ORIENTATION ON ELECTRON TRANSPORT SYSTEM ACTIVITY MEASUREMENT IN DIFFERENT ORGANISMS

(training for Indonesian-Dutch Snellius II-expedition in 1984-'85)

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by

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1. INTRODUCTION

Two processes are very essential for life on earth, those are the production of organic matter by photosynthesis and the degradation of organic matter by respiratory processes.

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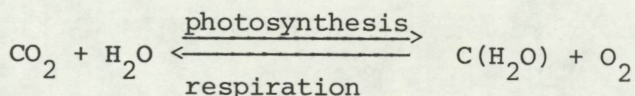

$$CO_2 + H_2O \underset{\text{respiration}}{\overset{\text{photosynthesis}}{\rightleftarrows}} C(H_2O) + O_2$$

So the carbon-cycle is closed and this is a sine qua non for life on earth.

In marine environments the photosynthesis can only occur in the upper layers because there is (sun)light, in the so-called euphotic zone. In this zone as well as below this euphotic zone degradation of organic matter and oxydation of other reduced compounds can occur by biological activities. In ecology we are very interested in the biogeochemical cycles and there is a need for measurements of the different metabolic activities.

Primary production can be measured by the incorporation of radioactive bicarbonate in organic matter (Steeman-Nielsen, 1952) but the respiration is a more complicated problem. There is a large scale of organic compounds in the biosphere and also there are different types of respiration. Respiration on oxygen but also, denitrification (= respiration on nitrate), manganese reduction, nitrate ammonification iron reduction, sulphate reduction and methane formation are types of respiration. We neglect fermentation because the reduced end products of fermentation will be respired somewhere in the ecosystem. So degradation of organic matter is done by respiration and respiration means electron transport along an electron transport chain to an electron acceptor.

In ecology an increasing stream of publications appears about the measurement of electron transport system (ETS) activities as a relative measurement for respiration (see e.g. Vosjan, 1982; Packard c.s., 1983). That is because for understanding an ecosystem we need information not only

about biomasses but also on fluxes. For the fluxes we need measurements of metabolic rates. One of these is the ETS activity measurement. The aim of this research is to prove that the ETS measurement can be used for different organisms and different systems, and that also the process of methane formation is included in the measurement of ETS-activity in field observations.

2. METHODS

The ETS-activity method is the measurement of electron transport along the electron transport chain at substrate saturation. This means we measure a Vmax. As substrates NADH, NADPH and sometimes also succinate are used together to saturate the transport chain. As e-acceptor INT (2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride), which has a high affinity for electrons, is used. The site of action of INT is the cytochrome-b-coenzymeQ complex in the electron transport chain (Packard, 1971). The reduction of 1 mol INT to formazan means the acceptance of 2e, this is equivalent to the reduction of ½ mol O_2. Formazan concentration can be measured easily by spectrophotometry. The extinction coefficient of INT-formazan is at a wavelength of 490 nm: $15.9 \cdot 10^3$ $M^{-1} \cdot cm^{-1}$. During this orientation we used different organisms, from vertebrate to bacteria and also water and sediment samples. If the samples were homogenised an ultra-turrax homogenizer was used. We followed the method described by Olanczuk-Neyman & Vosjan (1977) which is a modification of the method of Packard (1971). ETS-activity is expressed as equivalent formazan (= 2e=½ O_2) per quantity per time.

3. RESULTS AND DISCUSSION

In our orientation about ETS-activity in different organisms we started with a vertebrate, a little fish: *Pomatoschistus lozanoi*. Oxygen consumption of four *Pomatoschistus* species was already studied at the Netherlands Institute for Sea Research by Fonds and Veldhuis (1973), that is why we choosed this organism. Of course respiration is, as in other organisms, related to body weight, temperature and other fysiological contitions. We did not study this aspects. We only wanted to prove that the ETS-method is possible for different kinds of organisms.

So we homogenized a little fish with an ultra-turrax homogenizer, e.g. a fish of 400 mg in 20 ml homogenate buffer. In 0.1 ml of this homogenate it is easily to measure the activity after an incubation time of 10 to 20 minutes at 20°C. We found activities in equivalent formazan of the electron transport system of about 30 $\mu mol.g^{-1}.h^{-1}$.

