References and Notes

1. This ephemeris, not yet described in the literature, is a numerical integration fitted to both optical and laser data. It was developed by J. D. Mulholland and P. L. Bender (Joint Institute for Laboratory Astrophysics) as a part of the Apollo Lunar-Laser Ranging Experiment.
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4. We thank the following persons for their aid and cooperation: L. DiPalma, J. T. Williams, and J. Wohn (Smithsonian Astrophysical Observatory); W. Liller and M. Mattei (Harvard College Observatory); D. H. Eckhardt (Air Force Cambridge Research Laboratory); J. G. Williams (Jet Propulsion Laboratory); E. C. Silverberg (McDonald Observatory); and C. O. Alley (University of Maryland). This work was supported in part by contract NASW-2014 and grant NGR 44-012-219 from the National Aeronautics and Space Administration.

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Inorganic Nitrogen Removal from Wastewater: Effect on Phytoplankton Growth in Coastal Marine Waters

Abstract. *Algal bioassays were used to demonstrate the high efficiency of a combined tertiary wastewater treatment and marine aquaculture system in removing inorganic nitrogen, and to show that the coastal waters off Woods Hole, Massachusetts, are limited in nitrogen for marine phytoplankton growth. When nutrients were removed from secondarily treated domestic wastewater through assimilation by phytoplankton in an outdoor growth pond, the pond effluent, in varying dilutions with seawater, could not support more phytoplankton growth than the seawater alone. However, when nitrogen was added back to the mixtures of pond effluent and seawater, the phytoplankton growth response was similar to that with a mixture of wastewater and seawater. This is similar to the findings of other researchers, and suggests that nitrogen may be the key growth-limiting nutrient in many coastal marine waters. The combined tertiary treatment–marine aquaculture system appears to be an effective means of removing nitrogen from secondarily treated wastewater and controlling eutrophication of coastal marine waters.*

There is increasing evidence that nitrogen is the phytoplankton growth-limiting nutrient in many coastal and estuarine waters (*1, 2*). This is in contrast to the traditional view that phosphorus is often the growth-limiting nutrient in aquatic environments (*3*). As a result, considerable emphasis is curently being placed on finding ways to remove nitrogen from wastewaters in order to control and prevent further eutrophication of aquatic systems (*4*).

A novel approach to this problem, described by Ryther *et al.* (*5*), and one we are currently studying, involves combining a nutrient removal system with a marine aquaculture. In this combined system, nutrients (mainly nitrogen) are first removed from secondarily treated domestic wastewater (diluted with four parts of seawater) through assimilation by marine phytoplankton in an experimentally controlled outdoor growth pond. The phytoplankton, in turn, are removed from the system by filter-feeding herbivores such as oysters, clams, and mussels, all economically important shellfish. Any soluble inorganic nitrogen remaining in the wastewater, or regenerated by the herbivores, is removed by passage through a seaweed growth system. Details of the treatment process, and actual nitrogen removal efficiencies, will be reported elsewhere (*6*).

To demonstrate the high efficiency of the process in removing inorganic nitrogen, and to show that nitrogen was the growth-limiting nutrient in the coastal waters off Woods Hole, Massachusetts, at the time of our study, we have conducted a series of algal bioassays on samples from various components of the system.

In the bioassay reported here we compared the "algal growth potential" (*7*) of the influent and effluent samples from the phytoplankton growth system. Influent samples consisted of treated wastewater from the secondary wastewater treatment facility at Otis Air Force Base (Cape Cod, Massachusetts), diluted with four parts of seawater, and effluent samples consisted of the filtrate from the growth pond culture (Table 1). The pond influent and effluent samples were passed through Millipore filters (0.45 μm) and diluted with filtered seawater to simulate the actual dilutions that might occur when wastewaters are discharged in coastal waters. The dilutions used were 2 percent treated wastewater and 1 and 5 percent growth pond effluents. These samples (125 ml) and a seawater control sample were added to 250-ml flasks. We also enriched half of the 1 and 5 percent pond effluent samples with additional inorganic nitrogen as NH_4Cl. These samples then contained the same quantity of inorganic nitrogen that would be present if secondarily treated wastewater was used in place of the pond effluent at the designated dilutions with seawater. All samples (four replicates each) were inoculated with approximately 3500 cell/ml of a phytoplankton mixture (mainly the diatom *Chaetoceros simplex*) that had been taken the previous day from the growth pond. The flasks were incubated for 6 days at 22°C under fluorescent lighting ($\sim$ 5500 lumen/m^2), and in vivo chlorophyll measurements were made daily on a Turner model 111 fluorometer. The averaged results are reported as relative percentage values of the maximum seawater control value (Fig. 1).

