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Role of the denitrifying Haloarchaea in the treatment of nitrite-brines

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Summary. *Haloferax mediterranei* is a denitrifying halophilic archaeon able to reduce nitrate and nitrite under oxic and anoxic conditions. In the presence of oxygen, nitrate and nitrite are used as nitrogen sources for growth. Under oxygen scarcity, this haloarchaeon uses both ions as electron acceptors via a denitrification pathway. In the present work, the maximal nitrite concentration tolerated by this organism was determined by studying the growth of *H. mediterranei* in minimal medium containing 30, 40 and 50 mM nitrite as sole nitrogen source and under initial oxic conditions at 42 °C. The results showed the ability of *H. mediterranei* to withstand nitrite concentrations up to 50 mM. At the beginning of the incubation, nitrate was detected in the medium, probably due to the spontaneous oxidation of nitrite under the initial oxic conditions. The complete removal of nitrite and nitrate was accomplished in most of the tested conditions, except in culture medium containing 50 mM nitrite, suggesting that this concentration compromised the denitrification capacity of the cells. Nitrite and nitrate reductases activities were analyzed at different growth stages of *H. mediterranei*. In all cases, the activities of the respiratory enzymes were higher than their assimilative counterparts; this was especially the case for NirK. The denitrifying and possibly detoxifying role of this enzyme might explain the high nitrite tolerance of *H. mediterranei*. This archaeon was also able to remove 60 % of the nitrate and 75 % of the nitrite initially present in brine samples collected from a wastewater treatment facility. These results suggest that *H. mediterranei,* and probably other halophilic denitrifying *Archaea*, are suitable candidates for the bioremediation of brines with high nitrite and nitrate concentrations. [**Int Microbiol** 2012; 15(3):111-119]

Keywords: *Haloferax mediterranei* · Haloarchaea · respiratory nitrite pathway · assimilatory nitrite pathway · brines · denitrification · bioremediation

Introduction

High concentrations of nitrite in water are highly toxic to humans, fauna and flora and thus a matter of great concern [42]. Human consumption of drinking water containing high nitrite levels has been associated with gastric cancer, induced by the endogenous formation of genotoxic N-nitrous-compounds by bacteria in the human stomach [11,51]. Nitrite has also been identified as a possible cause of migraine headaches [24]. Furthermore, the passage of nitrite into the bloodstream results in the irreversible conversion of hemoglobin to methemoglobin, thus compromising the release of oxygen to tissues [50] and in turn causing respiratory deficiencies in aquatic and terrestrial animals, including humans [9,26,42].

Groundwater contamination with nitrite is typically due to the excess use of fertilizers in agriculture, the disposal of animal waste, or the improper disposal of industrial effluents [2]. The manufacturing of chemicals such as pesticides, herbicides, explosives and dyes usually generates effluents containing complex mixtures of salts and nitrate or nitrite [7,46]. While biological denitrification is a cost-effective and envi-

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ronmentally friendly method for the removal of these nitrogenous compounds [53], most microorganisms are very sensitive even to low nitrate and nitrite concentrations [7]. The negative effect of these nitrogen compounds is mainly due to the extreme toxicity of nitrite and nitric oxide upon nitrite reduction [15]. Chen et al. [17,18] reported that most heterotrophic denitrifiers are inhibited by 200 mg nitrite/l (around 4 mM), probably due to the formation of the protonated species nitrous acid. Other researchers have reported the toxicity of nitrous acid (HNO_2), which is consistent with the deleterious effect of low pH on denitrification [1,5]. In addition, the denitrifying capacity of many microorganisms can be limited by the high salt concentrations in wastewater, which cause a loss of enzymatic activities and eventually lead to plasmolysis [25].

The recognition of *Archaea* as a Domain of life [52] and the characterization of the ecology, biochemistry, and molecular biology of its members have brought about new approaches with which to better understand evolution and ecosystem dynamics. On the basis of their metabolism and nutritional or physicochemical requirements, *Archaea* are usually classified as methanogens, thermoacidophiles, halophiles, or alkalophiles. The capability of the respective species to proliferate under such a huge range of extreme conditions as well as their mechanisms supporting genetic plasticity [14,16] make them good candidates for research in fields such as astrobiology, microbial ecology, evolution, biochemistry, molecular biology and biotechnology [23,39,45].

