

# Interdependent environmental factors controlling nitrification in waters

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**Abstract** In many areas of the world limited water resources have led to increased use of recycled water for agricultural and industrial purposes. Prior to releasing reclaimed water to consumers, excessive concentrations of inorganic nitrogenous compounds (ammonium, nitrite and nitrate) must be removed, and the water has to meet sanitary standards. The dynamics and abundance of the different nitrogenous compounds depend on the nitrification process (microbial oxidation of ammonia and nitrite). This is a key process in the nitrogen cycle and the autotrophic nitrifying bacteria catalyzing it are found in soils, sediments, wastewater, freshwater and marine water. Nitrification is a two-step process: first ammonia oxidizers convert ammonia to nitrite and then nitrite oxidizers convert nitrite to nitrate. An efficient nitrification process requires linked balanced activity of the two bacterial groups. Environmental factors that control nitrification affect ammonia and nitrite oxidizers differentially and thus disrupt the linkage between the two steps of the process. The effects of various environmental factors on the two bacterial groups and on the overall nitrification process are discussed. Light was identified to be a major factor inhibiting nitrification in a wastewater reservoir in Israel. Especially, nitrite oxidation was hindered causing the accumulation of nitrite during late spring and summer.

**Keywords** Nitrification; wastewater reservoir; nitrite accumulation; light; inhibition of nitrite oxidizers

## Introduction

The levels of nitrogenous compounds (ammonium, nitrite and nitrate) are critical parameters determining the quality of wastewater for its use in agriculture. This depends on microbial oxidation of ammonium to nitrate (nitrification) (Halling-Sorensen, 1993). Nitrate is subsequently reduced to atmospheric nitrogen ( $N_2$ ) by denitrifying bacteria (Robertson *et al.*, 1991). Two distinct groups of chemolithotrophic bacteria are responsible for the oxidation of ammonia to nitrate. The ammonia-oxidizing bacteria convert ammonia to nitrite that is subsequently oxidized to nitrate by the nitrite-oxidizing bacteria. If nitrogen removal fails, the nitrogenous compounds passing into waterways might leach to groundwater and may cause serious environmental and medical problems (Argaman, 1991).

Incomplete nitrification resulting in accumulation of nitrite was reported in activated sludge (Alleman, 1984; Randall and Buth, 1984), wastewater reservoirs (Azov and Tregubova, 1995), rivers (Smith *et al.*, 1997; von der Wiesche and Wetzel, 1998) and fish ponds (Hargreaves, 1998). A number of mechanisms have been found to inhibit nitrification processes in waters (e.g., Olson, 1981; Abeliovich and Vonshak, 1993; Diab *et al.* 1993; Fdz-Polanco *et al.*, 1994; Hargreaves, 1998). However, the effects of interdependent environmental factors on the overall nitrification process is still not completely clear.

In the present study we report the observations obtained by closely monitoring the concentrations of nitrogenous compounds and the effects of fluctuating environmental conditions in a wastewater reservoir.

## Methods

**Sampling site.** The Ma'ale Kishon reservoirs are part of a wastewater reclamation plan which has been described elsewhere in detail (Rebhun *et al.*, 1987; Azov and Shelef, 1991;

Azov *et al.*, 1992). The reservoir is subdivided into two connected basins (southern and northern reservoir), the flows into and from the basins can be separately regulated. Secondary effluents are pumped into the reservoir throughout the year whereas water is withdrawn only during the irrigation season (late spring and summer).

*Field sampling.* Water samples were drawn at 1m depth intervals, from both the southern (S) and northern (N) reservoirs and brought to the laboratory for analysis. Temperature, oxygen concentration, and pH were monitored on site along the water column at 1m intervals. The reservoirs were sampled weekly and data obtained during the period from January to December 1996 will be presented.

*Light measurement and attenuation coefficient calculation.* Light intensity ( $I$ ) was measured along the water column (at 10 cm intervals) in the reservoirs throughout the study using a LI-1000 data logger (LI-COR, Inc.) with an underwater light sensor.

The measurements were used to calculate the diffused light attenuation coefficient ( $\alpha$ ) according to the Beer-Lambert law:

$$\alpha = -1/x \ln I/I_0$$

where:  $x$  = depth in cm,  $I$  = the light intensity measured in a given depth, and  $I_0$  = the light intensity at the surface ( $x=0$  cm).