The addition of filtered pond effluent to seawater had no effect over that of the seawater control in stimulating phytoplankton growth. The total inorganic nitrogen concentration ($\Sigma N = NH_4{}^+\text{-N} + NO_2{}^-\text{-N} + NO_3{}^-\text{-N}$) of the pond effluent was 5.13 μg-atom/liter. When the pond effluent was mixed with Woods Hole seawater ($\Sigma N = 2.38$ μg-atom/liter) at the designated dilutions, the nitrogen concentrations of the mixtures were virtually the same as that of the seawater alone.

Phosphate removal in the growth system was not nearly as complete as nitrogen removal because there was a gross excess of phosphorus over nitrogen in the secondarily treated wastewater used in this study, relative to that normally required by phytoplankton. This situation appears typical. For example, Ryther and Dunstan (*1*) pointed out that the nitrogen/phosphorus ratio in coastal marine waters, and in secondarily treated wastewater, is usually lower than in marine phytoplankton. Thus, more phosphorus is available in treated wastewater than can be utilized by the phytoplankton. In our study only 35 percent of the inorganic phosphorus was removed in the growth pond, so that the final phosphorus concentration in the pond effluent was 18.36 μg-atom/liter. Thus, the phosphate concentration of the samples increased significantly as more pond effluent was added to the seawater.

Table 1. Nitrogen and phosphorus concentrations of seawater off Woods Hole, Massachusetts, and of influent and effluent samples from a controlled outdoor phytoplankton growth pond. Nitrogen removal in the growth pond was 95 percent complete, whereas only 35 percent of the phosphorus was removed. The nitrogen/phosphorus atomic ratio of the pond influent was about 5/1. This ratio represents the lower limit of the nitrogen/phosphorus ratio found in marine phytoplankton (*1*), and thus accounts for the excess phosphorus relative to nitrogen found in the pond effluent.

Sample	Nutrient concentration (μg-atom/liter)				
	NH_4^+-N	NO_2^--N	NO_3^--N	ΣN	PO_4^{3-}-P
Seawater control	0.78	0.06	1.54	2.38	0.49
Pond influent (secondarily treated wastewater diluted with four parts of seawater)	115.50	5.70	20.50	141.70	28.17
Pond effluent	0.76	2.36	2.01	5.13	18.36

A comparison of the assay results for the 2 percent wastewater samples with those for the 1 percent and 5 percent pond effluent samples containing added nitrogen demonstrated that the phytoplankton growth response was similar for all three samples. However, it was four times greater than the response for the seawater control and pond effluent samples (Fig. 1). The phosphorus concentration in the 2 percent wastewater samples was considerably higher than in the pond effluent samples in either dilution, with or without NH_4^+-N added back. Yet only in samples in which ΣN was increased above the background seawater value did significant phytoplankton growth occur (Fig. 1). Also, although the nitrogen concentration in the three high-nitrogen samples varied from almost 15 to 75 μg-atom/liter, there was no significant difference in phytoplankton growth in these samples. Apparently, above a ΣN concentration of 15 μg-atom/liter some other factor became growth-limiting. It is possible that inorganic nitrogen was not limiting at even slightly lower levels.