Halophiles have been isolated, or at least identified by denaturing gradient gel electrophoresis (DGGE), from marshes, coastal salty ponds or inland salterns exploited for NaCl extraction, as well as from halite evaporites [21,37], salty soils and seas [33]. Halophilic archaea from these environments can grow in the presence of high NaCl concentrations (12–30 % salt, corresponding to 2–5 M NaCl). Haloarchaea counteract these high concentrations by accumulating K^+ in their cytoplasm, which enable the continued function of the cellular machinery [40].

Comparative molecular analyses of halophilic microbial communities have revealed that similar environments select for specific microbial lineages [44]. Accordingly, it has been found that the major microbial populations in these salty ecosystems are not only members of the Haloarchaeabut also of the extreme halophilic *Bacteria* [3]. Since denitrification carried out by Haloarchaea is not inhibited in the presence of high salt concentrations, denitrifying haloarchaeal species provide good model organisms with which to optimize the removal of nitrogenous species (mainly nitrate and nitrite) from waste water and brines. Moreover, denitrifying haloarchaeal consortia with nitrifying communities could further improve bioremediation processes in wastewater treatment plants.

Haloferax mediterranei is an extreme halophilic archaeon able to grow in an unusually wide range of NaCl concentrations (1.0–5.2 M) [49]. It is also known as a denitrifier, based on its capability of reducing nitrate and nitrite under oxic and anoxic conditions [8]. In the presence of oxygen, this haloarchaeon uses nitrate and nitrite as nitrogen sources for growth [35,36]. Under oxygen scarcity, it uses both ions as terminal electron acceptors [29,34]. With the objective to determine the feasibility of using *H. mediterranei* in wastewater treatment or brines bioremediation, we analyzed the capacity of a representative strain to remove high nitrite concentrations from salted water at relatively high temperature (42 °C). In similar studies, measurement of enzyme activities were shown to be a good index of a biological rate [6]. In this study, quantitative determination of nitrate reductase and nitrite reductase (involved in the assimilatory and respiratory pathways) helped us to understand the role of these enzymes in the entire process of nitrogen removal by *H. mediterranei*.

Materials and methods

Growth of *Haloferax mediterranei* in salt media with high nitrite concentrations. *Haloferax mediterranei* (ATCC 33500^T) cultures were grown in a 25 % (w/v) mixture of inorganic salts (25 % SW) as described by Rodríguez-Valera [43]. This minimal medium also contained: 0.005 g $FeCl_3$/l, 0.5 g KH_2PO_4/l, 5 g glucose/l and different nitrite concentrations (30, 40 and 50 mM KNO_2). The pH value of the culture media was adjusted to 7.3 using KOH or HCl.

Each medium was inoculated with 10 ml of stationary-phase cells adapted to a nitrite concentration lower than the one tested, in order to analyze the capability of *H. mediterranei* to adapt to higher nitrite concentrations. Under these conditions, the experiment reproduced the conditions at wastewater plants, where microorganisms used for bioremediation are not usually pre-adapted to the wastewater chemical characteristics. *H. mediterranei* was grown at 42 °C (optimal temperature for growth) in a 2-l flask in a Biostat B fermenter (B. Braun Biotech International), with continuous pH and temperature control and under initial oxygenic conditions. Growth was monitored by measuring the optical density at 600 nm. All the cultures were carried out in quadruplicate, and the data points plotted are the average of the results obtained from them.

Growth of *Haloferax mediterranei* in brine samples. Samples were obtained from a wastewater treatment plant located in Vinalopó Valley, Alicante, Spain. Nine samples were collected, three on April 2007 and six on September 2008. Culture media were prepared by replacing the 25 % mixture of salt water with the brine samples (brines were previously autoclaved to prevent the growth of the microorganisms inhabiting the brines at the wastewater treatment plant), which were supplemented

with: 0.005 g $FeCl_3$/l, 0.5 g KH_2PO_4/l and 5 g glucose/l. Each medium was inoculated with 5 ml of stationary-phase cells. The growth conditions were the same as described for the ATCC strain. Growth was also monitored by measuring the optical density at 600 nm. The data points plotted are the average of the results obtained from the nine cultures cited above.

