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Growth of *Spirulina platensis* in Effluents from Wastewater Treatment Plant of Pig Farm

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Spirulina platensis **NIES 39 was grown in effluents from a wastewater treatment plant utilizing activated sludge process at a pig farm to reduce pollutants and to produce feed resources. The addition of 2 g/*l* NaCl was required for the growth of *S. platensis* in the effluents with about 100 mg/*l* ammonia nitrogen concentration. The growth was stimulated by the addition of 2 g/*l* sodium bicarbonate and 4 mg/*l* ferrous sulfate. The microorganism grew well at pH 8~11, and the optimum pH for the growth was 10. The algal concentration of 1.1 g/*l* was attained after 8 days of growth at the optimum condition with the removal of 95% ammonia nitrogen and 34% phosphorus.**

Spirulina platensis is a blue-green alga which belongs to the family of Oscillatoriaceae. It is a large-size microscopic, multicellular alga which grows in shallow ponds of high salinity and alkalinity in tropical countries.

Spirulina platensis has been used as food since ancient times in some regions of Africa and Mexico. Nutritional studies, carried out by the Institute Français du Petrole (IFP), showed that *Spirulina* is one of the richest protein sources ever found (6) and the protein also contains all essential amino acids in a proportion comparable to other conventional protein foods and satisfies the composition recommended by FAO with the exception of methionine (4). *Spirulina* as a food source also supplies a considerable amount of fat, carbohydrate, vitamins, and calories. Tests carried out on rats and chickens showed that *Spirulina* has a high nutritive value and a good digestibility (3).

Considering the nutritional values of *Spirulina* and the relative simplicity in cultivation and harvesting of this microorganism because of its large size compared to other microalgae, several pilot plant studies have been performed in France, Algeria, and Mexico to produce *Spirulina*. The microorganism were cultivated in synthetic media made up mainly of aqueous mineral salt solutions and a carbon source from combustion gases containing carbon dioxide.

From another aspect, one of the major problems in the pollution of inland waters today is the excessive enrichment of waters by nutrients, especially nitrogen and phosphorus compounds, which support the growth of undesirable algae blooms. These blooms, upon dying, cause taste and odor problems in lakes or rivers. Most of the nutrients come from industrial or domestic sewage since the conventional treatment processes are relatively ineffective in removing these nutrients. Normally, only 20 to 40% of nutrients are removed from sewages unless special procedures are adopted for their removal. One of the possible methods for removing large amounts of nutrients from waste waters is through biological removal using algal culture.

In this study *Spirulina platensis* was grown in effluents from a wastewater treatment plant utilizing activated sludge process at a pig farm and factors influencing the growth were investigated. The two objectives of this study were: a) to treat the effluents for nutrient removal (i.e., nitrogen and phosphorus) and b) to produce a biomass with good nutritional characteristics suitable for animal consumption.

MATERIALS AND METHODS

Materials

Spirulina platensis NIES 39 was supplied from the National Institute for Environmental Studies, Japan.

The effluent sample was obtained from a wastewater treatment plant utilizing activated sludge process of a pig farm in Gyonggi Province.

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Preparation of Algae Inoculant

The *Spirulina* was cultivated in a synthetic medium (1) for 5~7 days. It was filtered with a 300 mesh nylon net, suspended in a small amount of distilled water, and then used as a concentrated inoculant. The size of the inoculant was 0.05 as optical density at 560 nm.

Cultivation of *Spirulina*

Cultivation was done with 1 *l* round-bottom flask which was filled with 800 *ml* cultivation medium. After inoculation, the flask was placed in a 30℃ water bath and illuminated with four 60 W fluorescent lamps. The microorganism was grown for 8 days with aeration rate of 1 vvm.

Analytical Methods

The growth of *Spirulina* was detected by measuring optical density at 560 nm on a Varian 634 spectrophotometer. Cultivation medium was used as blank after removal of *Spirulina* by filtration through a Toyo 5A filter paper. Algae suspension which had an OD_{560} value of 1.0 contained about 790 mg algae per liter (5).

The algal biomass was collected by filtration and it was washed several times with distilled water to remove dissolved salts. The clean samples were then dried to constant weight at 105℃ for the dry weight determination.

