Exploring ETS activity as a measure of potential respiration

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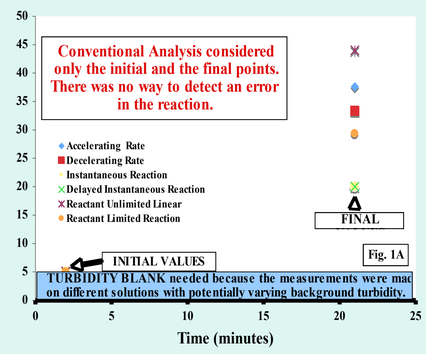
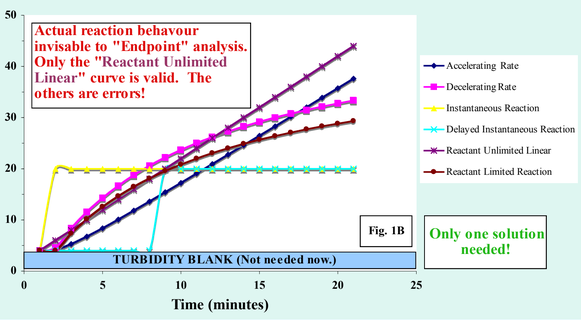
Endpoint Analysis Pitfalls



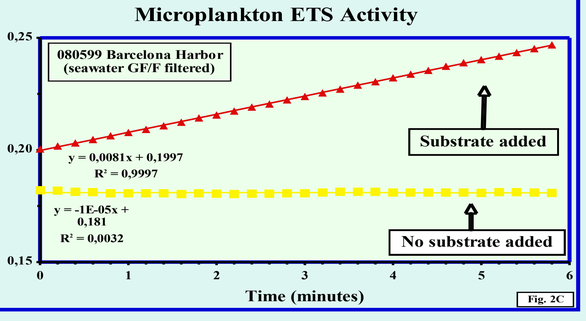
Two separate Solutions needed!

20 min Time Course reveals errors!

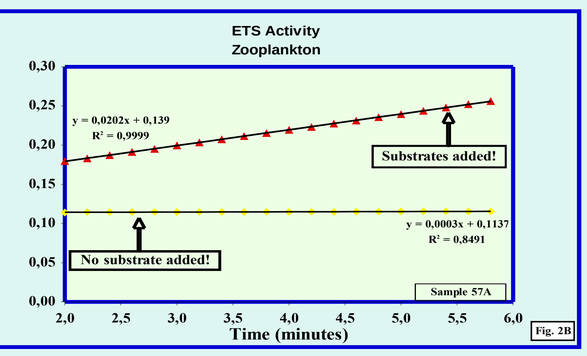


Only one solution needed!

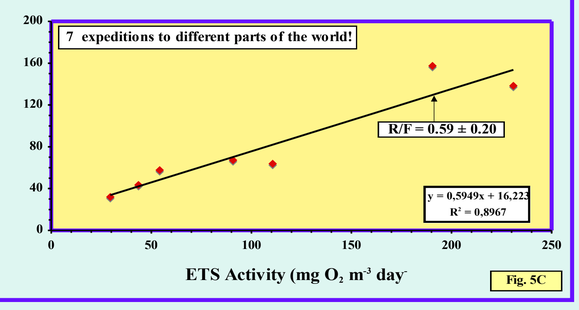
Microplankton ETS Activity



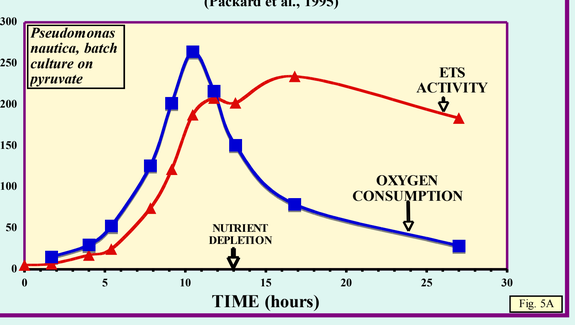
ETS Activity Zooplankton

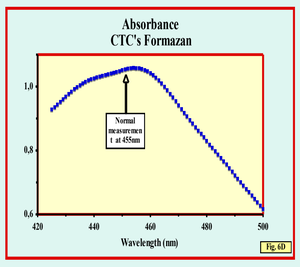


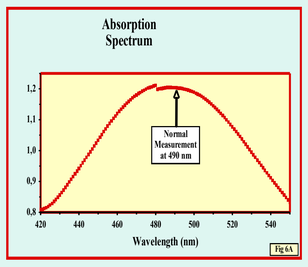
R/ ETS by Arístegui & Montero (1995)



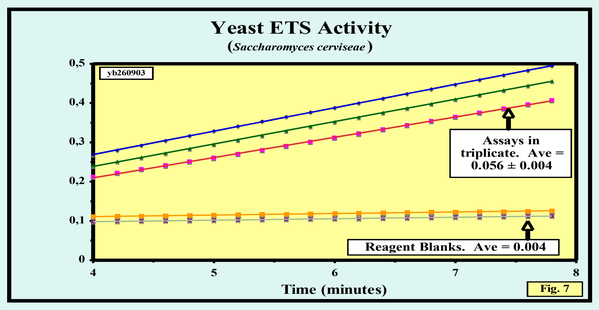
ETS & Respiration time course
(Packard et al., 1995)