For the chemical characterization procedures, 1 ml from each sample was used to determine the inorganic ion composition and concentrations (Na^+, Ca^{2+}, Mg^{2+}, Cl^-, SO_4^{2-}), following UNE standard methods (Spanish Association for Standardization and Certification [http://www.aenor.es/desarrollo/normalizacion/normas/]: UNE 34204:1981; UNE EN77041:2002; UNE 34233:1984. The data summarized in Table 2 correspond to the average of the results obtained from triplicates.

Determination of nitrite and nitrate concentrations in the culture media. Nitrite concentrations in the media were determined spectrophotometrically after a 40-fold dilution of 25 µl of the medium using the diazo-coupling method [47]. Since nitrite can be spontaneously oxidized to nitrate under the initial oxic conditions of this study, the nitrate concentrations in the media were estimated by the UV method after a 50-fold dilution of 1 ml of the medium [31].

Nitrate and nitrite reductase assays. Aliquots (25 ml) of cell suspensions were harvested at different times during the culture period by centrifugation at 10,000 rpm at 4 °C for 40 min in a MPW-350 R centrifuge. The supernatant (corresponding to the extracellular medium) was collected for enzymatic assays. The freshly harvested cells were first resuspended in a 50 mM phosphate buffer, pH 7.3, containing 2.5 M $(NH_4)_2SO_4$ and then disrupted by sonication at 150 W for eight periods of 3 min each at 4 °C in a Virsonic 475 ultrasonic disintegrator. The suspension was centrifuged at 13,000 rpm for 10 min. The pellet (membranes) and supernatant (cytoplasmic fraction) were collected separately and used as the source of enzymes. In order to promote membrane solubilization, the pellet was resuspended in a 100 mM Tris buffer, pH 8, containing 2 M NaCl and 20 % Triton.

Nitrite and nitrate reductase activities were assayed following published colorimetric methods using reduced methyl viologen as artificial electron donor [35,36]. The enzymatic activity units are expressed as µmol nitrite consumed per min or µmol nitrite produced per min for nitrite and nitrate reductase activities, respectively. All the assays were carried out in triplicate and against a control assay without the enzyme. The protein concentration was estimated by the Bradford method [10], with bovine serum albumin (fraction V) as the standard.

Results and Discussion

Growth of *Haloferax mediterranei* in salt media containing high nitrite concentrations. Previous studies described the resistance of *H. mediterranei* to very high nitrite concentrations (up to 10 and 40 mM without and with pH control, respectively) compared with other prokaryotes [8,31,33,34] when the cells are grown at 37 °C in brines prepared in the lab. Thus, we sought to determine the highest nitrite concentration tolerated by this haloarchaeon without effects on its denitrifying capacity. The experiment was carried out at a higher temperature (42 °C) using brines either prepared in the lab or collected from wastewater plant treatments. A temperature of 42 °C was chosen for two reasons: it is the optimal temperature for *H. mediterranei* growth in the natural environment (salty solar ponds) of this archaeon; and the rate of denitrification by *H. mediterranei* is higher at high temperatures, due to the thermostability of the enzymes involved [8,29].

Given that the maximum tolerance for the majority of microorganisms analyzed to date ranges from 2 to 5 mM NO_2^- [46], the concentrations chosen for this study (30, 40 and 50 mM KNO_2) can be considered extremely high. We hypothesized that the nitrite tolerance of *H. mediterranei* should be higher based on its high efficiency as a denitrifier and the location of NirK (catalyzing the second step of denitrification). This enzyme, which is synthesized in the cytoplasm, is exported to the membrane and subsequently detected within the culture media. The kinetic parameters of this enzyme as well as its location could explain the high rates of nitrite reduction in *H. mediterranei* (B. Zafrilla personal communication; work under submission).

To avoid the deleterious effect of low pH on denitrification, due to the formation of HNO_2, the experiments were carried out in a Biostat B fermenter with a continuous pH control system. In all cases, nitrate was detected in the culture media as a product of the spontaneous oxidation of nitrite to nitrate, in the presence of oxygen. Since nitrate can also be used as a nitrogen source for cell growth or as a terminal electron acceptor when oxygen is scarce [29], both nitrate and nitrite were quantified at different times during the culture growths.

