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ORIGINAL PAPER

# Enhanced haloarchaeal oil removal in hypersaline environments via organic nitrogen fertilization and illumination

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**Abstract** Hypersaline soil and pond water samples were mixed with 3 % crude oil, some samples were autoclaved to serve as sterile controls; experimental samples were not sterilized. After 6-week incubation at 40 °C under light/dark cycles, the soil microflora consumed 66 %, and after 4 weeks the pond water microflora consumed 63 % of the crude oil. Soil samples treated with 3 % casaminoacids lost 89 % of their oil after 6 weeks and water samples lost 86 % after 4 weeks. Samples treated with casaminoacids and antibiotics that selectively inhibited bacteria, lost even more oil, up to 94 %. Soil–water mixtures incubated under continuous illumination lost double as much more oil than samples incubated in the dark. The soil–water mixture at time zero contained $1.3 \times 10^4$ CFU $g^{-1}$ of hydrocarbon-utilizing microorganisms which were affiliated to *Halomonas aquamarina, Exiguobacterium aurantiacum*, *Haloferax* sp., *Salinococcus* sp., *Marinococcus* sp. and *Halomonas* sp. After 6-week incubation with oil, these numbers were $8.7 \times 10^7$ CFU $g^{-1}$ and the *Haloferax* sp. proportion in the total microflora increased from 20 to 93 %. Experiments using the individual cultures and three other haloarchaea isolated earlier from the same site confirmed that casaminoacids and light enhanced their oil consumption potential in batch cultures.

**Keywords** Bioremediation · Crude oil · Haloarchaea · Hypersaline environments · Light · Nitrogen fertilization

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## Introduction

Like the nonextreme environments, hypersaline habitats are regularly exposed to pollution, including hydrocarbon pollution (Lefebvre and Moletta 2006). Conventional "nonextremophilic" hydrocarbon-utilizing microorganisms (for reviews see Rehm and Reiff 1981; Radwan and Sorkhoh 1993; Van Hamme et al. 2003; Rosenberg 2006; Radwan 2009) are obviously not suitable for bioremediation of oily hypersaline environments (Pieper and Reineke 2000; Oren 2002). In the latter environments only extreme halophilic microorganisms, including haloarchaea could be effective.

Within the past 20 years, some important papers have been published on hydrocarbon-utilizing microorganisms in saline and hypersaline environments (Kulichevskaya et al. 1992; Oren et al. 1992; Emerson et al. 1994; Daane et al. 2001; Margesin and Schinner 2001; Garcia et al. 2004; Nicholson and Fathepure 2005; Al-Awadhi et al. 2007; Zhao et al. 2009; Al-Mailem et al. 2010a, b; Gayathri and Vasudevan 2010; Tapilatu et al. 2010; Zhuang et al. 2010; Bonfa et al. 2011; Ghevariya et al. 2011). The hydrocarbons comprised aliphatics and aromatics, and the microorganisms included bacteria, actinomycetes and archaea. There is however, some variations in the literature reports regarding the relation between hydrocarbon biodegradation and salinity. Kerr and Capone (1988) found that salinity did not influence microbial hydrocarbon-biodegradation, whereas Diaz et al. (2000) and Yang et al. (2000) reported that this activity was enhanced by salinity increase. Riis et al. (2003) and Kleinsteuber et al. (2006) reported the adaptation of hydrocarbon-utilizing microbial consortia to high salinity. These variations may, at least in part, be due to the complexity and variability of the microbial consortia involved in hydrocarbon-biodegradation.

In an earlier study (Al-Mailem et al. 2010a), our group reported on four haloarchaeal strains from a hypersaline (about 4 M NaCl) coastal area of the Arabian Gulf which optimally degraded aliphatic and aromatic hydrocarbons in batch cultures containing 3 M NaCl. In another paper (Al-Mailem et al. 2010b), we studied and demonstrated the potential of this hypersaline area for oil phytoremediation using halophilic microorganisms, including haloarchaea. The results of these studies encouraged us to do the current work on the bench-scale hydrocarbon-bioremediation of hypersaline soil and water samples from this area as affected by two environmental parameters; organic nitrogen fertilization and illumination. In preliminary experiments, these factors revealed to have dramatic effects on the oil-bioremediation efficiency.