*Chemical analysis.* Ammonium, and nitrite were assayed according to Standard Methods (APHA, 1995), and nitrate concentration was assayed with NAS reagent. Total carbon (IC, TOC, DOC) was assayed with a DC-190 High Temperature TOC analyzer (Rosemount analytical).

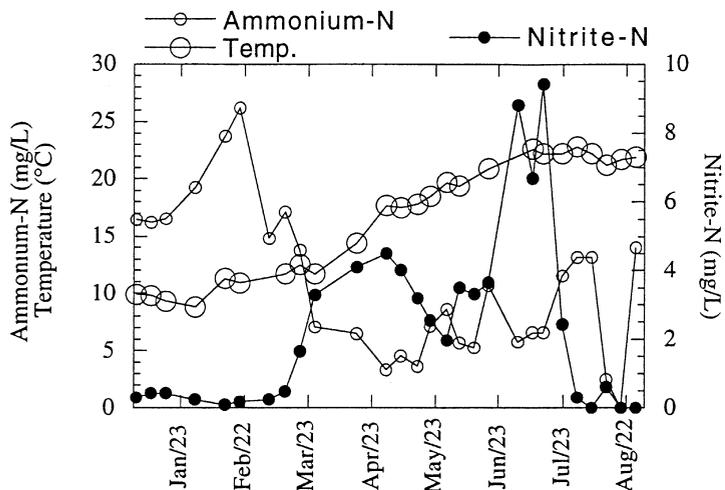
*Bacterial counts.* Counts of viable ammonia- and nitrite-oxidizing bacteria were determined by the MPN method, in triplicate (Schmidt and Belser, 1982).

*Statistical analysis.* Statistical analysis was carried out using StatView software (Abacus Concepts Inc.).

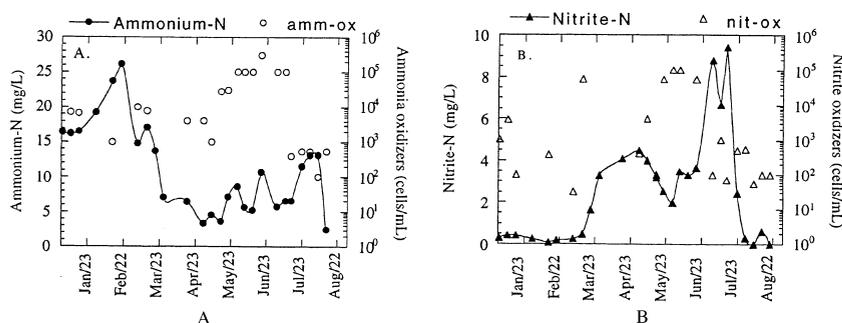
## Results and discussion

The distribution of nitrifying bacteria along the water column was homogenous and so were the concentrations of ammonium, nitrite, and inorganic and dissolved organic carbon. Unlike the other measured parameters, oxygen concentration decreased along the water column sometimes dropping below 0.5 mg/L close to the bottom. Yet, oxygen concentration was usually higher than the half saturation constant  $K_m$  (Painter, 1970) for either the ammonia or the nitrite oxidation in the open water. Moreover, we did not find a correlation between nitrite accumulation and low oxygen concentrations ( $R^2=0.16$ ). Therefore, oxygen limitation could not be the reason for an incomplete nitrification at the oxygen concentrations measured in the reservoir.

Typical changes in N-ammonium, N-nitrite and water temperature during 1996 in the northern reservoir are presented in Figure 1. Similar patterns were previously reported for the upper water layer at distributed sites at the reservoir (Azov *et al.*, 1992; Azov and Tregubova, 1995; Institute of Environmental Engineering and Water Resources, 1985-1996). As expected, concentration of N-ammonium increased during the winter months when the water temperature was about 10–12°C and nitrifying bacteria are not active. Starting in March, a rapid decrease in ammonium concentrations was observed. High



**Figure 1** Changes in temperature and ammonium and nitrite concentrations in the northern reservoir (1996)



**Figure 2** Concentrations of ammonium and nitrite and nitrifying bacteria counts (MPN) (A) ammonium and ammonia oxidizers (B) Nitrite and nitrite oxidizers

concentrations of nitrite were detected for about five months, indicating that the rate of ammonia oxidation was higher than that of nitrite, resulting in unbalanced nitrification.