The results obtained from the cultures with 30 and 40 mM nitrite (Fig. 1A,B) showed that both nitrite and nitrate were completely exhausted when cell growth reached the stationary phase. The consumption rate of these ions increased substantially once the oxygen concentration drastically decreased (oxygen concentration < 30 %). This observation was consistent with the ability of *H. mediterranei* to use nitrite and nitrate as nitrogen sources not only for growth but also as terminal electron acceptors under anoxic conditions. Consequently, this haloarchaeon is of great interest for bioremediation processes. The profile of the growth curves summarized in Fig. 1 is similar to the profiles expected during diauxic growth, in which a substrate is first exhausted (nitrite in the experiments here presented) thus slowing down the growth of the cultures. Shortly before the onset of this effect, a second substrate begins to be used (nitrate), again speeding growth. This pattern is also supported by changes in enzymatic activities related to nitrate and nitrite reduction as discussed later.

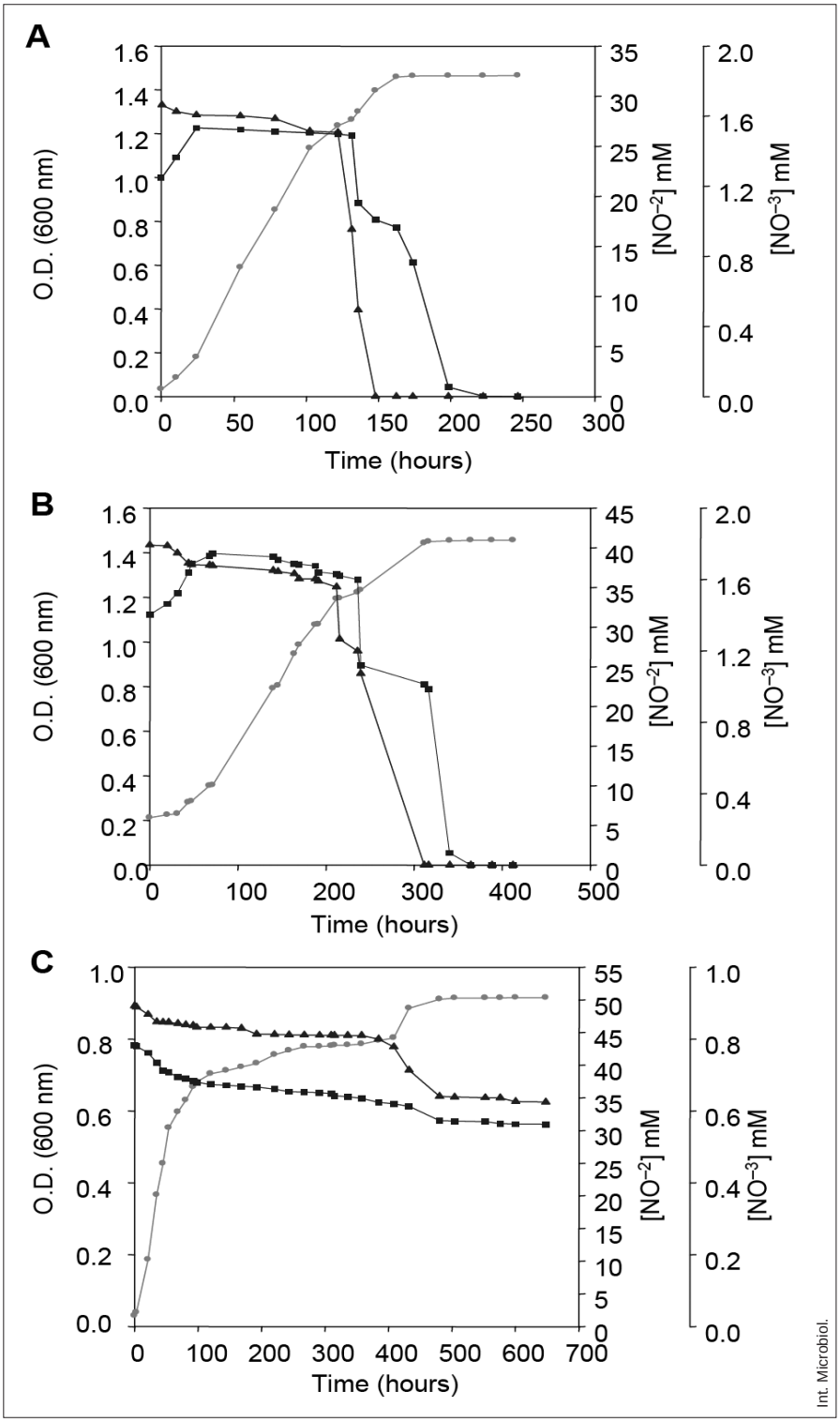

Fig. 1. *Haloferax mediterranei* growth in salt media (25 % SW) with 30 mM (**A**), 40 mM (**B**) and 50 mM (**C**) nitrite as nitrogen source. Optical density at 600 nm (circles), nitrite concentration within the medium (triangles), nitrate concentration within the medium (squares). The average value of the error was ± 0.01 mM of absorbance units for nitrate and nitrite concentrations or optical density, respectively.

Haloferax mediterranei was able to grow in media supplied with nitrate in concentrations up to 50 mM (Fig. 1C). The cultures grew exponentially for 120 h of batch culture, followed by an extensive phase of approximately 288 h in which the optical density (OD) remained around 0.7; then the culture slightly increased until reaching a second stationary phase (OD around 0.9). This initial behavior may have been due to the cells used as inoculum, which were well

adapted as their metabolic pathways had been induced for a long time, favoring cell proliferation. However, growth was inhibited earlier than expected on the basis of a previously reported study conducted by Martínez-Espinosa and coworkers [34], in which cultures reached an OD_{600} of around 2. Factors such as the incubation temperature (42 °C in this study and 37 °C in the study in [34]) and the characteristics of the inoculum (10 ml of inoculum, with an OD around 2.4 in this study, and 25 