## Materials and methods

### Sampling

Hypersaline soil, with white salt crusts and red pond water samples were collected in sterile containers in June (2011) from the supertidal "sabkha" coastal area at Al-Khiran, south of Kuwait. The samples were transported to the laboratory (8 °C) and processed the same day. Description of this hypersaline area and the prevailing environmental parameters was done earlier (Al-Mailem et al. 2010a).

### Bench-scale oil-bioremediation

In these bench-scale experiments, the effects of organic nitrogen fertilization and illumination on hydrocarbon-bioremediation were studied. Antibiotics which selectively inhibit bacteria, but not haloarchaea (Al-Mailem et al. 2010a) were added in some treatments to inhibit the halophilic bacteria. To create more specific conditions for the haloarchaea, the incubation temperature was kept at 40 °C. Confirming earlier reports on the rather high temperature requirement of haloarchaea (Lizama et al. 2001; Fab et al. 2008), we found earlier that the haloarchaea used in this study grew best at 40–45 °C (Al-Mailem et al. 2010b).

To study the effect of casaminoacids, freshly collected soil samples containing 3.9 M NaCl were artificially polluted with 3 % (w/w) crude light Kuwaiti oil and mixed mechanically for 3 h. The oily soil was divided into 1 kg piles, some of which were sterilized by autoclaving and used as a sterile control. The other experimental piles were treated differently, some piles were left as such, as non-sterile controls. Other non-autoclaved piles were provided with 3 % casaminoacids (Fluka, USA), and still others were provided with casaminoacids and a mixture of the antibiotics chloramphenicol, cyclohexamide, nalidixic acid, penicillin, streptomycin, tetracycline, 50 mg $Kg^{-1}$, each. After mechanical mixing for another hour, the piles were incubated at 40 °C under 14 h illumination, 81 μmole $m^{-2}$ $s^{-1}$/10 h dark cycles (simulating field conditions).

Freshly collected pond water samples (2.8 M NaCl) were dispensed into sterile 500 ml conical flasks, each receiving 100 ml. The water samples were artificially polluted with 3 % oil. Controls were prepared as described above and some experimental flasks were also provided with casaminoacids and antibiotics. Incubation was at 40 °C under light/dark cycles as described above.

At time zero and in 2-week interval, three 100 g soil samples collected at random from the soil piles, and three replicate water flasks were taken for residual oil recovery and determination, as described below.

To study the effect of light intensity on oil-bioremediation in water–soil mixtures, 5 g aliquots of freshly collected soil were dispensed in sterile conical flasks containing 100 ml aliquots of freshly collected water. Each flask received in addition 3 % crude oil and 0.2 % casaminoacids. The flasks were sealed and some were sterilized by autoclaving to serve as sterile controls, whereas others were coated with 5 layers of aluminum foil, to serve as dark-incubated controls. Still other flasks were subjected to the light intensities of 18, 39 and 81 μmole $m^{-2}$ $s^{-1}$. Triplicates were prepared throughout, and the flasks were incubated at 40 °C for 3 weeks. Residual hydrocarbons were recovered and quantitatively determined as described below.

### Hydrocarbon attenuation by pure cultures

The cultivable halophilic oil-utilizing microorganisms in a hypersaline soil–water mixture were counted, isolated, purified, characterized by 16S rDNA sequencing, and the effects of casaminoacids and illumination on their oil-consumption potential studied. This experiment comprised also three oil-utilizing haloarchaea that were isolated from the same sampling site and identified earlier (Al-Mailem et al. 2010a).

The conventional dilution-plating method was used for microbial counting. Ten g fresh soil was suspended in 90 ml fresh pond water and the suspension shaken at 170 rpm for 15 min to release soil adhering bacteria. Dilution (1:10, v/v) series were prepared using sterile pond water. Dilution aliquots, 0.25 ml, were plated, and the mineral medium used (Mevarech and Werczberger 1985) had the following composition (g $l^{-1}$): 240 NaCl; 30 $MgCl_2 \cdot 6H_2O$; 35 $MgSO_4 \cdot 7H_2O$; 7KCl; 2 $NH_4NO_3$; 20 g agar; pH 7.0. Oil vapor volatilizing from 3 ml oil impregnated filter papers fixed in the Petri-dish lids was the only carbon and energy source available to the

microorganisms. Dishes were sealed with cello tape and incubated at 40 °C for 3 weeks. Three parallel plates were prepared for every dilution. The colony forming units (CFU) were counted, purified and representatives characterized by sequencing their 16S rRNA coding genes.

