## DETERMINATION OF MICROBIAL ACTIVITY IN ESTUAIRE AND MARINE SEDIMENTS

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**Summary:** The results are presented concerning the determination of microbial activity in a few Po River delta sediments and Adriatic Sea sediments off the Po River mouth by applying a simple method based on the reduction of the dye resazurin.

**Résumé :** L'activité microbienne dans quelques sédiments du delta du Pô et du littoral adriatique d'en face, a été déterminée par une méthode simple qui utilise la réduction du colorant résazurine. Les résultats sont discutés.

A simple method has been set up in order to evaluate microbial activity in aquatic sediments. It is based on the reduction of the blue dye resazurin which turns irreversibly pink by means of cellular electron transport chains and reduced chemical compounds which may be present in the sample. The addition of m-cresol (100 µl), which acts as metabolic inhibitor, to an aliquot of the same sediment sample allows one ro measure microbial metabolism by difference. Small aliquots (about 1 g FW) of homogenized natural sediment collected by a gravity corer are mixed with 1 ml of filtered seawater and 1 ml of resazurin solution (10 mg in 100 ml of filtered seawater), with and without m-cresol. After incubation (1 h at 20° C), the samples are filtered and the liquid passed through the filter is made up to 250 ml with filtered seawater. The unreduced resazurin which remains in the solution is measured at 600 nm and compared to a reference curve. Since the reduction of resazurin involves 2 electrons, assuming that it is entirely due to aerobic respiration, the µmoles of disappeared resazurin may be converted to µmoles of oxygen by dividing for 2, thus obtaining a measure of microbial activity in the sediment under examination. This method has been applied to the study of marine sediments during the campaigns carried on by our laboratory.

In this paper the results are presented for five samples taken in the Basson Inlet (Po River delta) and three samples taken in the Adriatic Sea off the Po River mouth, at the end of June. As far as the Basson Inlet is concerned, microbial activity in the first 5 mm varied according to granulometry and hydrodynamic energy. In fact, in the sandy station the value of resazurin reduction was 2.8  $\mu g/h/g$  DW, in the muddy stations subjected to moderate hydrodynamic action the values ranged from 18.5 to 26.5  $\mu g/h/g$  DW, and in the sheltered muddy station, covered with abundant vegetal remains, the value was 157  $\mu g/h/g$  DW. The resazurin reduction was also determined in the subsurface layer (around 2-3 cm) where the brown colour changes to the grey colour indicating roughly the transition from oxidizing to reducing conditions.

In situ Eh measurements by a probe (Idronaut, Milan) confirmed a drop at that layer from about 360 mV to about 50 mV, except in the sandy station where no significative drop was observed. The transition layer is thought to be a site of high microbial activity (Novitsky and Kepkay, 1981). The activity values we found are one order of magnitude higher than those in the surface layer (except the sheltered station) and were similar for the muddy stations ranging from 169 to 195  $\mu$ g of reduced resazurin/h/g DW. In the sandy station, where no redoxcline was apparent, the subsurface resazurin reduction was the same as in the surface layer (3.2  $\mu$ g/h/g DW).

As far as the marine samples are concerned, they were collected at three water depths: 10, 20 and 30 m. In the surface layer of all the 3 sediments the values of resazurin reduction were similar and higher than those obtained in the surface layers of the delta sediments, ranging from 112 to 222  $\mu g/h/g$  DW. When expressed as oxygen consumption values, they are relatively high, but are in the range of the benthic community metabolism values found by various authors, as compared by Es (1982). The subsurface measurement is available only for 30 m station and it is about twice the surface value (306 versus 163  $\mu g/h/g$  DW).

The advantages of the resazurin reduction method are: simplicity, relative speed, inexpensiveness, sensitivity. They are particularly appreciable in the field, when it is necessary to process many samples quickly. However, care must be taken when considering the possible enhancement of microbial metabolism due to incubation temperature which may be higher than environmental temperature, and to sample stirring prior to incubation. Furthermore, it must be pointed out that the present method allows the assessment of the metabolic activity of the aerobic and facultative anaerobic bacteria, but not that of the strict anaerobic bacteria, at least in the described experimental conditions. Since the metabolism of the latter is important in the subsurface layers and in reducing environments (Jorgensen, 1977), we are studying the possibility of treating and incubating the samples also under anoxic conditions.

## **REFERENCES**

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