ml of inoculum with an OD around 2.4 in [34]), might have influenced the results. Although the optimum growth temperature reported for *H. mediterranei* is 45 °C [27,49], oxygen solubility decreases at high temperature. Since in this study the cultures were initially aerobic, temperatures around 40–45 °C might have affected cell growth during the early hours of incubation. As for the removal of nitrogen compounds, as little as 30 % of the nitrite and nitrate present in the culture had been consumed by the end of the stationary phase, indicating that the assimilative and respiratory pathways were negatively affected by the high nitrite concentration. Therefore, although a nitrite concentration of 50 mM is still tolerated by *H. mediterranei* under pH control, it may be on the threshold of toxicity for this organism.

From the growth curves (Fig 1), it was possible to determine the specific growth rate (μ) and cell doubling time (t_d) for cultures under each of the assayed conditions. In medium containing 30 mM nitrite, a μ of 0.019 h^{-1} was determined, which was higher than the value at 40 mM nitrite (0.0060 h^{-1}). Th t_d was 35 h, around three times lower than in 40 mM medium. Exponential growth was reached after 175 or 300 h of incubation in presence of 30 and 40 mM nitrite, respectively. These findings suggest that *H. mediterranei* better tolerates 30 mM than 40 mM nitrite, probably because the latter begins to be slightly toxic for the organism. Incubations in medium with 50 mM nitrite resulted in the highest μ ($0.0544h^{-1}$) and the lowest t_d (12.74 h). This does not imply, however, that these conditions were optimal for the growth of this organism; on the contrary, they were closer to a stress situation of nitrite toxicity at relatively high temperatures (42 °C). The rapid growth observed over the first hours of incubation was due to the conditions of the inoculum, as mentioned above. A comparison of the above results with those reported in complex medium with 0.5 % yeast (0.107 h^{-1} and 6.47 h) [G. Bravo, PhD Thesis, University of Alicante, 2011], leads to the conclusion that growth in minimal medium with high nitrite concentrations is slower than in rich culture medium, as was expected. Figure 1 suggests that *H. mediterranei* exhibits diauxic-like growth, supporting a change in metabolism from aerobic nitrite reduction (nitrite was used as nitrogen source for growth) [35,36] to anaerobic denitrification (nitrite was used as final electron acceptor) [29,34].