The GenElute Bacterial Genomic DNA Miniprep Kit (Sigma-Aldrich, St. Louis, MO, USA) was used to extract total genomic DNA and the 16S rRNA genes were amplified by polymerase chain reaction (PCR) using the primers 0018F (5′-ATTCCGGTTGAGCC TGCC) and 1518R (5′-AGGAGGTGAGCCAGCCGC). The PCR products were purified using the QIA quick PCR purification kit (Qiagen, Valencia, CA, USA) in order to remove the Taq polymerase, primers, and dNTPs that might interrupt the sequencing procedure. Partial sequences of the 16S rRNA encoding genes were obtained by applying the BigDye version 3.1 Terminator Kit (Applied Biosystems, Warrington, UK); 20 ng of the DNA template was added to 8 μL of BigDye version 3.1 terminator; 1 μL of the primers was added to the mixture and the final volume was brought up to 20 μL with sterile molecular water. The vials were incubated in a GeneAmp PCR system 9700 thermocycler (Applied Biosystems, Foster City, CA, USA). The standard PCR program was conducted; it consisted of 1 cycle of denaturation for 5 min at 94 °C, annealing at 65 °C for 1 min, followed by 25 cycles with denaturation temperature at 55 °C for 30 s and extension step at 72 °C for 30 s followed by 7 min at 72 °C. Purification was done by adding 2 μl of 3 M sodium acetate pH 5.2 and 50 μl of 100 % ethanol to each sample followed by incubation for 20 min at room temperature before centrifugation for 20 min at 13,000 relative centrifugal force (RCF). The pellet was washed with 50 μl of 70 % ethanol and recentrifuged at room temperature for 5 min at 13,000 RCF. The pellet was left to dry in the hood for 10 min in the dark and 20 μl Hi-Di formamide (Applied Biosystems, Foster City, CA, USA) was added to each sample prior to the denaturing step, where samples were incubated in a thermocycler for 2 min at 94–95 °C before being loaded in the 3130*xl* genetic analyzer (Applied Biosystems, Foster City, CA, USA). Sequencing analysis version 5.2 software (Applied Biosystems, Foster City, CA, USA) was used to analyze the results. Sequences were subjected to basic local alignment search tool analysis with the National Center for Biotechnology Information (NCBI, Bethesda, MD, USA) GenBank database (Altschul et al., 1997). The sequences were deposited in the GenBank database under the accession numbers in Table 1. For further confirmation of the strain identities, the sequences were compared with actual type strain sequences accessible through the "Ribosomal Database Project".

To study the effect of casmino acids on the potential of individual pure cultures for crude oil attenuation, 50 ml aliquots of the above mineral medium Mevarech and Werczberger (1985) containing 2 % crude oil were dispensed in 250 ml conical flasks. A similar set of flasks containing in addition 0.2 % casaminoacids was prepared. Each flask was inoculated with 1 ml of cell suspension, 1 loopful of 48 h microbial biomass in 10 ml sterile 3 M NaCl solution. Three replicates were prepared throughout. The sealed flasks were incubated at 40 °C in the dark for 3 weeks.

To study the effect of light, flasks containing the mineral medium, crude oil and casaminoacids were prepared and inoculated as described above. The flasks of one set were coated, each with 5 layers of aluminum foil (dark incubated), and the others were left uncoated (light incubated). Three replicates were prepared for each treatment, and the flasks were incubated at 40 °C and continuous illumination, 81 μmole $m^{-2}$ $s^{-1}$, for 3 weeks.

