

Full Length Research Paper

Biological Wastewater Denitrification by Thermophilic Bacteria

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ABSTRACT

The biological denitrification of wastewater was studied by two thermophilic strains (*Bacillus* sp.) isolated from hot spring (Hammam Essalhine, Khenchela). The kinetics of denitrification was followed at 55°C anaerobically using (5g/l) of KNO₃ as final electron acceptor. The determination of NO₃⁻ was performed by colorimetric method using sodium salicylate. The results showed the ability of the thermophilic strains to reduce NO₃⁻ to NO₂⁻ at much faster rate (14 to 17h) compared with a mesophilic strain *Enterobacter cloacae* (45h) isolated from activated sludge of a wastewater treatment plant. The study of the influence of temperature on nitrate reductase activity showed a maximum denitrification at 60 °C. These thermophilic denitrifying bacteria may be useful in processes of treatment of wastewater, to reduce the high concentrations of nitrates by a biological process.

Key words: Pollution, sewage treatment plants, denitrification, bacteria, thermophily

1. INTRODUCTION

Nitrate contamination of water is widespread in the region of Khenchela (eastern Algeria). Increased levels of nitrate have negative effects on health and environment. Biological denitrification has been shown to be one of the most advanced methods for removing nitrogen in low-cost way. (Rezaee A. *et al.*, 2008).

Denitrification by bacteria is generally regarded as being a result of anaerobic nitrate respiration (Payne W. J., 1973; Knowles R., 1982). Moreover, high temperatures promote more this process influencing the dissolved oxygen concentrations. Biological denitrification is inhibited when the dissolved O₂ is above 0.2 mg/l (Nozawa T. *et al.*, 1988). In this paper, we demonstrate the nitrate removal process by two thermophilic bacterial strains which thrive at high temperatures (Horiike T. *et al.*, 2002).

2. MATERIALS AND METHODS

To study the biological denitrification kinetics, three batch cultures were performed in 500ml flasks containing a synthetic medium with similar composition of wastewaters. Containing (g L⁻¹): KNO₃, 5; KH₂PO₄, 1; NaCl, 1; MgSO₄, 0.2; CaCl₂, 0.02, trace elements solution, 1mL L⁻¹, the pH was adjusted to 7 ± 0.04. After sterilization at 120 °C for 30 min, glucose was added as carbon source (5 g L⁻¹) (Balch W.E. *et al.*, 1979; Mammeri L., 2007).

Each flask was inoculated with one of the selected bacterial strains. The three strains are characterized by their ability to reduce nitrate through bacterial enzyme, nitrate-reductase (Fig.1, Table 1).

Flasks were then incubated anaerobically in shaking water bath.

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Samples were taken every 2 hours to determinate the growth rates by measuring the optical density at 600 nm using a UV/ VIS spectrophotometer (SHIMADZU). Nitrate rates were determinate by colorimetric method

using salicylate sodium (Kariminiaae et al., 2004; Rodier, 2009).

The nitrate-reductase activity of *Bacillus sp. II* was tested at different temperatures (20-80°C) (Garcia J. L., 1977).



Figure 1: Microscopic appearance of the three nitrate-reductase strains. (a), *Bacillus sp. I*; (b), *Bacillus sp. II*; (c), *Enterobacter cloacae*.

Table 1: Characteristics of the three bacterial strains studied.

| | <i>Bacillus sp. I</i> | <i>Bacillus sp. II</i> | <i>Enterobacter cloacae</i> |
|--------------------------|--|--|--|
| Growth T° | 55 °C | 60 °C | 30°C |
| Gram stain | + | + | - |
| Nitrate-reductase | + | + | + |
| Isolation site | Thermal water (Hammam Essalhine) -Khenchela- | Thermal water (Hammam Essalhine) -Khenchela- | Activated sludge (Treatment plant) -Khenchela- |
| Incubation T° | 55 °C | 55 °C | 30 °C |

3. RESULTS AND DISCUSSION

The results show the ability of the three bacterial strains studied to reduce NO_3^- to NO_2^- at different rates. This is explained by decrease of nitrate rate over time of incubation, reaching minimum concentrations (Fig. 2, 3 and 4) (Jutharat et al., 2007).