Taking into account that halophilic *Archaea* are the main population inhabiting most of the extreme saline ecosystems, these results also support the ability of Haloarchaea to sustain the N-cycle in extreme environments.

Nitrite and nitrate reductase assays in cultures with high nitrite concentrations. Nitrite and nitrate reductases activities were analyzed at different growth stages. The measurement of the enzymatic activities provided insight into the influence of high nitrite concentrations on the kinetics of assimilation and denitrification during growth of the cultures in greater detail than the limited nitrate and nitrite degradation profiles [22].

The specific nitrite and nitrate reductase activities during cell growth in media with different nitrite concentrations are shown in Table 1. At concentrations of 30 and 40 mM nitrite, the activity patterns were similar. During the first hours of incubation, enzyme activities were low, especially in the case of nitrate reductase in the cytoplasmic and membrane fractions (Nas, assimilatory nitrate reductase, and NarGH, respiratory nitrate reductase, respectively). When the cultures reached the mid-exponential phase, the nitrate and nitrite reductase activities of the crude extract increased significantly. At that stage, the cultures were growing aerobically, indicating that the assimilative pathway actively provided nitrogen for cell growth. Once the oxygen was completely consumed (data not shown), the activities of membrane nitrate reductase and nitrite reductase of the extracellular medium (NirK: respiratory nitrite reductase) increased, which coincided with the rapid consumption of NO_2^- and NO_3^- (Fig. 1A,B). This sequence of events suggested that both ions also act as terminal electron acceptors throughout the denitrification process, when assimilatory enzymatic activities are considerably lower due to inhibition of the assimilatory nitrate pathway under oxygen-scarce conditions [38]. Changes in enzymatic activities would account also for the diauxic pattern seen in the growth curves. This pattern is consistent with the presence of two substrates and two types of metabolisms (aerobic and then, at the end, anaerobic).

In medium containing 50 mM nitrite (Table 1), a few hours after culture inoculation, the nitrite and nitrate reductase activities of the cytoplasmic fraction reached very high values, unlike in the other media containing lower nitrite concentrations. The high-level activity occurred during exponential growth of the culture (Fig. 1C). Subsequently, growth stabilized and the activities of these enzymes decreased. The

Table 1. Specific nitrite and nitrate reductase activities* obtained during the growth of *Haloferax mediterranei* in salt medium (25 % SW) with: (A) 30 mM, (B) 40 mM, and (C) 50 mM nitrite

Time (h)	OD (600 nm)	Specific NiR activity.* Cytoplasmic fraction	Specific NaRGH activity.* Membrane	Specific Nas activity.* Cytoplasmic fraction	Specific NirK activity.* Extracellular medium
			(A) 30 mM		
24.0	0.1805	0.1855	0.1317	160.4	12.01
78.5	0.8519	1.1918	0.1345	567.5	12.33
122.0	1.2368	0.1152	2.3965	389.6	737.8
222.5	1.4644	0	0	0	0
			(B) 40 mM		
71.5	0.358	0.17653	0.1243	150.7	11.79
145.5	0.804	1.0734	0.1271	506.3	11.26
212.0	1.194	0.1021	2.1461	366.5	716.4
364.5	1.455	0	0	0	0
			(C) 50 mM		
45.0	0.4533	0.6126	0.1923	220.20	10.04
220.5	0.7564	0.3423	0.1934	150.33	10.47
408.5	0.8030	0.1601	1.2235	90.23	172.60
504.5	0.9138	0.1423	0.7843	52.41	114.00
648.0	0.9149	0	0	0	0

*Specific activity (mU/mg) values are reported as an average of at least three trials and are reproducible within 10 % error.

nitrate reductase activities of the membrane extract and medium remained almost constant until the rapid decrease in oxygen, as described for the other nitrite concentrations. Once growth re-stabilized (OD = 0.91), the activities were insignificant (Fig. 2).

In general, the highest activities were measured in the nitrite reductase assays. High concentrations of nitrite relative to nitrate may have a positive effect on the activities of the assimilative and respiratory nitrite reductases [29,35,36], which would explain the preferential consumption of nitrite (Fig. 1). In medium containing 30 mM nitrite, the highest enzymatic activities were detected, suggesting that this concentration is better tolerated by *H. mediterranei* and that higher concentration began to exert toxic effects on the cells, especially in the case of 50 mM nitrite.