## Determination of hydrocarbons and pigments

The residual hydrocarbons in all studied samples were recovered after 3-week incubation by three successive 25 ml aliquots of pentane. The combined extract was completed to 75 ml and 1 μl was analyzed by gas–liquid chromatography (GLC), using a Chrompack (Chrompack, Middelburg, The Netherlands) CP-9000 instrument equipped with a FID, a WCOT-fused silica CP-SIL-5CB capillary column, 15 m × 0.25 mm, and temperature program

**Table 1** The 16S rDNA sequencing of microorganisms from hypersaline soil–water mixture

| Micro-organism codes | % of the total | Subdivision | Nearest GenBank match | Bases compared | % similarity | GenBank accession numbers |
|---|---|---|---|---|---|---|
| OA1 | 24 | Gammaproteobacteria | *Halomonas aquamarina* | 515 | 99 | JQ350847 |
| OA2 | 22 | Firmicutes | *Exiguobacterium aurantiacum* | 510 | 98 | JQ350848 |
| OA3 | 20 | Halobacteria | *Haloferax* sp. | 494 | 97 | JQ350849 |
| OA4 | 14 | Firmicutes | *Salinicoccus* sp. | 520 | 99 | JQ350850 |
| OA5 | 11 | Firmicutes | *Marinococcus* sp. | 520 | 99 | JQ350851 |
| OA6 | 9 | Gammaproteobacteria | *Halomonas* sp. | 498 | 97 | JQ350852 |

which raised the temperature from 45 to 310 °C at 10 °C $min^{-1}$. The peak areas of residual hydrocarbons were compared to the areas of the autoclaved (=time zero) control peaks enabling the calculation of decrease percentages.

For the determination of pigments, 100 ml aliquots of 3-week liquid cultures of haloarchaea grown at different salinities were extracted with acetone, methanol and hexane, and the pigments determined spectrophotometrically (UV–Vis-Scanning Spectrophotometer, Japan) by measuring the absorbance at 490 nm (Khanafari et al. 2010). The pigment concentrations were calculated according to Calo et al. (1995).

## Results and discussion

The typical GLC profiles in Fig. 1 show that, without any treatment (rows I), the indigenous soil and water microflora brought about considerable attenuation of the oil hydrocarbons. Yet, after 6 and 4 weeks, soil and water still contained, 34 ± 2 and 63 ± 1 %, respectively, of the time zero hydrocarbons (calculation based on total peak area reduction). Treatment with casaominoacids (rows II) resulted in 89 ± 3 % removal of oil in soil after 6 weeks and 86 ± 4 % removal in water after only 4 weeks. Surprisingly, the treatment with antibiotics in addition (rows III) enhanced the hydrocarbon removal even more, also in early phases of bioremediation, namely after 2 and 4 weeks. In preliminary experiments, we confirmed earlier results that the antibiotics used in this study inhibit the bacteria listed in Table 1 but not archaea (Denner et al. 1994; Grant et al. 2001; Stan-Lotter et al. 2002) including the haloarchaea we isolated earlier (Al-Mailem et al. 2010a, b). This implies that the hydrocarbon attenuation activities are exclusively, or mainly due to haloarchaeal partners, with little or no contribution of bacteria. The selective conditions prevailing during this experiment, namely the high salinity, relatively high temperature and the presence of antibiotics consolidate that mainly haloarchaea contributed to hydrocarbon consumption. The GLC profiles of hydrocarbons recovered at the ends of the incubation periods from previously autoclaved samples were similar to those recovered at time zero, indicating that no hydrocarbons were consumed in sterile controls.

An interesting side result of this experiment is that bioremediation occurred in water more effectively than in soil. It took aquatic microorganisms only 4 weeks to remove hydrocarbons almost completely; the soil microorganisms needed more, namely 6 weeks. This is probably because the hydrocarbon substrates are more available to the microorganisms in water than in soil. The fact that the antibiotic addition promoted the hydrocarbon attenuation in casaminoacid-treated media may be due to their role in inhibiting bacteria that might have competed with the archaea, especially for molecular oxygen (see relevant interpretation below).

The GLC profiles in Fig. 2 show how light affected the efficiency of bench-scale bioremediation of hydrocarbons in a hypersaline soil–water mixture. It is obvious that the hydrocarbon consumption increased with increasing light intensity. Calculations based on the total peak areas revealed that, in dark incubated cultures, only 13 ± 1 % of the available hydrocarbons was consumed. The consumption values increased to 24 ± 1, 35 ± 2 and 40 ± 2.0 % under the illumination intensities of 18, 39 and 81 μmole $m^{-2}$ $s^{-1}$, respectively. The GLC profiles of hydrocarbons recovered after 3 weeks from previously autoclaved controls were similar to those recovered at time zero, indicating that no hydrocarbons were consumed.