Yeast ETS Activity
(*Saccharomyces cerviseae*)



INTRODUCTION Is the ocean net autotrophic or heterotrophic? Is the biosphere losing nitrogen to anammox and denitrification or is it in balance with nitrogen fixation and anthropogenic inputs? These questions cannot be resolved without accurate measurements of the physiological processes involved and yet our methodology lags far behind our curiosity and the demand to monitor our rapidly changing planet. This situation cries out for methods development. For this reason, as in Gomez et al (1996), we continue to explore the use of the respiratory electron transport system (ETS) as a measure of potential respiration (Φ) and a proxy for respiration (R).

Here we demonstrate: (1) how kinetic ETS assays document reaction-rate time-courses, cuts reaction-time in half, and eliminates turbidity blanks; (2) why substrate blanks do not measure ETS activity and why a Michaelis-Menten V_{max} should be measured instead; and (3) the biochemical meaning of R/Φ in seawater. We also present new data on: (4) the molar specific absorption coefficient for the tetrazoliums, INT and CTC (17.8 and 22.9 absorbance units (mM INT formazan)$^{-1}$ (1 cm cuvette)$^{-1}$, respectively). Finally we propose using baker's yeast (*Saccharomyces cerviseae*), with an ETS activity (20°C) of 25.1 ± 5.1 µmol e^- min^{-1} (mg dry weight)$^{-1}$ as a biological standard.

METHODS ETS assays reported here are based on the Packard and Williams (1981) method with the simplifications: (1) that turbidity and substrate blanks have been replaced by a reagent blank (an ETS assay with <1/1000 part of the sample's biological material); (2) that succinate as a reactant (Savenkoff et al, 1995) and cyanide as an inhibitor of cytochrome oxidase, have been eliminated. All measurements were made kinetically according to F. D. King (personal communication) with a computer-controlled spectrophotometer using automatic sample changing and temperature control. Homogenates were kept at 0-4°C and were measured within 20 min after being removed from either liquid nitrogen or -80° storage.

RESULTS **Fig.1A-1C** show that kinetic analysis offers the advantages of: (1) detecting errors during the ETS reaction; (2) halving the time of the analysis; (3) increased sensitivity and resolution through better slope definition and higher data acquisition rate. **Figs. 2A-2C** shows that ETS activity cannot be measured in zooplankton, phytoplankton, or seawater (microplankton) without adding substrates (NADH and NADPH). **Fig. 3** illustrates why an irreproducible result is obtained if substrate is not added to an enzyme assay based on disrupted cells. **Fig. 4** compares the currently used reagent blank with an ETS assay done on a seawater sample.

To reconcile known variability in the ratio of respiration (R) to potential respiration (Φ) in bacteria (**Fig. 5A and 5B**) with the use of a constant R/Φ in oceanographic fieldwork (**Fig. 5C**) one can consider a kinetic formulation of respiration (**Box 1**). Upon doing this, it becomes apparent that R/Φ in the field (Arístegui and Montero, 1995) can be an oceanographic average of the expression, [NADPH][NADH]/(K_b+[NADPH][NADH]), as explained in **Box 1**. This is an unproven, but testable hypothesis.

ETS activity, as currently measured, is based on a specific absorbance (A_{490}) of 15.9 absorbance units mM^{-1} in a 1 cm cuvette of the INT formazan at a wavelength of 490nm. Here we show that the peak at 490 nm in phosphate buffer is correct (**Fig. 6A**), but that A_{490} may be 17.8 (**Fig. 6B**) rather than 15.9. We also tested another tetrazolium salt (CTC) to see if it could replace INT as an electron acceptor. We found its absorption maximum at 455nm (**Fig. 6C**) and its A_{455} to be 22.9 absorbance units mM^{-1} (**Fig. 6D**).

Box 1 **f respiration (R) in seawater, phytoplankton, bacterioplankton, and zooplankton can be represented by the equation:**

$$R = \Phi[NADPH][NADH]/(K_b+[NADPH][NADH]),$$

where Φ = potential respiration, NADH and NADPH are the principal reactants (substrates) for the ETS, and

$$K_b = (K_{NADH})(K_{ia}) + (K_{NADPH})[NADH] + (K_{NADH})[NADPH],$$

where K_{ia} is the dissociation constant for the ETS-NADPH complex, K_{NADH} is the affinity of the ETS for NADH, and K_{NADPH} is the affinity of the ETS for NADPH;

