

NITRATE REDUCTASE FROM THE MARINE CYANOBACTERIUM *OSCILLATORIA LAETEVIRENS* (CROUAN) GOM.

B. J. MEHTA and V. D. CHAUHAN

Central Salt and Marine Chemicals Research Institute, Bhavnagar 364 002, India.

THE enzyme nitrate reductase catalyses the first step in the assimilation of nitrate, namely the reduction of nitrate to nitrite. In the cyanobacteria *Anabaena variabilis* and *Synechocystis* sp., the effect of nitrogen source on the activity of assimilatory nitrate reductase has been studied. In these species, nitrate behaves as a nutritional inducer of nitrate reductase¹⁻⁷, and enzyme activity is repressed during growth on ammonia¹. The growth rate of the marine cyanobacterium *Oscillatoria laetevirens* (Crouan) Gom is related to the source and concentration of nitrogen supplied. Cultures that received nitrate showed higher growth rate than those that received ammonia and nitrite⁸. Studies on uptake kinetics have revealed that the culture has high affinity for nitrate⁸. Hence it was of interest to study nitrate reductase in nitrate-grown marine *O. laetevirens*.

O. laetevirens was isolated from coral stone collected from the intertidal region of the Porbandar coast (21°38' N, 69°37' E). The culture was grown using seawater medium under controlled conditions, as described earlier⁸.

Nitrate reductase activity was assayed by the method of Lowe and Evans⁹. A known amount of fresh algal culture mass was washed 3-4 times with sterile distilled water containing 2.5% NaCl and then incubated in sterile culture medium containing 5 mM KNO₃, pH 7.5. Samples were taken after 18 h of incubation in controlled conditions, and the nitrite formed was measured by a diazo coupling method. Absorbance at 540 nm of the pink colour developed was measured after 15 min. The reagent blank was 1 ml medium instead of culture supernatant. NaNO₂ was used as standard. Protein content of the algal culture was estimated by the method of Lowry *et al.*¹⁰ using bovine serum albumin as standard.

Table 1 shows nitrate reductase activity of *O. laetevirens* in culture. A gradual increase in the enzyme activity was seen up to 11 days of incubation, and then there was no significant change in the activity. Cultures were grown in different concentrations of nitrate and enzyme activity was determined on day 11. The enzyme gave a Michaelis-Menten pattern (figure 1a). The

Table 1 Nitrate reductase activity in marine *O. laetevirens* in culture

| Days of incubation | Nitrate reductase activity (nmol NO ₂ produced/mg protein) |
|--------------------|---|
| 4 | 4.675 |
| 8 | 7.400 |
| 11 | 11.800 |
| 16 | 9.650 |

Lineweaver-Burk plot gave K_m 0.181 mM and V_{max} 11.11 nmol/mg protein (figure 1b).

In higher plants the role of nitrate as an inducer of the enzyme has been well studied¹¹⁻¹⁵. Increase in the level of nitrate reductase in the presence of nitrate implies *de novo* synthesis of the enzyme¹³⁻¹⁵. Studies with cycloheximide and other inhibitors of protein synthesis have shown that in both higher plants and eukaryotic algae, the enzyme is synthesized on 80 S cytoplasmic ribosomes¹⁶⁻²³. Nitrate reductase activity of the prokaryotic *O. laetevirens* grown in the presence of the protein synthesis inhibitor chloramphenicol was studied. At 6 µg/ml chloramphenicol added at the start of incubation, there was an inhibition of growth. When the antibiotic was added after two days of growth enzyme activity fell