No publications related to the effect of light on hydrocarbon attenuation by microorganisms were found in the available literature. The only heterotrophic microorganisms known to benefit metabolically from light energy are the red pigmented archaea. Hypersaline environments suffer from oxygen lack due to the limited oxygen solubility at high salt concentrations. We showed earlier that the dissolved oxygen content in the studied soil and water may fall down to only 2.1 mg $Kg^{-1}$ (Al-Mailem et al. 2010a). El-Sayed et al. (2002) found that low oxygen tension under illumination relatively enhanced red pigmentation in *Halobacterium salinarum*. Haloarchaea in such environments would use the red pigment-mediated ATP synthesis to meet the shortage of ATP produced via oxidative phosphorylation caused by low oxygen tension. In this way, these organisms can save the little oxygen still available for the initial step of attack on the hydrocarbon substrates, which needs molecular oxygen (Klug and Markovetz 1971; Rehm and Reiff 1981). This may explain why light enhanced hydrocarbon consumption, and imply that only or mainly archaea are involved in this consumption. This speculative interpretation is supported by several facts. The water sampling sites and archaeal colonies on agar medium subjected to 81 μmole $m^{-2}$ $s^{-1}$ light were red pigmented. The red color of hypersaline ponds, although reportedly is mainly due to haloarchaea (and sometimes to *Dunaliella* cells as well), may also arise, albeit to a minor extent, from halophilic bacteria e.g. *Salinibacter* (Oren and Rodriguez-Valera 2001). Furthermore, the hydrocarbon consumption was not inhibited, but it was even enhanced by the antibiotic treatment, at the rather high temperature of 40 °C (Fig. 1).

The above speculation received more support and consolidation by the results gained on pure cultures from the hypersaline soil–water environment. The untreated

