

**Contributors:** J.D.L. van Bleijswijk, G. Muyzer

A role of microbial mats in the biodegradation of oil in the Arabian Sea was suggested when about one year after the massive oil spills in the Gulf war of 1991, cyanobacterial mats developed on top of the oil. Yet, oxygen producing phototrophs (cyanobacteria and algae) are generally believed to play a major role in the breakdown of oil. Probably they do not themselves breakdown the oil components on a large scale but they create favourable conditions for oil degradation by bacteria, because they influence oxygen and nutrient concentrations, pH and the physical characteristics of the mats.

Detailed studies on the biodiversity of microorganisms in marine mats were still lacking. Even less was known about the oil tolerance of individual species. NIOZ provided data on species richness and species composition of cyanobacteria and algae that were present in selected microbial mats exposed to different levels of oil pollution. The research is part of the European project on the Role of Microbial Mats in Bioremediation of Hydrocarbon Polluted Coastal Zones (MATBIOPOL). In this integrated program, studies on microbial diversity and activities in response to oil pollution are combined with studies on behavior and degradation of hydrocarbon molecules in microbial mat systems. Knowledge of the biodiversity of microorganisms in mats and identification of oil sensitive, oil tolerant and oil loving species and communities may help to select effective strategies for the bioremediation of oil pollution in coastal zones.

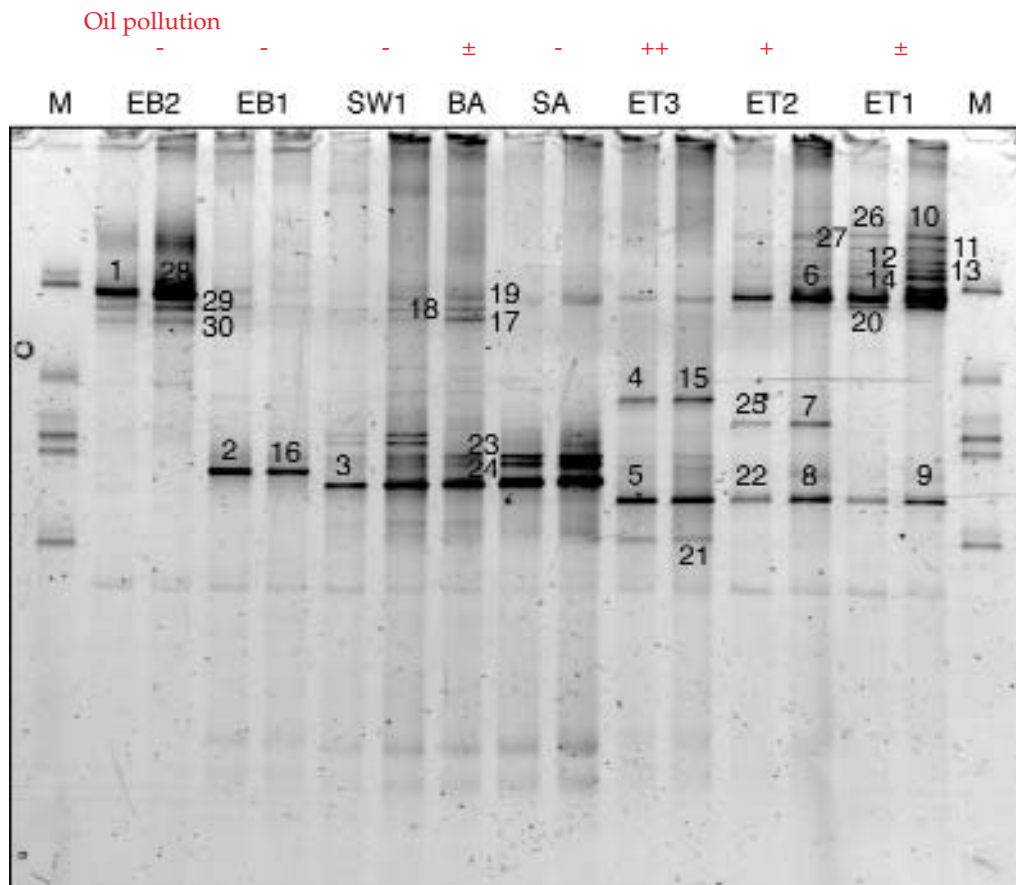
A DNA fingerprint was determined of nine selected microbial mat communities that were exposed to different levels of oil pollution. PCR-DGGE-Sequencing analysis of 16S rRNA gene fragments revealed information on the species richness of the community and on the identity of the organisms that were present in the samples (see figures).

