Microbiological investigation of the surface water of the Laguna Veneta (North Italy) in relation to light gas-oil biodegradation

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Pollution by hydrocarbons is a serious problem in the Laguna Veneta (North East Italy) because one of the busiest oil port in Italy is located in this area; also, the lagoon receives polluted waters from an heavily industrialized and populated hinterland (FOSSATO, 1990).

Microbial degradation of hydrocarbons, universally recognized as a natural remediation, is the most important biological mechanism in oil decontamination of marine waters.

The aim of this study has been: 1) to assess the distribution of oil-degrading microorganisms in the lagoon; 2) to evaluate the oil degradative potential of surface waters; 3) to isolate strains with efficient oil degradative activity and 4) to test the hydrocarbon-degrading activity of axenic cultures in laboratory condition.

Surface waters from three different stations from the central area of the lagoon (A,B,C, from outer to inner part) were sampled aseptically in November 1990 and in June 1991 with a Schomaker sampler.

Basic physical and chemical parameters were determined in each station and in each period.

Enumeration of total aerobic heterotrophic bacteria was carried out by direct epifluorescence method (HOBBIE *et al.*, 1977). Oil degrading bacteria were determined by the MPN technique (MILLS *et al.*, 1978) and by the Spread Plate Technique. To isolate oil degrading strains, colonies which were different for colour, size and morphology were streaked twice and stored at -80°C in 25 % glycerol.

The microbial activity was determined by oxygen consumption in presence of hydrocarbons. Water samples from each station with and without 0.2 % of light gas-oil were incubated at 22°C. Oxygen consumption was determined by Winkler's method after 7 and 14 days in November 1990, and after 5, 10 and 15 days in June 1991.

To determine the oil degrading activity, at each station 8 flasks with 200 ml of water were spiked with 0.2 % of light gas-oil. Four flasks were sterilized before this addition. The percentage of oil consumed after 5, 10, 15 and 30 days of incubation at 22°C was determined by FIP gas chromatography, after extraction with n-hexane.

To test the oil degrading activity of axenic cultures, the strains best growing on plates were cultured in flasks until stationary phase, then the consumption of gasoil was determined by gas chromatography. The microbial activity was also tested by oxygen consumption measured by Clark's probe.

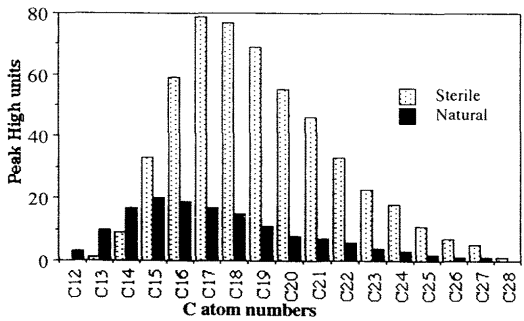
The concentration of heterotrophic and oil degrading bacteria was higher in June than in November. A higher percentage of oil degrading bacteria has been also found in June, probably due to better temperature conditions (ATLAS and BARTHA, 1972). GALASSI and CANZONIER (1977) also found a higher hydrocarbon degrading activity in the Southern part of the Laguna Veneta in warmer season.

The percentage of oil degrading bacteria, considered as a biological index of hydrocarbon pollution (WALKER and COLWELL, 1976) was not significantly correlated with the expected pollution gradient from station A to station C. The threshold of hydrocarbon concentration for induction of oil-degrading activity in bacteria has not been well investigated and no significant correlation has been often found in the environment between the two parameters (LEAHY and COLWELL, 1990).

The oxygen consumption in water samples spiked with light gas-oil was significantly enhanced in all samples, with the highest oxygen depletion in surface water of the station C (June 1991).

The highest oil degradation activity was also found in the same station after 30 days of incubation, resulting in more than 80 % depletion of the n-paraffins (Fig.1).

Fig.1 Degradation pattern of the n-paraffins, determined by gas chromatography, in natural and sterile water samples from the Laguna Veneta after 30 days of incubation at 22°C.



In total, sixty three strains of bacteria were able to grow in presence of oil, the number of isolates being higher in June and mainly in the station C. The strains were mostly gram-negative.

Among the four strains (B1, B4, C3, C4) tested for *in-vitro* light gas-oil degradation, the best hydrocarbon degrading activity has resulted in a depletion of 44.6 % of the gas-oil n-paraffins, i.e. in a consumption of 0.77 µg/mg protein/ml and 5.5 µl $02/10^9$ cells/h by strain C3.

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