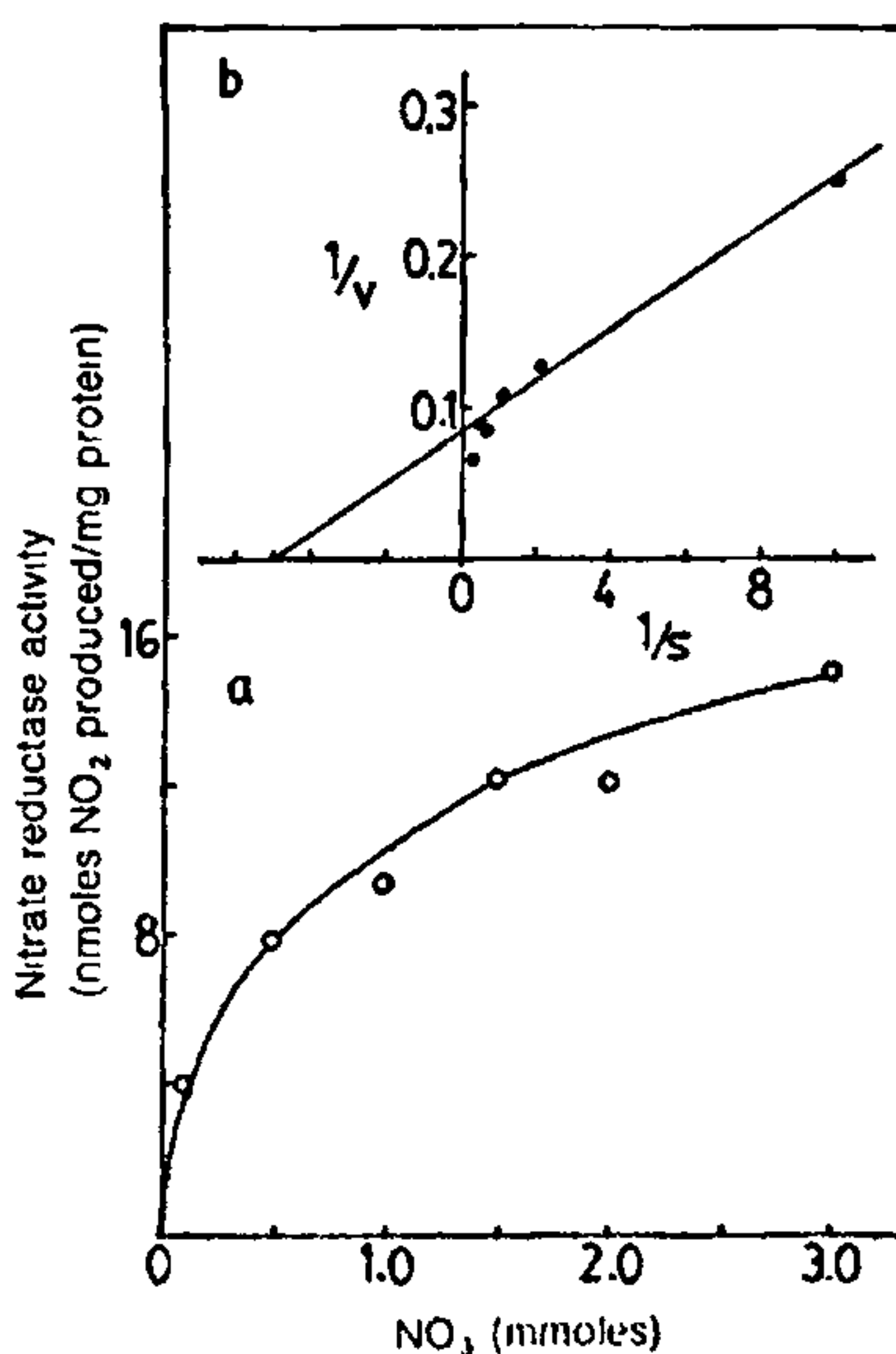


Figure 1. Substrate concentration vs velocity (a) and Lineweaver Burk (b) plots for nitrate reductase activity in *O. laetevirens*.