The DGGE banding patterns were unique for every site we sampled, reflecting differences in environmental parameters on a large scale (different locations) and on a smaller scale (different sites on the same location). By focussing on the mats in Etang de Berre (ET1, ET2, ET3), which were subjected to the same climatic conditions (temperature, light intensity) and only differed in the degree of oil pollution, we can observe trends that are related to oil pollution.

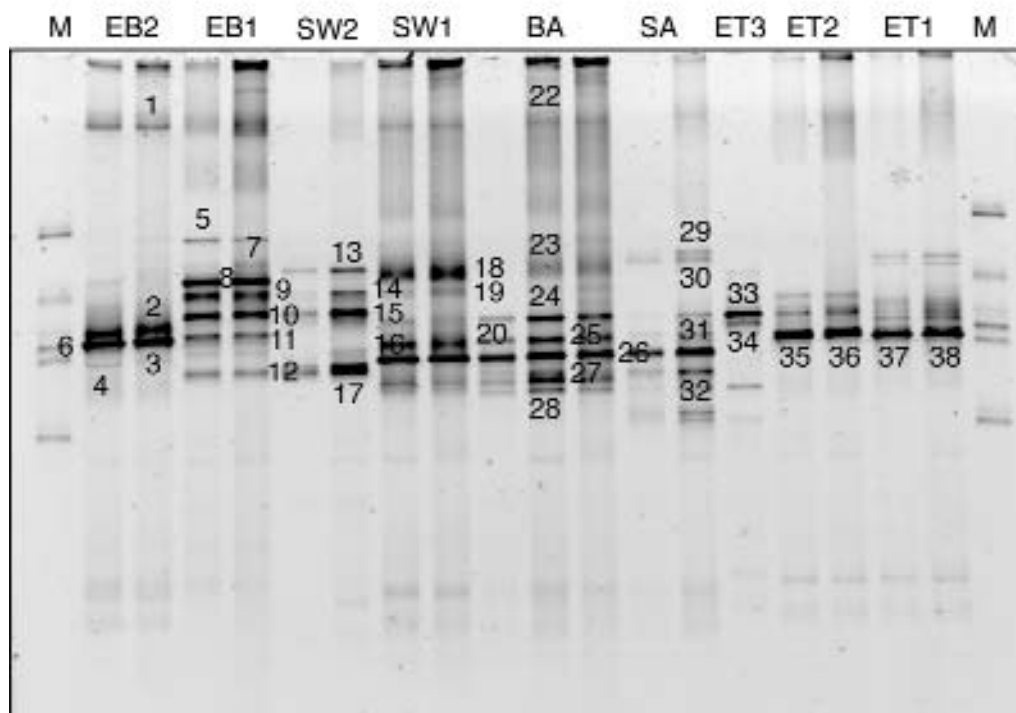
Diatom algae (e.g. *Skeletonema sp.*) were abundant in the slightly polluted mat, present but not abundant in the medium polluted mat and absent in the heavily polluted mat. The cyanobacteria *Phormidium sp.* and *Planktotrix sp.* were present in slightly and medium polluted mats but could not persist in the highly polluted mat. The only oxygenic phototrophs that were able to cope with the heavy oil pollution were affiliated to the cyanobacterium *Oscillatoria sp.*, and to the Prymnesiophyte alga *Isochrysis sp.* From culture work it is known that *Isochrysis galbana* is able to take up naphthalene. There were no field data of Prymnesiophyte algae as indigenous parts of microbial mat communities. According to the common idea, these pelagic algae bloom in the overlaying waters and reach the microbial mat surface due to sinking. However, a study of lipid biomarkers in microbial mats by MATBIOPOL colleagues did indicate the presence of indigenic alkenone containing algae, such as Prymnesiophyceae, but efforts to isolate these organisms from mats have not been successful.