Various processes may lead to nitrite accumulation: (i) incomplete denitrification, blocked at the nitrite stage (under anaerobic conditions); (ii) inhibition of nitrite oxidation (under aerobic conditions); (iii) a sudden increase in the rate of ammonia oxidation without a parallel increase in subsequent nitrite oxidation rate.

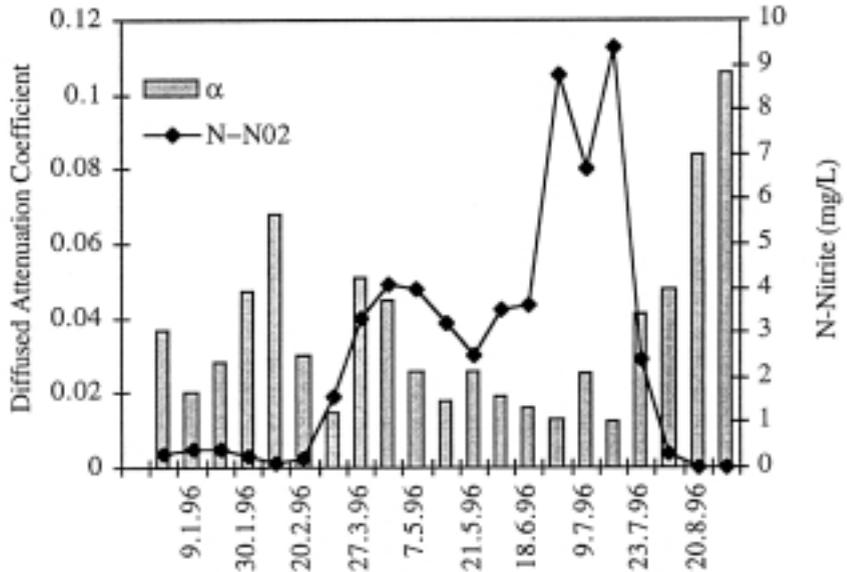
As previously reported (see Abeliovich, 1987), ammonia- and nitrite-oxidizing bacteria were present in the water throughout the year (Figures 2A and B). The decrease in ammonium concentration began about two months earlier than the increase in the number of ammonia oxidizers. Low concentrations of ammonium and high counts of ammonia oxidizers were observed during the summer months indicating that the bacteria were growing and active during that period of time (Figure 2A).

A different pattern was recorded for nitrite-oxidizing bacteria: although the number of nitrite oxidizers increased during spring and remained high throughout June, high concentrations of nitrite accumulated from March through July (Figure 2B). These observations indicate that high bacterial counts do not always reflect high activity, and that the activity of nitrite oxidizers was inhibited.

Nitrite oxidation was impeded although the water temperature seemed to be adequate for biological activity (Figure 1). Fdz-Polanco *et al.* (1994) showed that rising temperature dif-

**Table 1** Correlation between nitrite accumulation and some physicochemical parameters.  $\alpha$  = attenuation coefficient (see Materials and Methods)

Month	Nitrite mg/L	$\alpha$	Temp. °C	pH	DOC mg/L
Jan.- Feb.	Low (0–0.3)	High >0.03	10–13	7.4–7.9	15–25
Mar.-Apr.	Low (0.3–0.5)	High >0.03	13–18	7.2–7.6	15–20
May-Aug.	High (1.5–10)	Low <0.03	20–26	7.7–8.1	10–15



Further stepwise regression analysis performed with the data collected for temperature, oxygen, ammonia, pH light, DOC, phosphate and counts of ammonia and nitrite oxidizing bacteria indicate that temperature and light were the only major factors that significantly correlate with the activity of nitrifying bacteria over the year ( $R^2=0.74$ ). We summarized some corresponding parameters monitored in this study and the concentration of nitrite in the reservoir at different time periods during the year in Table 1.

## Conclusion

The observed dynamics of nitrite accumulation in the Ma'ale Kishon reservoir could thus be described by three regimes of environmental factors (Table 1):

- i. low temperatures prevent detectable nitrification;
- ii. rising temperatures support nitrification;
- iii. high temperatures, high light intensities and raised ammonia concentrations (pH) disrupt the balance between the two steps of the nitrification process resulting in nitrite accumulation.

## Acknowledgements

The authors would like to thank the Federal Ministry of Education, Science, Research and Technology of Germany (BMBF) and the Ministry of Science and Arts of Israel (MOSA) for funding part of this work (grant GR 1211).

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