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Utilization of Different Nitrogen Sources by Cultures of *Scenedesmus acuminatus*

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Abstract

Nitrogen was provided by different organic sources to promote *S. acuminatus* growth in the laboratory conditions and comparison was made with the culture unit where nitrogen was provided by inorganic source. Protease peptone and several aquatic plant species such as parrot feather (*Myriophyllum*), water lily (*Nymphaea*) and common cattail (*Typha)* were used as organic nitrogen sources. The average nitrogen concentration in the muds (0.36%) was lower than the nitrogen contents of the aquatic plants (1.21%, 1.53%, and 1.96% for *Typha*, *Myriophyllum*, and *Nymphaea*, respectively). Consequently, the mud produced the least growth of *S. acuminatus* with the average algal growth of $109 \pm 1.52 \times 10^4$ individuals per ml. The mineralization rates of nitrogen from organic sources were adequate to support good *S. acuminatus* growth in cultures. The suitability of decaying plants as sources of nitrogen was generally greater in plants with higher nitrogen content. *Nymphaea* produced the best algal growth, while *Typha* and *Myriophyllum* supported little algal growth. Muds were poor nitrogen source for *S. acuminatus* and did not support the good algal growth.

Key words: Nitrogen removal, Nitrogen sources, *Scenedesmus acuminatus*, Bacterial Decomposition, Wastewater

Introduction

Nitrogen is a major nutrient affecting the productivity of aquatic ecosystems because it is an essential component of protein and other constituents of cellular protoplasm. Considerable quantities of dead phytoplankton cells, aquatic plants, fish fecal solids and other organic material settle to bottom of the aquatic resources. It is well known that inorganic nitrogen released during microbial decomposition of organic matter is important for the growth of plants in both aquatic and terrestrial ecosystems (Boyd, 1974). The rate of ammonia production from mineralization depends on temperature, pH, oxygen availability, and the quantity and quality of organic matter. Ammonia production obviously increases as the amount of organic material undergoing decomposition increases and more ammonia is released from high-quality, nitrogen-rich material than from nitrogen-poor material. Ammonia produced when organic matter is mineralized is then available for further use in biological processes, such as reassimilation by phytoplankton. The continuous internal recycling of nitrogen through the processes of assimilation by plankton, cell death, mineralization of organic nitrogen, and reassimilation by plankton is an important aspect of nitrogen dynamics in aquatic resources.

It is generally accepted that microalgae are highly effective in reducing BOD and the dissolved nitrogen and phosphorus loads of urban and agricultural effluents, which are the main causes of eutrophication of the receiving water bodies (Oswald, 1988; Smith, 1990; Talbot and De la Noue, 1993). The use of dense algal cultures may be cost-effective because, apart from the relatively simple technology involved, the dissolved nutrients are recycled into a valuable commodity, represented by the high protein content of the microalgae biomass (Becker, 1981; Lincoln and Earle, 1990).

In the 1980s, American scientists studied, at a laboratory scale, the possibility of recycling nutrients from secondarily treated domestic wastewater through artificial food chains. They found that species like *Scenedesmus* and *Daphnia magna* were suitable organisms, both for their high growth potentials and also for their resistance to handling in the low technology culture systems used (Kawasaki *et al.*, 1982). In a Norwegian experiment (Kallqvist *et al.*, 1996), the green algae *Scenedesmus acuminatus* grew well in water from a wastewater contaminated creek and dominated the primary ponds.

The present study was initiated to evaluate NH_4NO_3 as inorganic source and mud, aquatic plant residues, and organic compounds as organic sources of nitrogen for *S. acuminatus* culture, using semi-continuous cultures kept under a simulated light and temperature cycle.