Haloferax mediterranei is able to assimilate nitrate and nitrite in the presence of oxygen, due to the expression of two previously characterized enzymes: ferredoxin-dependent assimilatory nitrate reductase (Nas) and ferredoxin-dependent assimilatory nitrite reductase (NiR) [35,36]. Since both enzymes are found in the cytoplasm, their activities corresponded to those assayed in the cytoplasmic fractions, with the highest values determined in mid-exponential phase, when the cultures were growing aerobically. This agrees with the assimilative ability of this archaeon in its use of nitrite and nitrate as nitrogen sources for the production of biomolecules necessary for cell growth [6,34].

Under oxygen-limiting conditions, *H. mediterranei* uses nitrite or nitrate as electron acceptors in the respiratory chain in order to obtain energy [29,32]. The enzyme involved in the reduction of nitrate to nitrite is the respiratory nitrate reductase (NarGH) [7,32], and its activity was assayed on the membrane extracts. In the case of *H. mediterranei*, this membrane-bound enzyme has been purified as a heterodimeric protein composed of two subunits, NarG and NarH [29]. It has been suggested that the catalytic site is located on the outside of the membrane. In fact, the N-terminal region of NarG includes a typical twin-arginine signal

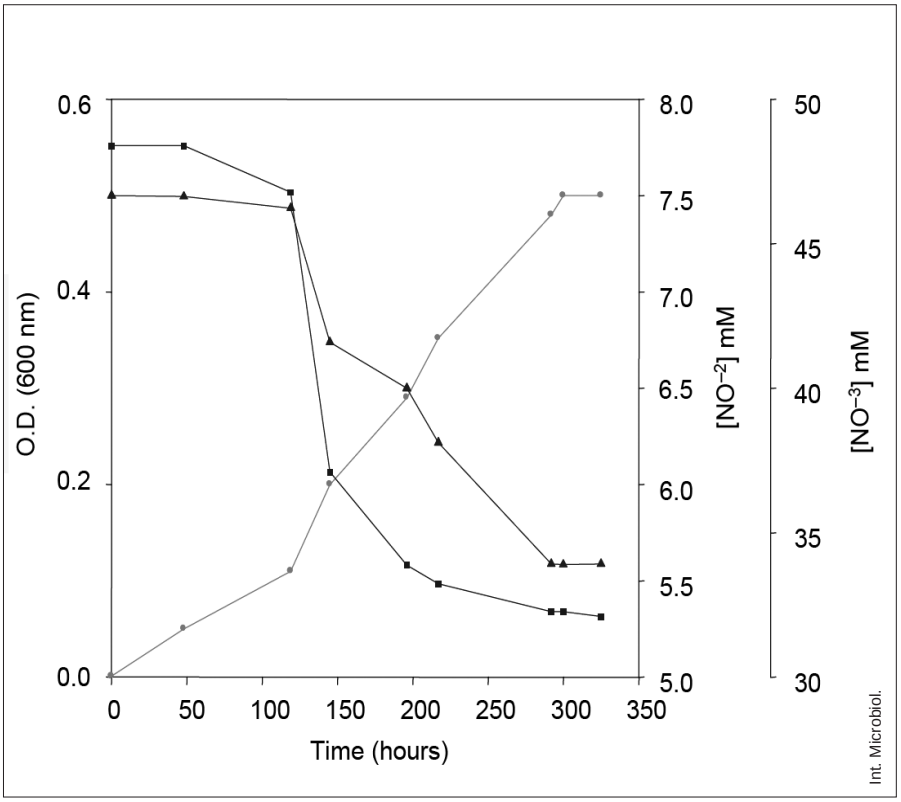

Fig. 2. Nitrite and nitrate degradation profiles during the growth of *Haloferax mediterranei* in a brine sample collected from a wastewater treatment plant. Optical density at 600 nm (circle), nitrite concentration within the medium (triangle), nitrate concentration within the medium (square). The average value of the error was ± 0.01 mM of absorbance units for nitrate and nitrite concentrations or optical density, respectively.

peptide for protein translocation across the membrane by the TAT export pathway (twin-arginine dependent translocase) and enzyme activity in situ was indeed obtained with both membrane permeable benzylviologen and membrane impermeable methylviologen [32].