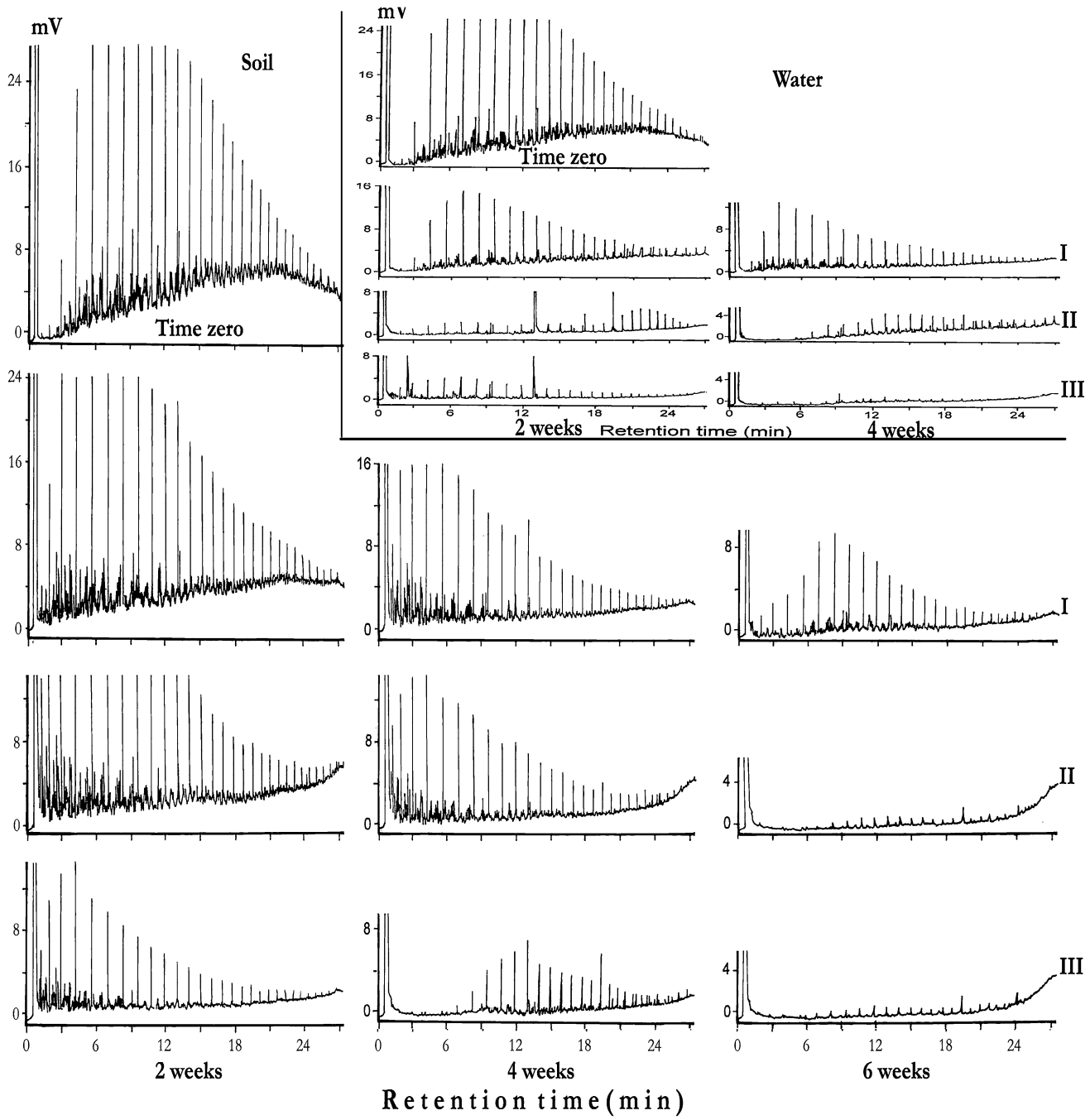


**Fig. 1** Typical GLC profiles of residual oil hydrocarbons recovered from hypersaline soil and water samples treated with casaminoacids alone and together with antibiotics. Soil and water samples were taken for analysis at time zero (controls) and every 2 weeks. Smaller peaks in the profiles mean more hydrocarbon attenuation. *I* Untreated soil/water. *II* Soil/water treated with casaminoacids alone. *III* Soil/water treated with casaminoacids and antibiotics

soil–water mixture contained $1.3 \times 10^4$ CFU $g^{-1}$ of cultivable hydrocarbon-utilizing microorganisms. These organisms were affiliated to 6 different species (Table 1) belonging to the subdivisions Firmicutes (the most dominant), Gammaproteobacteria and Haloarchaea. The latter subdivision, which was represented by *Haloferax* sp., made about one-fifth of the numbers of the cultivable hydrocarbon-utilizing microflora. Incubation with crude oil and antibiotics at 40 °C for 6 weeks raised the total number to $8.7 \times 10^7$ CFU $g^{-1}$, and the red pigmented haloarchaeal colonies made up 93 % of the total (Fig. 3b). This result is another confirmation of the speculation that haloarchaea are the major contributors to hydrocarbon attenuation in the studied hypersaline environments. However, should the