Materials and Methods

Mud and Plant Samples

Mud samples were obtained with an Ekman dredge from Lake Golbasi, Hatay, Turkey. Muds were dried in a furnace at 60ºC, pulverized with a mortar

and pestle to pass a sieve with 0.85 mm openings. Samples of phytoplankton, *S. acuminatus,* were concentrated by centrifugation of water from culture units in the laboratory. Macrophytes, including *Myriophyllum* (parrot feather), *Nymphaea* (water lily) and *Typha* (common cattail), washed free of mud and debris and dried in a furnace at 60 ºC, then dehydrated plant materials were pulverized to pass 0.85 mm screen. The nitrogen content of plant and soil samples was determined by the Kjeldahl technique (AOAC, 1960).

Algal Cultures

A pure culture of *Scenedesmus acuminatus* was provided by Mustafa Kemal University, Plankton Research Laboratory. This culture species was maintained under aseptic conditions in a nutrient solution of following composition; KH_2PO_4 – 50 mg/L, $MgSO_47H_2O$ – 50 mg/L, H_3BO_3 – 2.5 mg/L, $CaCl_22H_2O$ 25 mg/L, $CaCl_2$ 0.05 mg/L, NH_4NO_3 125 mg/L, $NaHCO_3$ 16 mg/L, $MnCl_24H_2O$ 1.4 mg/L. Because of large inorganic nitrogen in maintenance solution, 2 ml aliquots of *S.acuminatus* were transferred to fresh nutrient solution which contains no NH_4NO_3 for two weeks. Thus, cell numbers were also reduced from 600,000 cell/ml to 12,000 cell/ml by this transfer. Experimental cultures contained 50 ml experimental nutrient solution in 125 ml erlenmeyer flasks. All experimental flasks were plugged with cotton and set on a laboratory bench under a light intensity of 4,300 lux (18 h in light and 6 h in dark) at 24ºC and experiments were carried out for 6 days. *S.acuminatus* concentrations at the end of the experiment were enumerated with a Thoma Lam.

Experiments

Three treatments were used to triplicate each. Organic nitrogen sources were compared with inorganic nitrogen sources by applying different ratios each to culture flasks. Organic nitrogen sources provided for the experiments were protease peptone and three different species of aquatic plants, *Myriophyllum*, *Typha* and *Nymphaea*. Dry plant materials were added into 1 liter nutrient solutions, which do not include any nitrogen sources, to give the ratios of 50 and 100 mg organic matter per liter (Figure 1). At this stage, to evaluate muds as nitrogen sources, 0.5 g oven-dried muds were provided in each flask before 50 ml of nutrient solution, included dried plant materials at two different ratios, and were put in the culture flasks. *S. acuminatus* were inoculated into the cultures which contain muds and dried aquatic plants as nitrogen sources and seeded with 1 drop of the suspension of bacteria and fungi. Cultures included only dried mud but not any dehydrated plants were carried out as control group. Protease peptone, the other organic nitrogen source applied to cultures in the experiments, was applied under both sterile and non-sterile conditions to provide the ratios of 0, 5, and 10 mg nitrogen per liter (Figure 2). One drop of bacteria and fungi, provided by heat-killed *S. acuminatus* which had been allowed to decay in the dark, was introduced only into non sterile solutions.

The response of *S. acuminatus* to increasing inorganic nitrogen source was determined by varying the amount of NH_4NO_3 in the nutrient solution. Concentrations of NH_4NO_3 employed into solutions were 0, 2.5, 5, 7.5, and 10 mg per liter (Figure 3).

