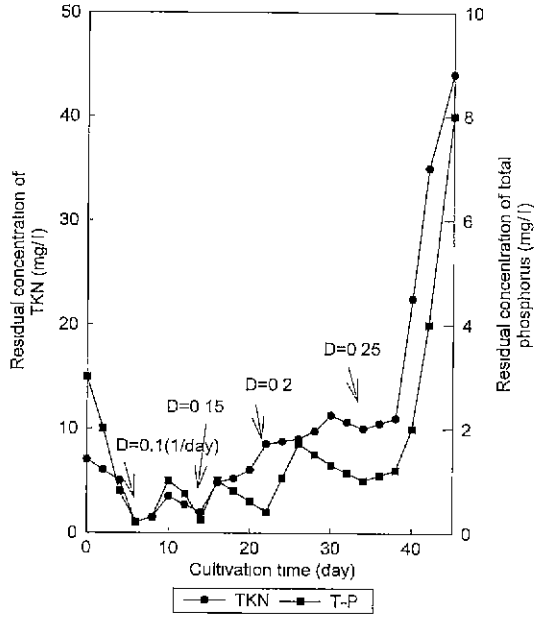

Fig. 9. Kinetics of removing TKN and T-P when adding 30% swine waste for continuous cultivations of *Spirulina platensis*. Arrows indicate points of dilution rates.

cultivation appears to be comparable to the increase of the cell density. The growth rate equaled the dilution rate when a steady state was reached. A chemostat culture is when the biomass concentrations and growth rates are maintained at a steady state and the chemical/physical environment of the cells also remains in a steady state. During continuous cultivation, as the cell growth increased, the dissolved oxygen also increased.

Table 5 is the result of various growth parameters obtained from continuous cultivations of *Spirulina platensis* in various dilution rates. The maximum specific growth rate was 0.23 (1/day) at a dilution rate of 0.20 (1/day). Figure 9 shows the nitrogen and total phosphorus removal in continuous cultivation. Both the total nitrogen and phosphorus removal rates were correlated with their respective loading rates. The increase of the TKN and T-P concentrations at the end of the cultivation indicated cell death due to accumulation of the toxic materials. In order to compare the removal efficiency with the growth of *Spirulina platensis*, Table 6 shows the results of the rate constants for removing total nitrogen, ammonium, total phosphate and orthophosphate. This data was used to compare the removal rate of inorganic and organic chemicals by the first-order reaction expressed as a linear relationship. At each dilution rate, the ammonium and orthophosphate removal rates were faster than those of total nitrogen and total phosphorus.

The quality of the protein, as determined by its amino acid composition shown in Table 7, was compared to that of commercial *Spirulina platensis*. As reported previously, the protein of *Spirulina platensis* showed much better nutritional value than those of the WHO pattern and other plant foods [10].

Table 6. Results of estimating rate constants* for removing TKN, NH_4^+-N, T-P, and PO_4^{3-}-P by cultivating *Spirulina platensis*.

Dilution ratio (%)	TKN (1/day)	NH_4^+-N (1/day)	T-P (1/day)	PO_4^{3-}-P (1/day)
10	0.17	0.23	0.14	0.22
20	0.10	0.12	0.11	0.14
30	0.09	0.11	0.06	0.09

*First-order removal rate constant (1/day).

Table 7. Comparison of amino acid profiles* in *Spirulina platensis* grown from swine waste and commercial health food.

Amino acid	*Spirulina* sp. grown from swine waste	Commercially available *Spirulina* sp.**
Aspartic acid	10.32	5.37
Threonine	5.04	4.19
Serine	5.58	5.42
Glutamic acid	13.53	16.03
Proline	5.34	3.69
Glycine	5.42	4.89
Alanine	7.45	12.64
Cystine	0.59	1.41
Valine	4.75	6.46
Methionine	2.25	0.89
Isoleucine	4.06	5.94
Leucine	7.58	9.21
Tyrosine	3.83	4.29
Phenylalanine	4.88	5.02
Histidine	2.26	2.01
Lysine	5.97	5.39
Arginine	4.25	1.03
Ammonia	6.90	6.14

*g/100 g protein.**Data from Earthrise company (U.S.A.) which produces the health food *Spirulina platensis* [4].

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