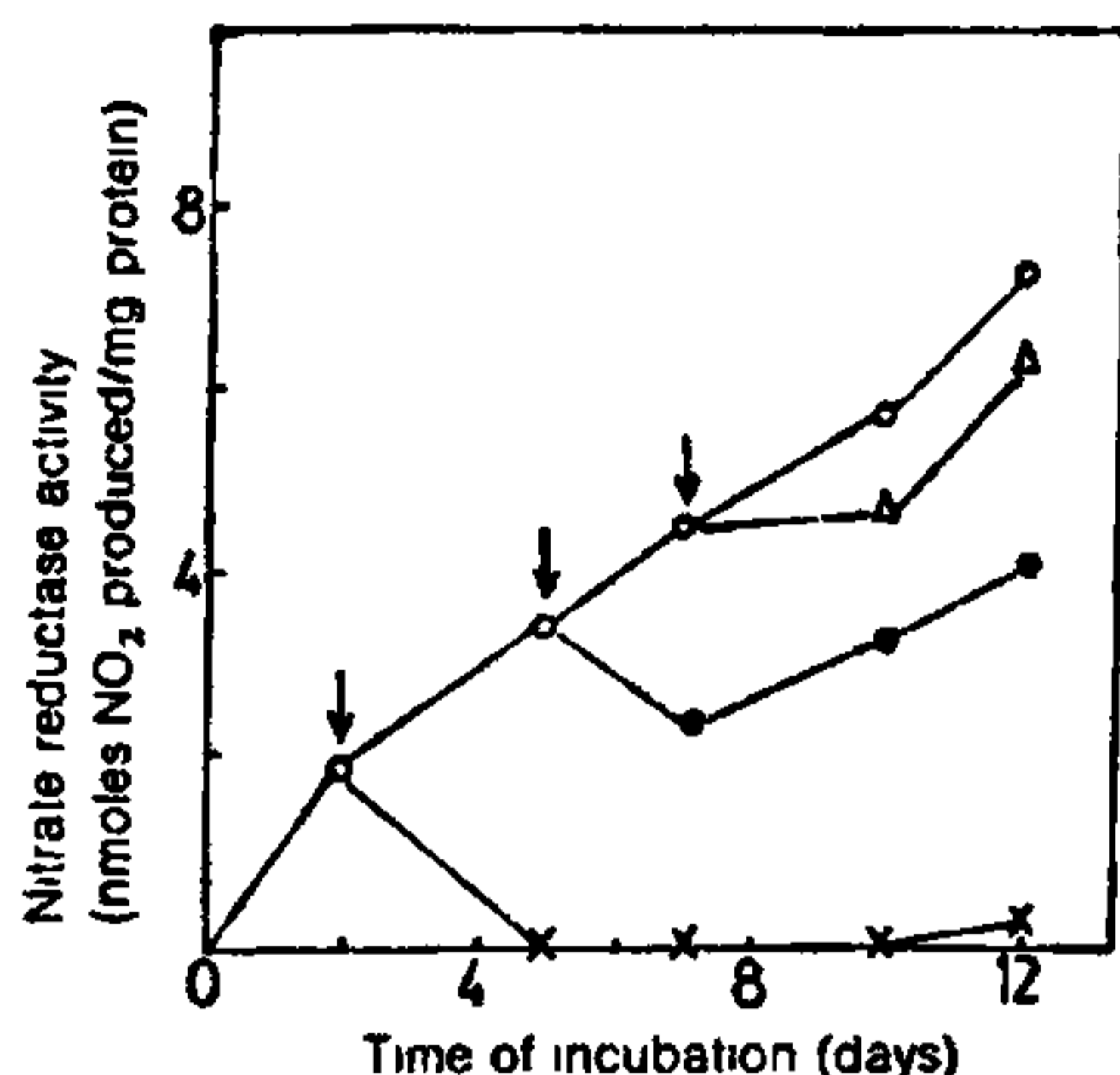


Figure 2. Effect of chloramphenicol on nitrate reductase activity in *O. laetevirens*: chloramphenicol absent (O-O), or added on day 2 (x-x), day 5 (●-●) or day 7 (Δ-Δ) of culture.

to zero by day 5 and was totally absent up to day 12; and then only 3.57% of the activity of the control culture could be observed (figure 2). When the antibiotic was added to five-day-old and seven-day-old cultures, about 55% and 85% enzyme activity respectively could be detected on day 12. These results indicate that most of the enzyme protein might be synthesized in the early stage of incubation, i.e. within the first five days. During this period the enzyme was sensitive to antibiotic-mediated inhibition. Once the rise in enzyme activity commenced, severity of inhibition was less. The results are similar to those for the eukaryotic *Chlorella vulgaris*, where cycloheximide inhibits the development of nitrate reductase activity only when added immediately after the transfer of cells from ammonia to nitrate medium, but not when added after the rise in enzyme activity has commenced¹⁷.

The present results show that nitrate reductase of *O. laetevirens* is an inducible enzyme.

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METABOLIC RESISTANCE TO METHYL PARATHION TOXICITY IN A BIVALVE, *LAMELLIDENS MARGINALIS*

C. RAVI SHANKARA, N. S. ASHOK BABU, K. INDIRA and W. RAJENDRA

Department of Zoology, Sri Venkateswara University, Tirupati 517 502, India.

INDISCRIMINATE use of pesticides is causing serious ecological imbalance, particularly in freshwater