Cells in each culture units were enumerated at

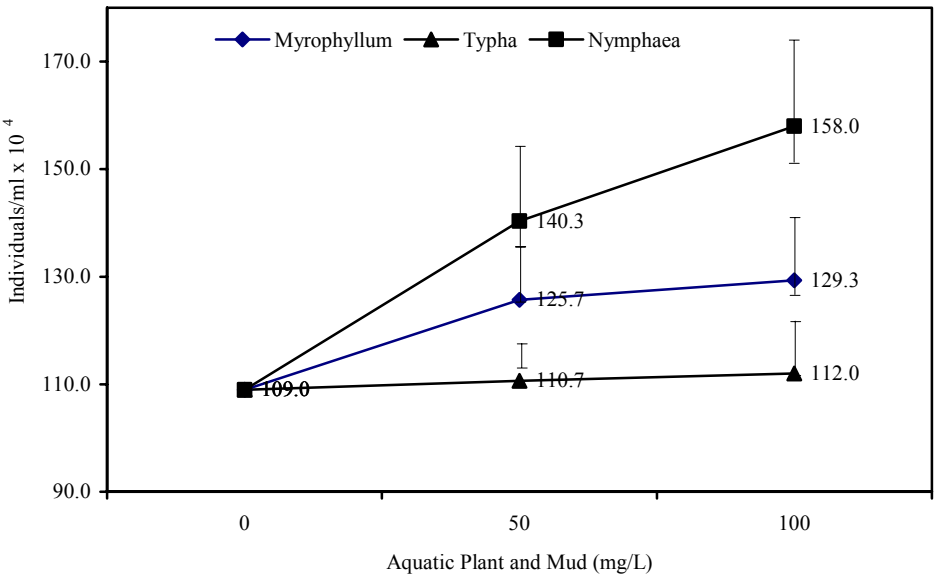

Figure 1. Growth of *S. acuminatus* in cultures where various concentrations of decaying aquatic plants and muds served as the sole source of nitrogen. Each point represents the mean (± SE) individuals in three replicates of 6-day-old cultures.

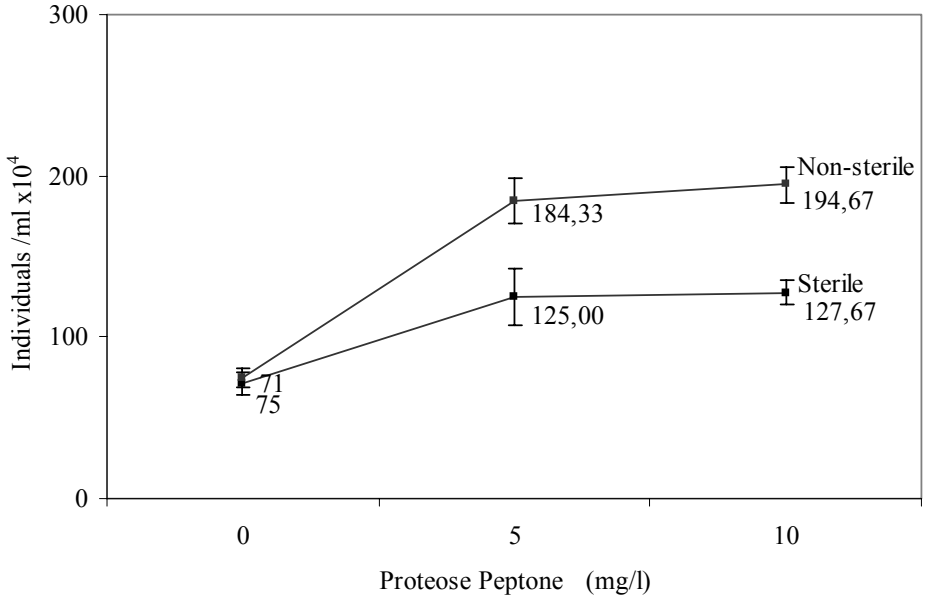

Figure 2. The growth of *S. acuminatus* in sterile and nonsterile media when protease peptone was the only source of nitrogen. Each point represents the mean (± SE) individuals in three replicates of 6-day-old cultures.