Ammonia nitrogen in the swine farm effluent was determined with ammonia electrode (Orion 95-10). Nitrate nitrogen was determined by the chromotropic method (7). The total amount of phosphorus was first converted to orthophosphate by acid hydrolysis. The orthophosphate content was then determined by using the vanadomolybdophosphoric acid colorimetric method (7). Analyses of K, Na, Ca, Mg, Fe and Mn were done with a Perkin-Elmer 2380 atomic absorption spectrophotometer.

Table 1. The element contents of the synthetic medium and pig farm effluent used for *S. platensis* culture

Element	Synthetic medium (mg/l)	Swine farm effluent[a] (mg/l)
N	206	114[b]
P	44.5	27.2
K	337.0	180
Na	2835.0	28
Ca	5.4	54
Mg	9.9	18
Fe	2.0	<0.1
Mn	0.25	0.3

[a] Effluent from wastewater treatment plant utilizing activated sludge process
[b] Ammonia nitrogen

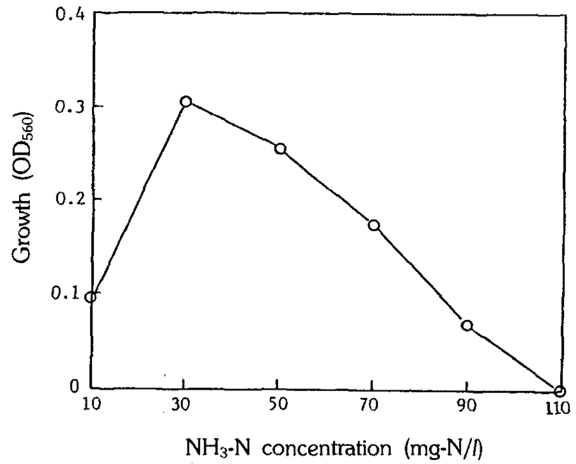

Fig. 1. Effect of ammonia nitrogen concentration in the diluted effluent of pig farm on the growth of *S. platensis*.

RESULTS AND DISCUSSION

Elemental Analysis of Synthetic Medium and Swine Farm Effluent

Since our intention was to substitute the synthetic medium with a farm effluent, we measured the quantity of essential elements in the effluent. Table 1 shows the contents of essential elements in the synthetic medium and the effluent. When compared with the elements in the synthetic medium, the Na and Fe content of the effluent is too low (Table 1) and therefore NaCl and $FeSO_4$ must be added to the effluent to ensure proper growth of *S. platensis*. The contents of ammonia and nitrate nitrogen in the effluent were 114 and 0.8 mg/*l* respectively.

Effect of Effluent Dilution on Growth

The growth of *S. platensis* was reduced at the ammonia nitrogen concentration higher than 50 mg/*l* when the microorganism was cultivated in the effluent of anaerobically fermented swine manure (1). The farm effluent was properly diluted to contain 10~110 mg/*l* ammonia nitrogen before use. As shown in Fig. 1, the growth was best at 30 mg/*l* ammonia nitrogen concentration, whereas the growth was obviously reduced at concentrations higher than 50 mg/*l*. When the ammonia nitrogen concentration exceeded 90 mg/*l*, the microorganism began to die.

Effect of Sodium Chloride at Various Ammonia Nitrogen Concentrations

Addition of sodium chloride is known to lessen the toxic effect of ammonia nitrogen on *S. platesis* cultivated in the effluent of anaerobically fermented swine manure

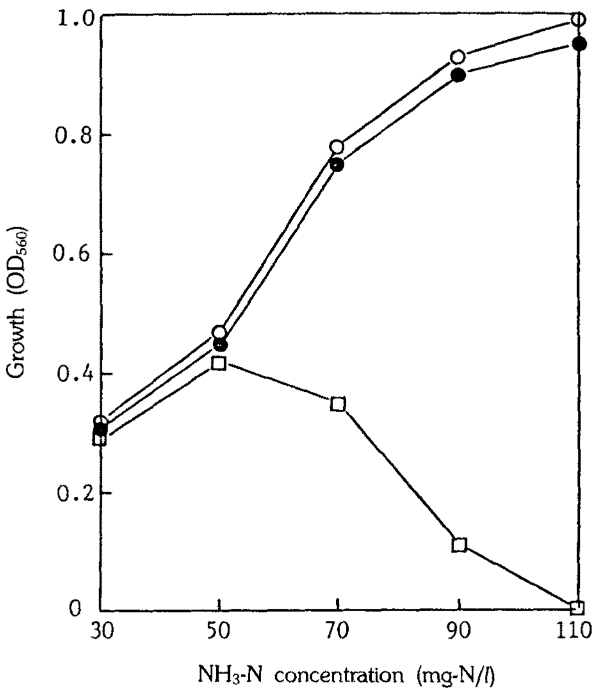

Fig. 2. Effect of NaCl concentration added on the growth of *S. platensis* at various ammonia nitrogen concentration.
NaCl concentration (g/l): (□) 1, (●) 2, (○) 3.