Fonds and Veldhuis (1973) found respiration rates of 5 to 80 $\mu mol.g^{-1}.h^{-1}$. The weight is expressed as g wet weight and respiration in equivalent formazan = $\frac{1}{2}O_2$. Also of an evertebrate, the bivalve *Macoma balthica* the ETS activity was measured. This organism was choosen because the oxygen uptake and energy metabolism was studied at the institute by de Wilde (1973, 1975). Medium sized organisms under natural conditions have an oxygen uptake of about 4.5 ml $O_2.g^{-1}.h^{-1}$ (g ashfree dry weight), this means per g wet weight about 40 $\mu mol.g^{-1}.h^{-1}$. The ETS activities we found were about 500 $\mu mol.g^{-1}.h^{-1}$.

This is very high in comparison with the fish. The fish we homogenized with endo-skeleton and from the bivalve we removed the exoskeleton. So it is clear that the factor oxygen

uptake/ETS activity is not the same after the different manipulations. Research should be done about this factor of respiration to ETS-activity for different organisms in different physiological conditions. Perhaps it is better to use a metabolic active organ as liver or hepato-pancreas for ETS-measurements as a relative measurement for activity of individual organisms or populations.

For one cellular organism we choosed yeast. Also with this organism (Saccharomyces) INT is easily reduced with NADH and NADPH as substrates. For microorganisms we did not homogenize the organisms, we expected that the formed formazan should come in solution with the Triton X-100 as a detergent. But it proved that substrate and INT entered the cells very rapidly and formazan was formed inside the cells and stayed there as a red precipitate. This could be easily observed by microscope. In the incubation solution the yeast cells formed a red coloured sediment. Trevors (1982), who studied tetrazolium reduction in Saccharomyces, to quantify the percentage actively respiring yeast cells, observed the same formazan granules inside the cells. That is why it is essential to homogenize this kind of organisms to extract the ETS-activity from the whole cells.

Bacteria can also be used for the reduction of INT. So far this is done with aerobic, nitrate reducing and sulphate reducing bacteria. We tried methane forming bacteria, because it is not known if the ETS-method is possible with this anaerobic organisms, which are present in anaerobic eco-systems with very low redox potentials. In our preliminary experiments we used consortia of anaerobic methane forming bacteria. From Dr van Andel (Laboratory for Microbiology of the University of Amsterdam) we got samples of methane producing cultures. It proved, that methane forming consortia of methane

producers, methane producing granules and also pure cultures of *Clostridium butyricum* have high activities in the reduction of INT with NADH and NADPH as electron donor. Values as high as 2000 µmole.g^{-1}.h^{-1} were found. Research about the biochemical background of this reaction has to be done. We concluded from these experiments that, if we study an ecosystem like anaerobic mud, in which methane formation occurs, the ETS method measures also activity of these anaerobic bacteria. The conversion of ETS-activity to activity of degradation of organic matter should be researched.

The activity of suspended matter of the Dutch Wadden Sea was also measured. By filtration water samples over a 0.2 µm filter and incubating the filter in the substrate solution at 20°C we found activities of 0.1 to 0.4 µmol.l^{-1}.h^{-1}. Depending on different environmental factors the activity in the ecosystem will change. For tidal effects on the ETS activity in the Dutch Wadden Sea, see Vosjan and Tijssen (1978) and Vosjan (1982).

In a sediment sample the vertical distribution of ETS-activity was studied. From the surface to deeper in the sediment the activity decreases. For literature about this see Vosjan and Olanczuk-Neyman (1977). The conversion from ETS-activity to degradation of organic matter is still a problem and needs further research. But it is clear that the anaerobic respiration processes as nitrate reduction, sulphate reduction and methane formation also are measured with the ETS-method. Trevors, Mayfield and Inniss (1982) measured ETS-activity in agricultural soils and found high correlations between oxygen uptake and ETS-activity in these soils and they recommend this technique as a rapid quantitative measurement for microbial ETS-activity in soil.

4. SUMMARY AND CONCLUSION

Preliminary experiments about ETS-activity were done in different organisms and different ecosystems; a vertebrate, an evertebrate, yeast, bacteria, methane forming consortia, suspended matter and sediment. It proved that the method is useful for the different systems but research has to be done about the conversion to respiration or to degradation rate of organic matter at in-situ circumstances.

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