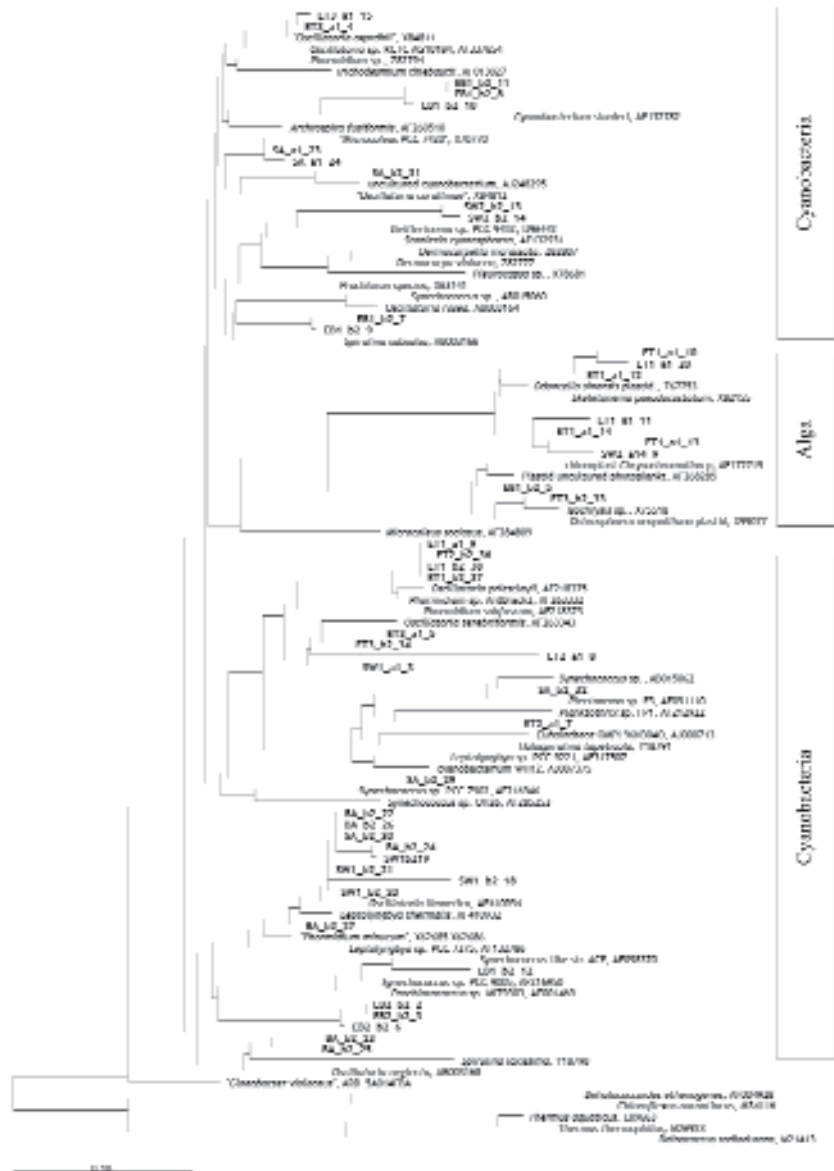
### Conclusions

Oil pollution affected the species composition of cyanobacteria and algae in microbial mats: Diatom algae (e.g. *Skeletonema sp.*) were abundant in the slightly polluted mat, present but not abundant in the medium polluted mat and absent in the heavily polluted mat. The cyanobacteria *Phormidium sp.* and *Planktotrix sp.* were present in slightly and medium polluted mats but could not persist in the highly polluted mat. The organisms that were able to cope with the heavy oil pollution were affiliated to the cyanobacterium *Oscillatoria sp.*, and to the Prymnesiophyte alga *Isochrysis sp.*



DNA fingerprint of cyanobacteria and algae in samples of microbial mats exposed to different levels of oil pollution (see top of figure: - not polluted; ± slightly polluted; + medium polluted; ++ heavily polluted). Samples: M=Marker; EB=EbroDelta, Spain; SW=SwanbisterBay, Orkneys; BA=Bay of Slick, Orkneys; SA=Saline de Giraud, Camarque; ET=Etang de Berre, Camarque. Two types of DNA fragments were analysed: A and B, as shown in the corresponding figures. Of every mat, two samples of double stranded DNA fragments were loaded on top of a gel and were forced by an electrical field to migrate downwards in an increasing gradient of urea/formamide. Bands indicate specific DNA fragments: different species in the sample show up as different bands in the figure. The blackness of the bands roughly corresponds to the quantity of the species present. The numbers in the figure refer to bands that were cut out the gel, sequenced and analysed. These numbers return in the figure of the phylogenetic tree.





Identification of unknown DNA sequences by comparison with known sequences from a global database. In the phylogenetic tree the length of the horizontal lines between two entries relates to sequence differences (scalebar = 10% difference): The position of an unknown sequence in the tree indicates the most similar species: e.g. on top of the tree: sequence ET3\_a1\_4 is nearly similar to the sequence of *Oscillatoria agardhii*. Sequences determined in our study are printed bold. Abbreviations refer to sample site and band number as presented in DNA fingerprint figures A and B (e.g. ET3\_a1\_4 is Etang de Berre site 3, figure A, band 4).