Regarding respiratory nitrite reductases, two different enzymes have been described in *Bacteria*: the homotrimeric copper-containing enzyme (NirK), and the homodimeric cytochrome cd1-nitrite reductase (NirS) [13,41]. Recently, NirK from *H. mediterranei* was characterized and purified. Apparently, its N-terminal region also includes a twin-arginine signal peptide, suggesting that this protein is exported outside the cell, as shown by B. Zafrilla (work submited) and confirmed in this study through the detection of nitrite reductase activity in the culture medium. The external location of the catalytic site of the NarGH complex and the reduction of nitrite by NirK outside the cytoplasmic membrane make physiological sense, as nitrite accumulation and therefore its toxic effects are avoided as is the energy cost of an active nitrite-uptake system. In this set of experiments, NirK activities were highest when the consumption rate of nitrite and nitrate began to increase and were almost twice those of NiR. The denitrifying and possible detoxifying role of NirK might explain the ability of *H. mediterranei* to tolerate high nitrite levels. The nitrite-detoxifying role of NirK also has been described in *Rhizobium* species [12], *Nitrosomonas europaea* [4], and *Nitrobacter winogradskyi* [47].

Nitrite and nitrate removal from wastewater samples. Table 2 summarizes all the physicochemical parameters quantified from the samples collected from contaminated brines. The results revealed that all the brine samples were similar in terms of physicochemical composition. For this reason, all samples were mixed to be used as culture media for the incubation of *H. mediterranei* cells. While the salinity of the brines can be considered, in theory, too low to support the growth of a halophilic *Archaea*, the growth of *H. mediterranei* cells in the presence of low NaCl concentrations has been reported [20].

Figure 1C displays the growth curve as well as the nitrate and nitrite consumption profiles. The concentrations of both ions decreased throughout the incubation period, indicating their use as nitrogen sources for growth (assimilatory nitrate pathway) and as electron acceptors when oxygen was depleted (denitrification). Consequently, 60 % of the nitrate and 75 % of the nitrite initially present in the brines were removed. These results suggest that *H. mediterranei,* and in general, halophilic (moderate or extreme) Archaea, are able to carry

Table 2. Physicochemical characterization of brine samples. Data correspond to the average of a set of variables measured in nine samples collected from a wastewater treatment facility located in Vinalopó Valley, Alicante, Spain

Variable	Concentration (g/100 ml)
Sodium	2.23 ± 0.1
Calcium	0.23 ± 0.05
Magnesium	0.17 ± 0.03
Chlorides	2.36 ± 0.09
Sulphates	0.87 ± 0.06
Turbidity (NTU)	5.14 ± 0.1
Conductivity (mS/cm)	12.59 ± 1.3
pH	8.10 ± 0.2
Nitrites	0.35 ± 0.04
Nitrates	3.00 ± 0.08

out denitrification, thus can provide excellent models to explore large-scale bioremediation processes to remove nitrogen compounds from brines.

This study shows that high nitrite concentrations could be removed from brines and confirm the results of previous studies [19,32,33]. However, in two of those reports [32,33] the brines were prepared in vitro (supplemented with nutrients such as casamino acids or glucose) and were not collected from wastewater plant treatments, as was done in this work.

In our study, *H. mediterranei* was able to grow in the presence of high salt and nitrite concentrations. Moreover, in the majority of cases, the archaeon was able to remove most of the nitrite and nitrate present in the medium, especially after induction of the denitrification pathway. To develop large-scale bioremediation processes using denitrifying Haloarchaea, it would be adequate to start the process under anoxic conditions in order to optimize the removal of nitrogen compounds. Although the experiments in this study were done at 42 °C, previous studies have demonstrated that denitrification also occurs at lower temperatures, thus allowing this group of microorganisms to be used for bioremediation over a wide range of temperatures (25–42 °C). Nitrogen removal (mainly nitrate and nitrite) by *H. mediterranei* is therefore a promising approach to recover the quality (at least in terms of nitrogen concentrations and the nature of the nitrogen compounds) of brines, wastewater and salty soils.

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Competing interests. None declared.

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