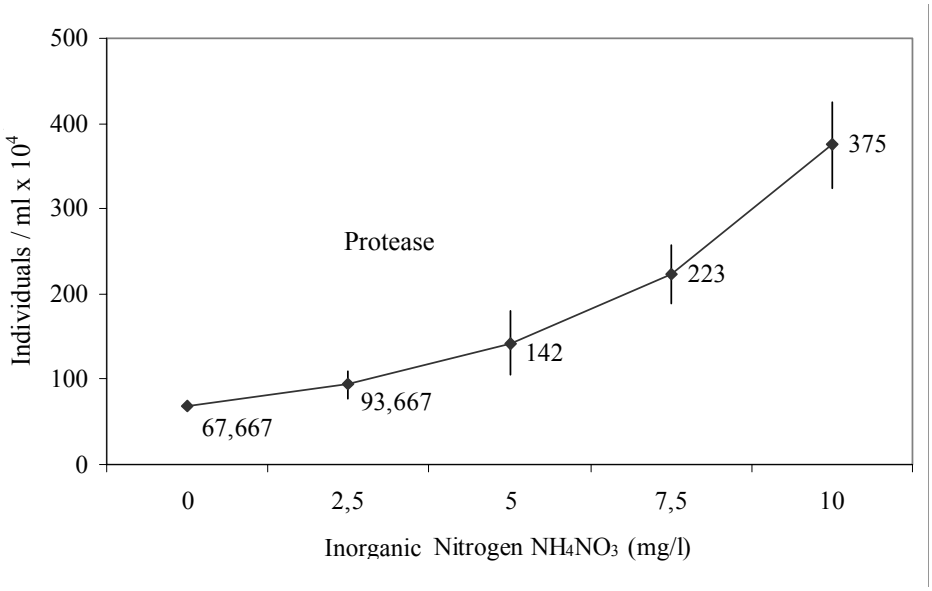

Figure 3. The growth of *S. acuminatus* at five different concentrations of inorganic nitrogen (NH_4NO_3). Each point represents the mean (± SE) individuals in three replicates of 6 day-old cultures.

the end of 6 day experiment periods. Differences between treatments were statistically analyzed by one way ANOVA. Sigma-Stat statistical software was employed to run statistical analyses (SPSS, 1997).

Results

The growth of *S. acuminatus* was the highest when *Nymphaea* provided as nitrogen source into culture at the concentration of 100 mg dried material per liter. Intermediate growth of *S. acuminatus* was provided when decaying *Myriophyllum* was the source of nitrogen. *Typha* produced the lowest algal individuals. The higher percentages of nitrogen in decaying plants yielded the higher nitrogen supply into culture media. The average nitrogen contents of dried plants were found as 1.21%, 1.53%, and 1.96% for *Typha*, *Myriophyllum*, and *Nymphaea*, respectively. The growth of *S.acuminatus* increased with increasing quantities of nitrogen added in dried plants increased. Dried plants, added into cultures in quantities of 50 mg/L and 100 mg/L produced yields

of *S. acuminatus* of 126 ± 11.05 x 10^4, and 129 ± 15.17 x 10^4 with *Myriophyllum*, 110 ± 3.28 x 10^4, and 112 ± 5.3 x 10^4 with *Typha*, and 140 ± 1.76 x 10^4, and 158 ± 7 x 10^4 with *Nymphaea*, respectively (Figure 1).

Utilization of protease peptone as nitrogen source in sterile condition was not as high as that of non-sterile condition where decomposer organisms were added to culture. Decomposer organisms in non-sterile conditions mineralized the inorganic nitrogen from protease peptone which was readily utilized by *S. acuminatus.* The growth of *S. acuminatus* significantly increased in both 5 mg/L and 10 mg/L concentrations by adding decomposer organisms. The average individuals of *S. acuminatus* per ml was 71 ± 6.6 x 10^4 (mean ± SE), 125 ± 17.1 x 10^4, and 128 ± 7.8 x 10^4 for the protease peptone concentrations of 0 mg/L 5 mg/L and 10 mg/L respectively in sterile condition. The protease peptone concentrations of 0 mg/L 5 mg/L and 10 mg/L in non-sterile condition yielded 75 ± 5.8 x 10^4 (mean ± SE), 184 ± 13.7 x 10^4, and 195 ± 1.3 x 10^4 individuals of *S.acuminatus* per ml, respectively (Figure 2).

