ORGANIC MATTER DEGRADATION IN THE VENICE LAGOON SEDIMENTS BY AEROBIC ETEROTHROPHIC MICROBIAL COMMUNITIES STUDIED WITH BIOLOG™.

F. Zecchini [1], R. Pastres [2], F. Baldi [1, 3*]

[1] Consorzio Interuniversitario Nazionale la Chimica per l'Ambiente, Via della Libertà, 5/12. I-30175 Marghera, Venezia, Italy
[2] Dipartimento di Chimica Fisica, Università Cà Foscari, Calle Larga S. Marta Dorsoduro, 2137. I-30121 Venezia, Italy
[3] Dipartimento Scienze Ambientali, Università Cà Foscari, Calle Larga S. Marta Dorsoduro, 2137. I- 30121 Venezia, Italy
* baldi@unive.it

Abstract
The sediments of Venice Lagoon differ greatly in their composition according to geographic location, yielding changes in microbial degradation of organic matter. The cellobiose (the dimer of cellulose) was chosen as model molecule to study the degradation of organic matter in the lagoon. Cellulose is quite hardly biodegradable and is abundant in the lagoon being the main component of algae wall. The microbial degradation of the algae biomass was the main cause of past anoxia in sediments and waters. The study of metabolic profiles coupled with statistical analysis (PCA) and respirometric tests characterised microbial populations according to their degrading activity.

Keywords: bacteria, Biolog Ecoplate, PCA, oxygen consumption, cellobiose

Venice and its Lagoon are under continuous surveillance by national and European scientific committees, this yielded an enormous database of physical, chemical, sedimentological and meteorological values. In the last ten years many studies regarding the biological diversity of upper organisms were carried out. Conversely, microbial populations and their activity are less investigated.

In the framework of CORILA project, microbial communities have been studied. The sediments in Venice Lagoon are heterogeneous and differ in the total content of carbon, nutrients, chlorophyll, and pollutants, also redox potential and pH are variable. So, changes in microbial degradation of organic matter was expected in different locations of the lagoon. Cellobiose was chosen as prototype molecule to study the degradation of the algae walls, mainly composed by cellulose. This polymer is relatively resistant to microbial attack in sea water under aerobic conditions and is the most abundant organic compounds in the lagoon. In the past, the microbial degradation of dead algae biomass was the main cause of extended anoxia in sediments and waters.

Metabolic profiles of microbial communities was investigated with Biolog-Ecoplate™ system, a 96 well-plate with 31 carbon sources plus one control with distilled water (three replicates); a non-coloured redox dye indicator turns to purple if the molecule is reduced (formazan). Among the carbon sources there were lipids, sugars, amino acid, metabolic intermediates and multifunctional compounds. Bacteria were removed from sediments by overnight (18 h) shaking in a slurry phase with 2 g of sediment in 24 ml of a very diluted medium containing only yeast extract (0.05 % w/v) and distilled water. After shaking, the sediment was allowed to settle, then 150 µl of the supernatant of the culture were used for inoculation of each well and the plate was incubated at 28°C for 72 h. The microbial oxidation in single wells was revealed and quantified by colour intensity using image analysis (Kodak DC 120 and NIH software). Seventeen sediment samples were collected in spring and summertime 2002. Twelve samples were from the North and Middle Venice Lagoon, four from a lagoon which lies adjacent to North of the delta of the Po river; one sample coming from an industrial area was used as an external control. Four stations were selected for thorough studies on the base of cluster analysis.

In general, each sediment presented a different microbial metabolic profile. Using the statistical analysis of principal components (PCA), appreciable differences were found in heterotrophic microbial communities of collected sediments if compared to those collected in summer. In particular four metabolic profiles were representative of all microbial populations. A scarce consume of carbon sources by bacteria in summer was due to nutritional stress, due to the lack of nutrients and carbon. A similar result was obtained in superficial waters containing oil-degrading bacteria in the Venice lagoon [1]. In fact, in summer oxygen consumption in the presence of amended hydrocarbons stopped after three days of incubation.

A different behaviour of active microbial populations (spring and summer) was observed in one sediment sample (SFR-154). An experiment was performed using this sediment in a gas-proof microcosm, where the microbial activity was continuously monitored for oxygen uptake for five days at 20°C using Oxytop™ system. Different experimental conditions were used: i) in natural conditions, ii) adding nutrients such as nitrogen (NH_4-NO_3, 1 g l^{-1}) and phosphorus (KH_2PO_4) 2 g.l^{-1}, Na_2HPO_4 (3 g.l^{-1}), iii) adding nutrients and cellobiose (2 g. l^{-1}, a β-linked glucose dimer of cellulose) as sole carbon source, iv) only with cellobiose after microbial adaptation to this molecule (2 g. l^{-1}).

The most important response to cellobiose additions was found in the sediment (SFR-154) collected in summer 2002 (Fig. 1), where Dese outflow enters the lagoon in front of Venice airport. Oxytop system showed an high O_2 consumption in natural sediments (without any addition), this levelled off after 2.5 days (line 1), due to a depletion of bio-available carbon sources. Addition of nutrients (line 2) alone was unsatisfactory, because microbial population were mainly carbon starving. On addition of cellobiose as carbon source, after three days of incubation, the microbial population was induced to consume this soluble sub-unit of cellulose (line 3). Further additions of cellobiose resulted in a lower carbon consumption, in this case probably due to nutrient depletion (line 4).

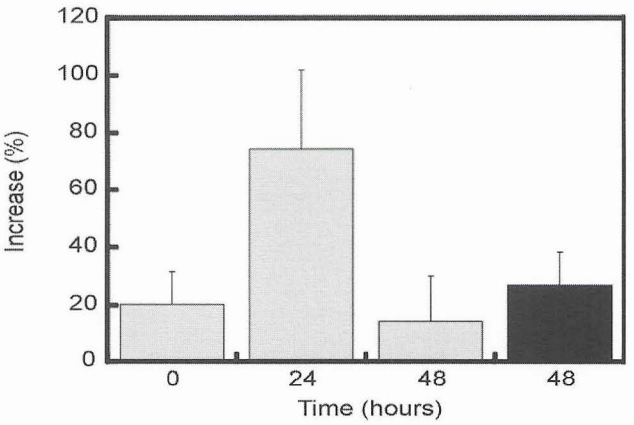

Fig. 1. Biological oxygen demand (5 days at 20°C). Cellobiose (line 3) stimulates microbial growth.

In conclusion the metabolic profile of the microbial populations changes according to period of collection. Variations in the metabolic profile in the studied sediments can be grouped in four typical profiles. In summer microbial communities seemed carbon and nutrients depleted, except sediments in the station SFR-154, which received freshwaters from Dese river. After addition of cellobiose to sediments, bacteria consumed the carbon source and nutrients quite quickly.

During summer, when both carbon sources and nutrients are lacking, heterotrophic microbial populations of the lagoon are less active respect to the ones coming from samples collected in spring at the same point.

References

1 - Baldi, F., Pepi, M., Fani, R., Di Cello, F., Da Ros, L. and Fossato, V., 1997. Complementary degradation of n-paraffins by aerobic gram-negative bacteria isolated from Venice Lagoon. *Croat. Chem. Acta*, 70: 333-346.