(1). As shown in Fig. 2, the addition of NaCl actually increased the growth of algae. In the effluent with 110 mg/l ammonia nitrogen, the algae grew well at concentrations of NaCl higher than 2 g/l, but were not able to grow at all at 1 g/l of NaCl.

Effect of Added Sodium Bicarbonate

Sodium bicarbonate of various concentrations, 0~5 g/l, was used as the supplemented carbon source for the *Spirulina*. The cultivation medium was an undiluted effluent with a NaCl concentration of 2 g/l. The result is shown in Fig. 3. The growth was maximum at 5 g/l of sodium bicarbonate, but it was not very different from that at 2 g/l. Therefore, 2 g/l of sodium bicarbonate was added in the latter experiments. The growth of the microorganism was maximum at 3 g/l of sodium bicarbonate added when the microorganism was cultivated in the effluent of anaerobically fermented swine manure (1).

Effect of Added Ferrous Sulfate

Since Fe content of effluent is very small, when compared with that of the synthetic medium, supplemental ferric salt was therefore necessary. The cultivation medium was an undiluted effluent with 2 g/l NaCl and 2 g/l $NaHCO_3$, and 0~10 mg/l of ferrous sulfate was added. The result is shown in Fig 4. The growth rate

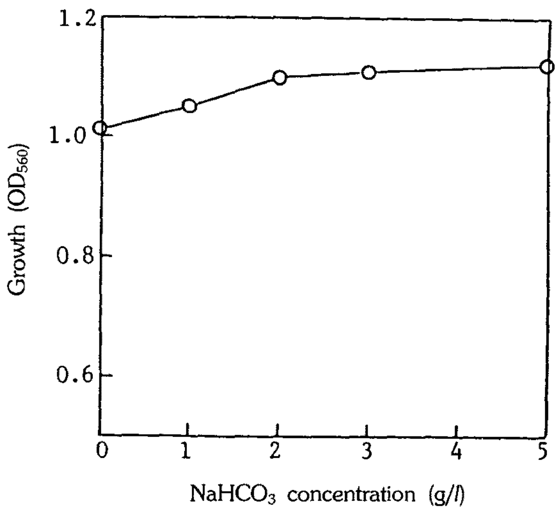

Fig. 3. Effect of $NaHCO_3$ concentration added in the treated effluent of pig farm on the growth of *S. platensis*.

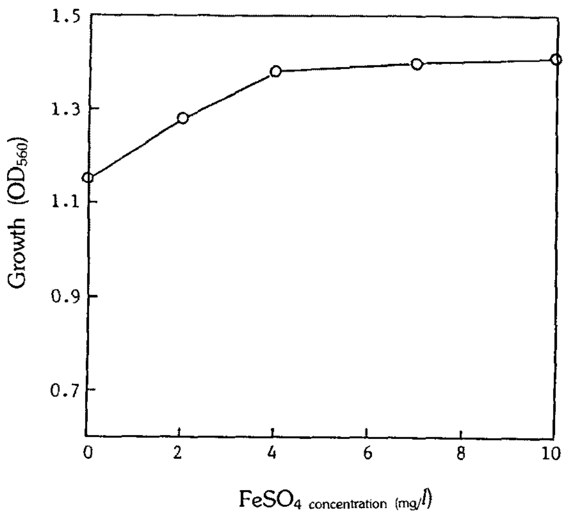

Fig. 4. Effect of $FeSO_4$ concentration added in the treated effluent of farm wastewater on the growth of *S. platensis*.

was high at ferrous sulfate concentrations higher than 4 mg/l. Therefore, 4 mg/l of ferrous sulfate was added in the latter experiments. Supplemental potassium salt was necessary when the microorganism was cultivated in the effluent of anaerobically fermented swine manure (1).