The first two graphs (Fig. 3 and 4) show an excellent removal of nitrate by the thermophilic strains (*Bacillus sp. I*, *Bacillus sp. II*) in a very short interval time (14-17 h) compared to the mesophilic strain (*Enterobacter cloacae*) which was unable to remove full nitrates after 45h of incubation (Fig. 4).

The study of the influence of temperature on the nitrate-reductase activity showed a higher denitrification at 60 °C (Fig. 5).

These results confirm the ability of thermophilic bacteria, isolated from hot springs, to denitrify (Chen *et al.*, 2002; Khelifi *et al.*, 2010).

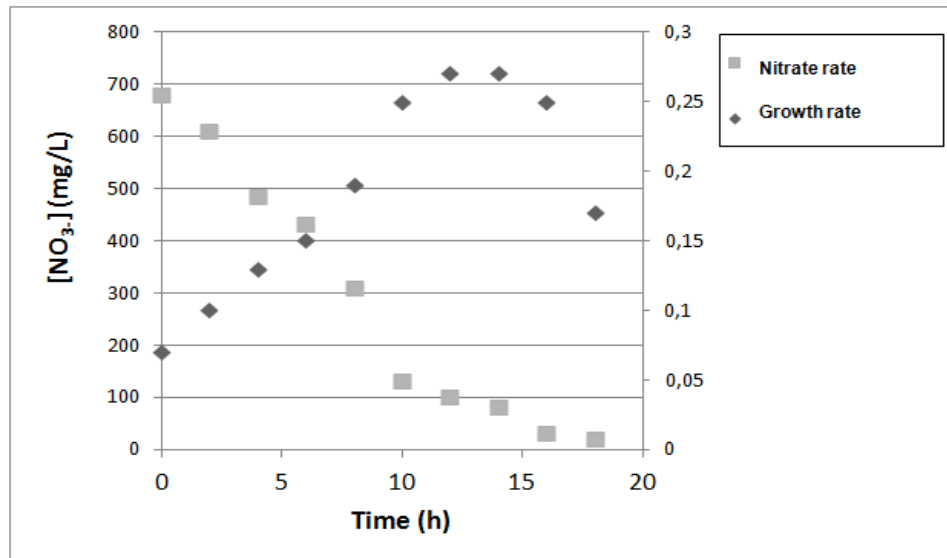


Figure 2: Kinetics growth and nitrate reduction by the thermophilic strain *Bacillus sp.I*.

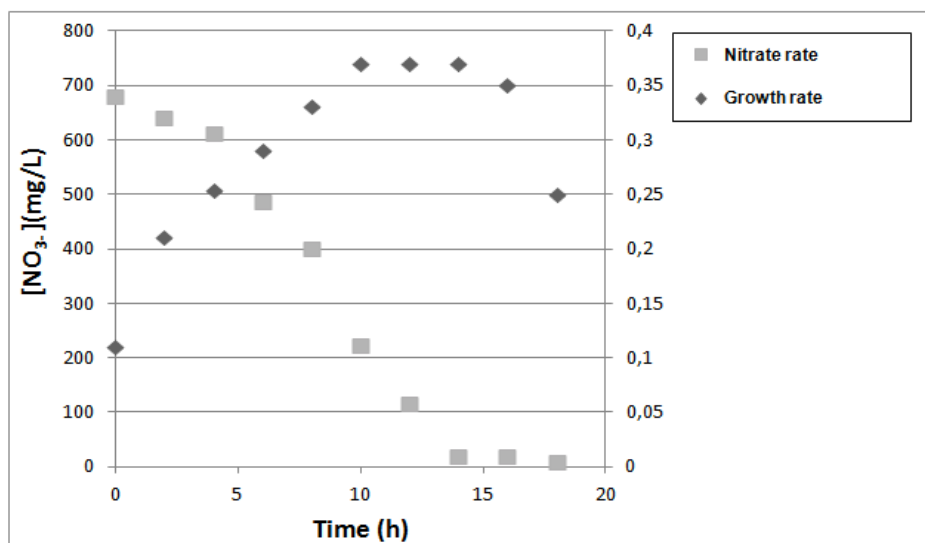


Figure 3: Kinetics of growth and nitrate reduction by the thermophilic strain *Bacillus sp.II*.