Maximum growth of *S. acuminatus* was provided by inorganic nitrogen treatment of 10 mg/L under the conditions of the present study (Figure 3). Protease peptone in non-sterile media produced greater number of individuals (184 x 10^4 ind/ml) than that of inorganic nitrogen as NH_4NO_3 (142 x 10^4 ind/ml) at 5 mg/l concentration while it was vice versa at 10 mg/l concentration.

The average nitrogen concentration in the muds (0.36%) was low when compared to the nitrogen contents of the aquatic plants. Consequently, lower growth of *S. acuminatus* with the average algal growth of 109 ± 1.52 x 10^4 individuals per ml was obtained in cultures where the only nitrogen was supplied in muds.

Discussion

It is well-known that under nitrogen limiting conditions, algae are better capable of utilizing alternative nitrogen sources (Flynn and Butler, 1986). Also, algae are known to increase excretion when stressed. To evaluate the effects of algae-bacterium interaction under nutrient-limited conditions, nitrogen-limited continuous cultures should be used to investigate the behavior of the mixed culture with amino acids as the sole nitrogen source (Ietswaart *et al.*, 1994). The nitrogen content of algae varies by species and physiological condition. Generally, nitrogen content increases with increased nutrient availability and growth rate. Nitrogen content can range from 4 to 9% (of dry weight) (Parsons *et al.*, 1961). Berg's (Berg *et al.*, 2003) results indicated that uptake of oxidized and reduced forms of nitrogen can be separated in time and space due to association with distinct phytoplankton groups.

The present study, focused on the utilization from different type of nitrogen sources by phytoplankton, confirmed that decomposers are important keystone in breaking down the plant materials. Protease peptone in non-sterile media produced greater number of individuals (184 ind/ml x 10^4 at 5mg/l, 195 ind/ml x 10^4 at 10 mg/l) than that of sterile media (125 ind/ml x 10^4 at 5 mg/l, 128 ind/ml x 10^4 at 10 mg/l). These results showed that nitrogen in organic matter released to water by decomposers. Bacteria and fungi are important decomposers of plant litter in aquatic systems (Webster and Benfield, 1986; Hieber and Gessner, 2002). Results of the present study reveal that the nitrogen of dead aquatic plants is readily available for use by algae. Nitrogen content of the aquatic plants effects its nitrogen release into culture. The nitrogen content of *Nymphaea* was higher than that of other macrophytes (*Myriophyllum* and *Typha*). Thus, better growth of *S. acuminatus* was already expected from the culture unit with *Nymphaea* compared to units with other macrophytes. Ultimately, much of the organic nitrogen either in culture or natural systems will be converted to inorganic nitrogen. However, inorganic nitrogen addition to readily organic nitrogen source of the culture or natural systems is likely an ecological advantage. Inorganic nitrogen (NH_4NO_3) was much superior to organic compounds as a source of nitrogen since all of the organic nitrogen was not mineralized and made available for algal growth at once.

After 6 days of incubation, the very low available dissolved inorganic nitrogen concentrations probably limited the growth of phytoplankton in the mud treatment. Results of the present study support the conclusion by some authors (Boyd, 1974; Fitzgerald, 1970) that muds do not supply sufficient nitrogen to produce good growth of algae in cultures under laboratory conditions. Organic nitrogen in muds is probably mineralized much slower than the nitrogen in the other types of organic matter tested in the present study. As Boyd (Boyd, 1974) mentioned, much of the labile organic matter is likely mineralized or released to the water in soluble organic matter before residues of aquatic plants are assimilated by sediment. The release of nitrogen from sediments certainly contributes to the pool of inorganic nitrogen available to algae and aquatic plants. The rate of mineralization, however, is slow and not enough to support high levels of algal productivity. Consequently, other sources of nitrogen are required if higher levels of productivity are attained.

The present study confirms that microalgae may be considered efficient nutrient removers. Their efficiency is caused in part by their active nutrient uptake and use for the synthesis of new biomass.

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