Thus, there appears to be no doubt that the addition of nitrogen, and not phosphorus, stimulated phytoplankton growth in our study. The similarity of our results for the coastal waters off Woods Hole with those for other waters off the eastern seaboard (*1*) and for coastal waters off southern California (*2*) suggests that nitrogen may be the key growth-limiting nutrient for many coastal marine environments. Nitrogen removal to reduce the algal growth potential of secondarily treated wastewater, and hence prevent eutrophication of coastal waters, may therefore have widespread applicability. The combined tertiary treatment–marine aquaculture system appears to hold promise as an effective method to combat this problem. Not only is the system capable of reducing the inorganic nitrogen in wastewater to lower concentrations than those found in coastal receiving waters, but it offers hope as an efficient process to eliminate the resulting algal biomass and recycle waste products through conversion to nutritionally valuable shellfish.

We are, however, fully aware of the possibility that, depending on the type of wastewater used, there may be a one-way transport of low-level refractory compounds [such as DDT and PCB's (*8*)], trace heavy metals, and pathogenic bacteria and viruses through our controlled food chain system with an ultimate accumulation in the herbivore growth portion. These and related problems should be examined so that a rational evaluation can be made concerning the overall suitability of the system for nutrient removal and waste recycling.

JOEL C. GOLDMAN
KENNETH R. TENORE
HELEN I. STANLEY
Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543

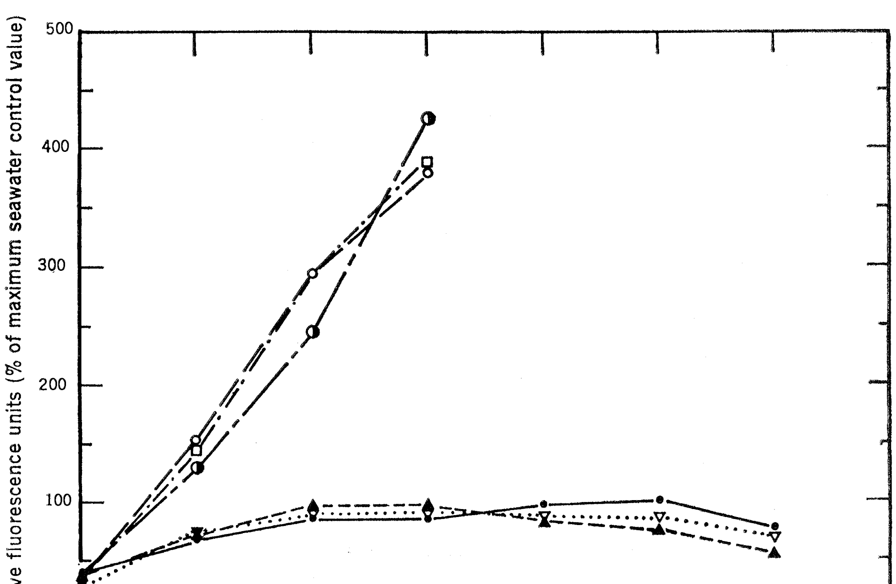

Fig. 1. Comparison of marine phytoplankton growth measured as chlorophyll fluorescence in seawater, for the coastal waters off Woods Hole, Massachusetts, and in seawater mixtures with secondarily treated domestic wastewater and phytoplankton growth pond effluent. Results are expressed as relative percentage values of the maximum seawater control value (100 percent on day 5). The following dilutions were made with seawater: (filled circles) seawater control; (filled triangles) 1 percent pond effluent; (open triangles) 5 percent pond effluent; (open circles) 2 percent secondarily treated wastewater; (half-filled circles) 1 percent pond effluent with NH_4^+-N added back (12.5 μg-atom/liter); and (squares) 5 percent pond effluent with NH_4^+-N added back (72.8 μg-atom/liter).

References and Notes

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7. Algal growth potential refers to the maximum algal biomass that can be produced in a natural water for a particular nutrient level. See W. J. Oswald and C. G. Golueke, *J. Water Pollut. Contr. Fed.* **38**, 964 (1966).
8. DDT is 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)-ethane and PCB's are polychlorinated biphenyls.
9. We thank J. H. Ryther, R. Vaccaro, E. J. Carpenter, and R. H. Backus for their critical review of this manuscript. Supported by NSF grant GI-32140. This is Woods Hole Oceanographic Institution Contribution No. 3021.

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