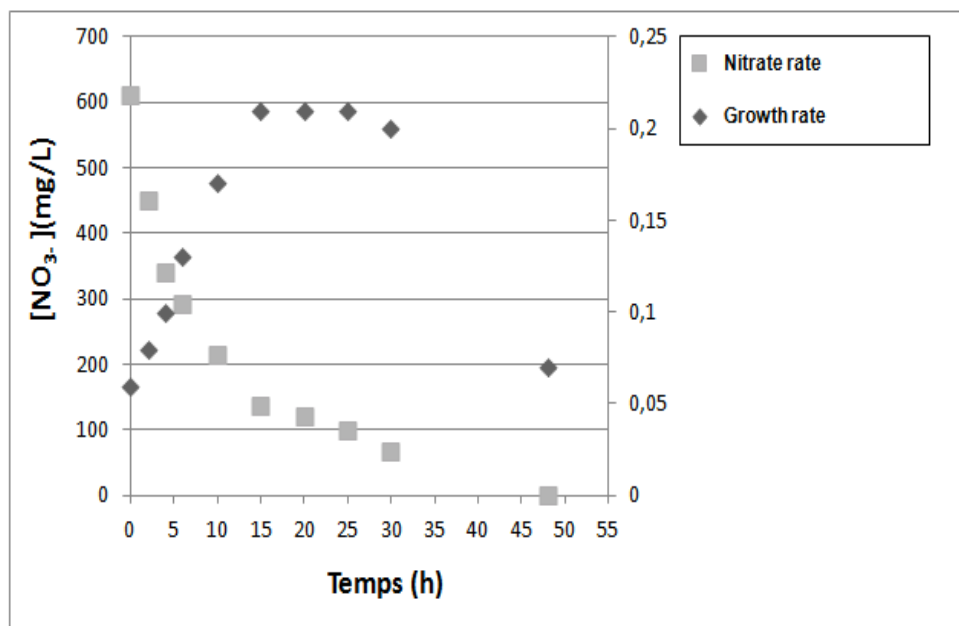


Figure: Kinetics of growth and nitrate reduction by the mesophilic strain *Enterobacter cloacae*.

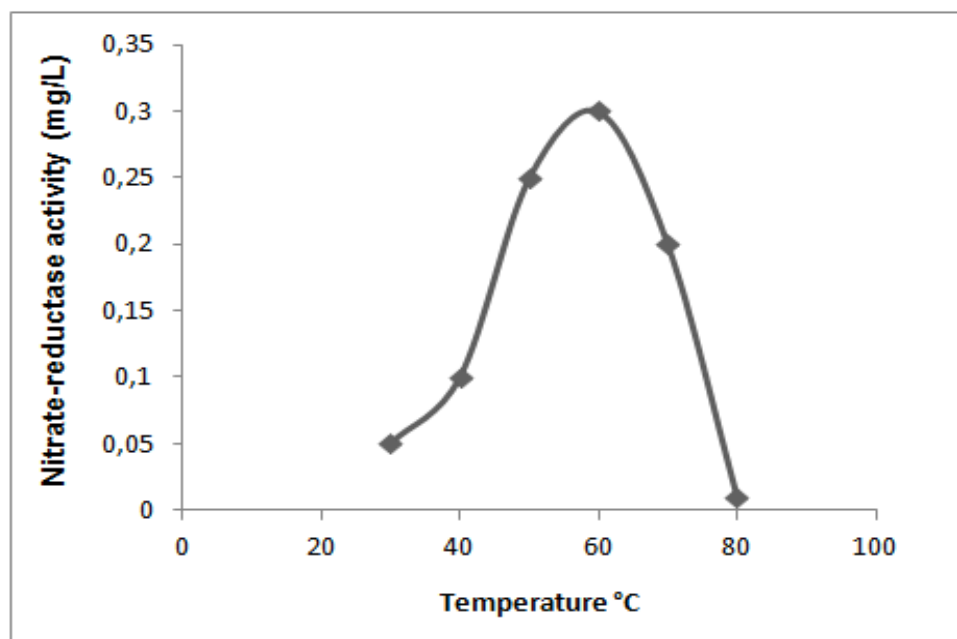


Figure: Dependence of the nitrate-reduction of *Bacillus sp. II* on the temperature.

4. CONCLUSION

Denitrification was studied at high temperatures using two bacterial thermophilic strains. The nitrate removal efficiency of these isolates was faster than that of control mesophilic

strain. These thermophilic bacterial strains can be very useful in processes of treatment of wastewater, by reducing the high concentrations of nitrates.

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