Effect of pH

The pH of the cultivation medium not only affects the growth of the *Spirulina*, but also determines whether there will be any growth of other microbes. For example, *Chlorella* has an optimum pH value of 6~7, which leads to easy contamination by many kinds of bacteria and

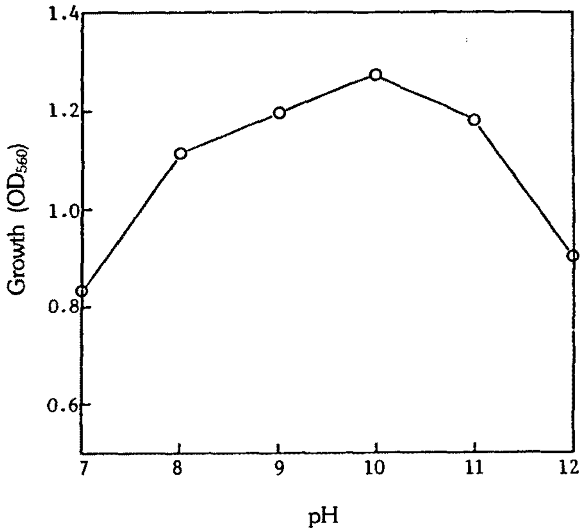

Fig. 5. Effect of pH of the treated effluent of farm wastewater on the growth of *S. platensis*.

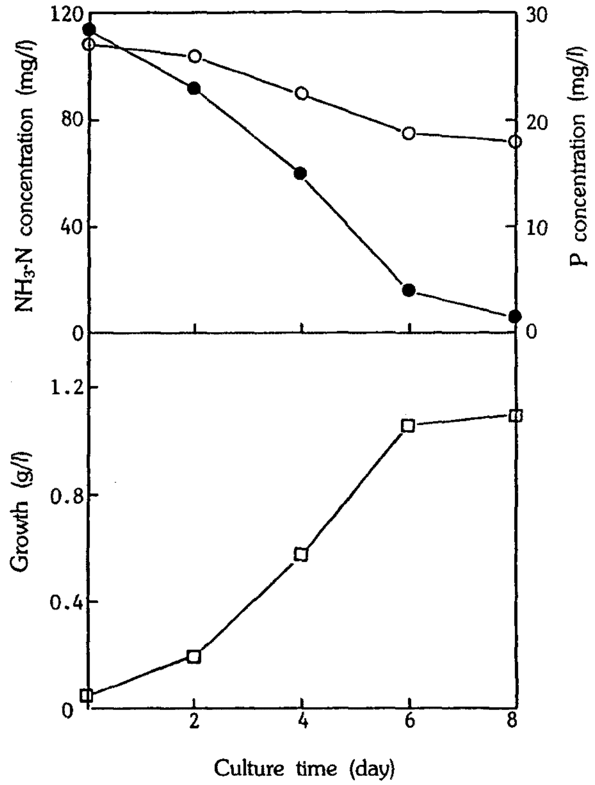

Fig. 6. Removal of ammonia nitrogen and phosphorus during the growth of *S. platensis* in the treated effluent of farm wastewater.
□, algal growth; ●, NH_3-N concentration; ○, phosphorus concentration.

microbes (2). In order to understand the effect of pH on the growth of the *Spirulina*, we used a series of cultivation media with different pH values. The cultivation medium was an effluent with 2 g/*l* NaCl, 2 g/*l* $NaHCO_3$ and 4 mg/*l* $FeSO_4$. The pH of each was adjusted every eight hours. Fig. 5 shows that the optimum pH for the growth is 10. However, the algae still grows well at the pH range of 8~11. The optimum pH for the growth of *S. platensis* was 9.0 when the organism was cultivated in the filtered effluent of anaerobically fermented hog manure (8).

The pH of the effluent medium was 8.6, and the pH increased to 8.2 during the cultivation without adjustment of pH. Serious contamination by other microbes was not seen in the microscopic examination during the growth.

Removal of Ammonia Nitrogen and Phosphorus by the Algal Growth

The effluent contained 114 mg/*l* ammonia nitrogen and 27.2 mg/*l* phosphorus (Table 1). Fig. 6 shows the removal of ammonia nitrogen and phophorus in the effluent with 2 g/*l* NaCl, 2 g/*l* $NaHCO_3$ and 4 mg/*l* $FeSO_4$ by the growth. The cell concentration of 1.1 g/*l* was attained after 8 days of growth. The removal of ammonia nitrogen was 95% after 8 days of growth, and the phosphorus content was 18 mg/*l* at this time (34% removal). The concentration of nitrate nitrogen was 0. 8~0.9 mg/*l* throughout the cultivation.

Acknowledgement

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