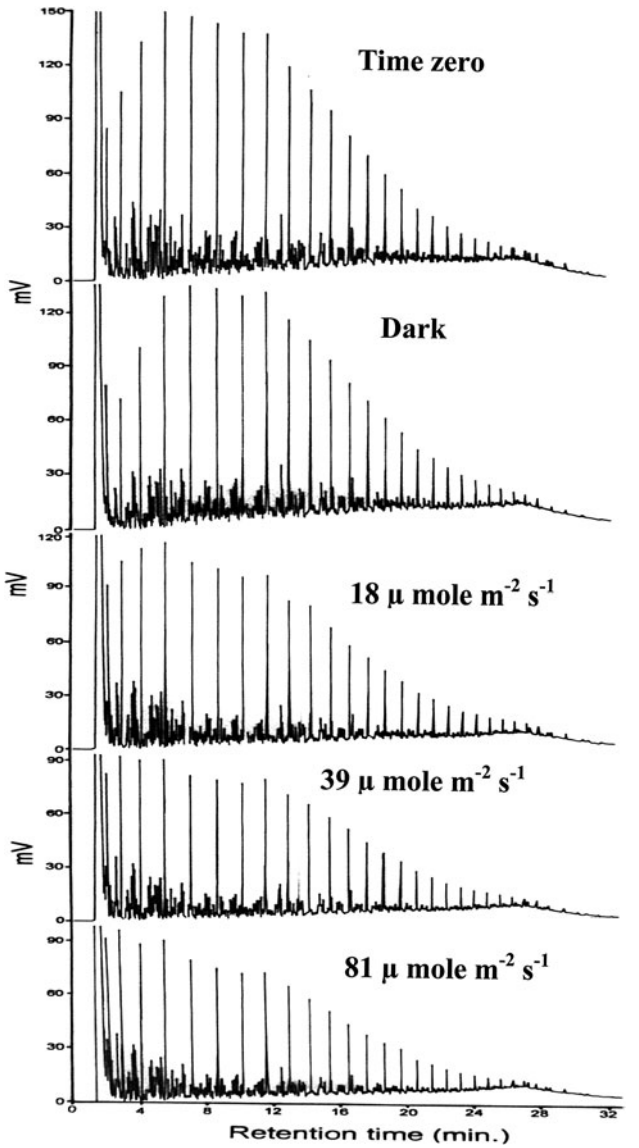


**Fig. 2** Effect of light intensities on crude oil consumption in fresh hypersaline soil–water mixtures. Incubation was for 3 weeks at 40 °C. Smaller peaks in the profiles mean more hydrocarbon attenuation

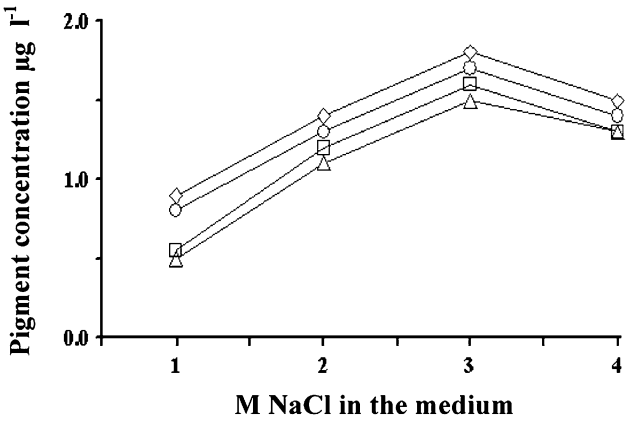


**Fig. 3** Effect of salinity on total pigment contents of individual 3-week haloarchaeal cultures. *Diamonds*, *Haloferax* sp. (isolated earlier); *Circles, Haloferax* sp. (isolated in this study); *Triangles*, *Halococcus* sp. (isolated earlier); *Squares*, *Halobacterium* sp. (isolated earlier)

salinity fall down in situ, e.g. after precipitation, the halophilic bacterial species may start to contribute more to bioremediation.

Results of experiments on individual pure isolates also support the assumption that haloarchaea in hypersaline, oxygen-poor environments may depend more on their pigments in ATP synthesis, thus saving the limited amount of oxygen for hydrocarbon oxidation. Firstly, confirming earlier results (Rodriguez-Valera et al. 1980), Fig. 3 shows that the pigment contents of the liquid cultures of the individual haloarchaea *Haloferax* sp. (isolated in this study) as well as *Haloferax* sp., *Halobacterium* sp. and *Halococcus* sp. (isolated earlier from the same site, Al-Mailem et al. 2010a), increased with increasing salt content of the medium. The highest pigment concentration was measured in the presence of 3 M NaCl. The pigment contents reflect the growth intensities of the haloarchaeal strains.

Secondly, the results in Table 2 show that pure archaeal isolates brought about more crude oil consumption under continuous illumination, 80 µmole $m^{-2}$ $s^{-1}$, than in the dark. There are no earlier reports in the literature directly related to this new finding which is quite interesting, not only from the basic but also from the practical points of view.

The need of the "Halobacteriacea" (Haloarchea) for complex nitrogenous compounds (Gibbons 1974) was the basis for studying the effect of casaminoacids on the hydrocarbon-consumption potential of the pure haloarchaeal isolates. For comparison, the pure halophilic bacteria isolated from the same site were studied. In this experiment, the media used already contained in addition inorganic nitrogen (0.2 % $NH_4NO_3$) enough for the consumption of the crude oil available (see the "Methodology" part). The N:C ratio for microbial growth is known to be about 1:25. The results in Table 3 show that, in the presence of casaminoacids, almost double as much oil was consumed by the individual organisms more than in the absence of this substance.

The lack of similar results in the literature makes the interpretation of this interesting and practically important finding difficult. However, the steadily increasing information in the field of nitrogen metabolism in haloarchaea (for reviews see Cabello et al. 2004; Bonete et al. 2008) may be relevant and useful. The question is why is the hydrocarbon consumption by haloarchaea enhanced by casaminoacids, although enough $NH_4NO_3$ was available;

**Table 2** Effects of light and salinity on crude oil consumption by extreme halophilic pure isolates

| Pure isolates | % of crude oil consumed | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 M NaCl | | 2 M NaCl | | 3 M NaCl | | 4 M NaCl | |
| | Dark | Light | Dark | Light | Dark | Light | Dark | Light |
| Haloarchaea[a] | | | | | | | | |
| *Haloferax* sp.[a] | 33 ± 2 | 47 ± 2 | 38 ± 2 | 51 ± 2 | 40 ± 2 | 53 ± 3 | 42 ± 2 | 56 ± 3 |
| *Halobacterium* sp.[a] | 27 ± 1 | 46 ± 2 | 31 ± 2 | 49 ± 2 | 34 ± 2 | 54 ± 3 | 37 ± 2 | 48 ± 2 |
| *Haloccoccus* sp.[a] | 24 ± 1 | 46 ± 2 | 39 ± 2 | 48 ± 2 | 44 ± 2 | 56 ± 3 | 49 ± 2 | 59 ± 3 |
| *Haloferax* sp.[b] | 25 ± 1 | 33 ± 1 | 41 ± 2 | 51 ± 2 | 43 ± 2 | 53 ± 2 | 39 ± 2 | 46 ± 2 |

Casaminoacids were added into the media throughout

Values are mean of 3 replicates ± standard deviation

[a] Isolated earlier (Al-Mailem et al. 2010a) from the same site

[b] Isolated in the current study

**Table 3** Effect of casaminoacids on crude oil consumption by extreme halophilic pure isolates

| Pure isolates | % Crude oil consumed | |
|---|---|---|
| | Without casaminoacids | With casaminoacids |
| Haloarchaea[a] | | |
| *Haloferax* sp.[a] | 16 ± 1 | 34 ± 1 |
| *Halobacterium* sp.[a] | 23 ± 1 | 41 ± 2 |
| *Haloccoccus* sp.[a] | 26 ± 1 | 49 ± 3 |
| *Haloferax* sp.[b] | 19 ± 1 | 32 ± 2 |
| Bacteria[b] | | |
| *Halomonas aquamarina* | 19 ± 1 | 37 ± 1 |
| *Exiguobacterium aurantiacum* | 17 ± 1 | 31 ± 1 |
| *Salinicoccus* sp. | 11 ± 1 | 28 ± 1 |
| *Marinococcus* sp. | 11 ± 1 | 24 ± 1 |
| *Halomonas* sp. | 16 ± 1 | 23 ± 1 |

Values are mean of 3 replicates ± standard deviation

[a] Isolated earlier (Al-Mailem et al. 2010a) from the same site

[b] Isolated in the current study

also nitrates were reported to be utilized by archaea (Bonete et al. 2008). In this context, the hydrocarbon-consumption potential of seawater bacteria (moderate halophiles) was enhanced by the addition of glucose and peptone (Radwan et al. 2000). The reason may again be the relative anaerobic conditions associated with high salinity leading to enhanced nitrate removal by denitrification. Literature reports show that archaea (Abell et al. 2010) including haloarchaea (Yoshimatsu et al. 2000) are active in denitrification (for review see Philippot 2002). In other words, the extreme halophilic microorganisms seem to need more nitrogen in their media than nonhalophilic microorganisms. This may also explain why the addition of casaminoacids enhanced the crude oil consumption. Furthermore, it should not be unusual for microbial cells to grow better in media containing amino acids compared to cells grown in media with nitrates. Energy to synthesize amino acids from nitrates is saved.

In conclusion, whatever the biochemical and physiological basis for the enhancing effect of light and casaminoacids on hydrocarbon consumption could be, this original result is obviously important from the academic and practical points of view. The selective conditions used in the current experiments suggest that mainly haloarchaea drove these bioremediation activities.

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