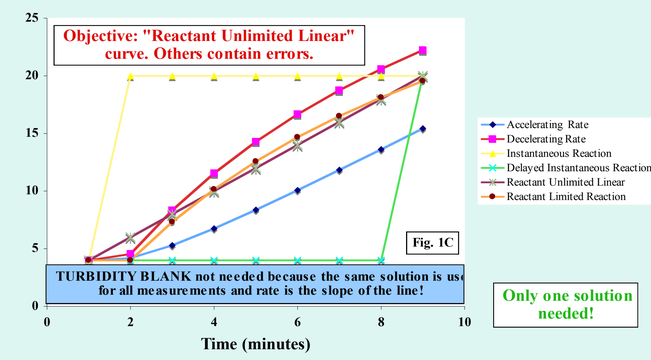
Then for seawater, reconciliation between Figs. 5A, 5B, and 5C is found when **[NADPH][NADH]/(K_b+[NADPH][NADH])** for seawater equals the slope of the calibration plot between seawater R and seawater Φ! In other words for a seawater average (Fig. 5C) according to Arístegui & Montero (1995),

[NADPH][NADH]/(K_b+[NADPH][NADH]) = 0.59 **Box 1**

CONCLUSIONS and SUMMARY

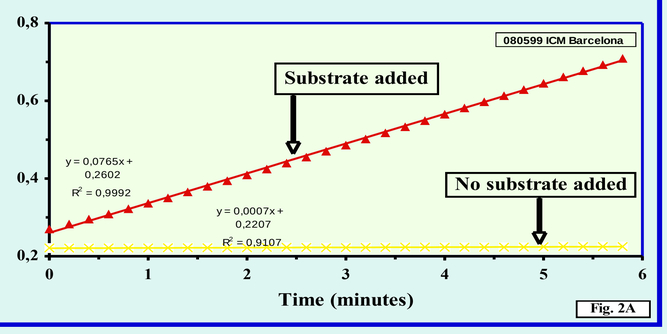
1. Kinetic analysis provides more sensitivity, reliability, information, and a higher data acquisition rate than end point analysis (**Figs. 1A-1C**).
2. An ETS assay measured without adding substrates (substrate blank) yields practically no activity and therefore cannot serve as a measure of Φ. Furthermore, it is not a measure of invivo ETS activity because the reactants, during homogenization, are driven far lower than their occurrence in the living organism (**Figs. 2-4**).
3. **Fig. 5** and **Box 1** argue that a measurement of R/Φ in the ocean is a measure of [NADPH][NADH]/(K_b+[NADPH][NADH]) in the plankton.
4. **Fig. 6** supports the use of INT as an efficient electron acceptor for the ETS.
5. **Fig. 7** illustrates the facility of using yeast as a biological standard for ETS measurements.

8 min Time Course saves time & reveals errors!

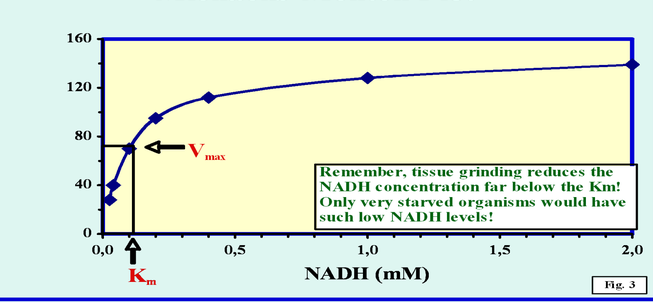


Only one solution needed!

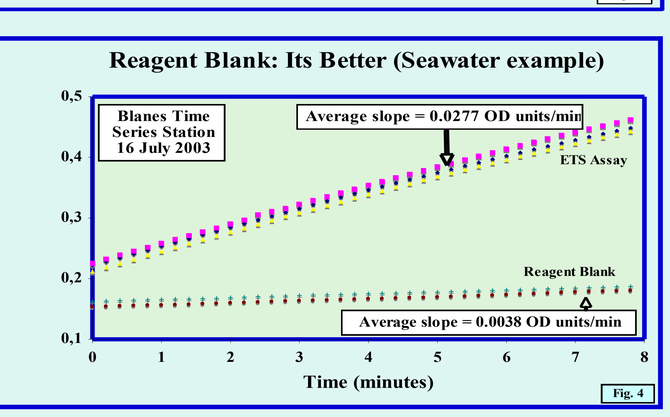
***Gyrodinium corsicum* ETS**



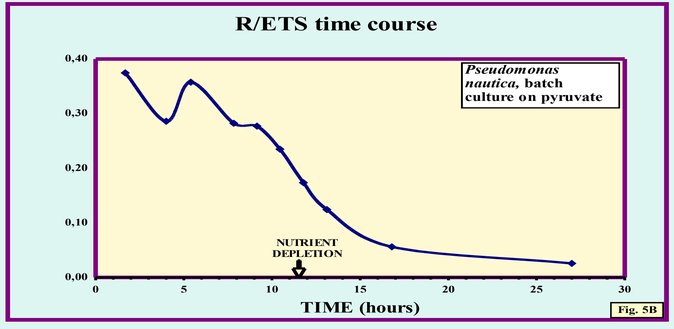
Michaelis-Menten Plot

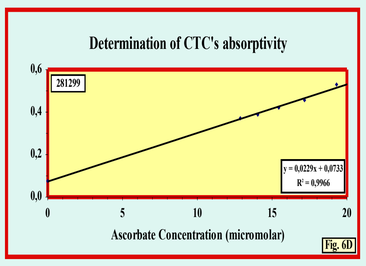


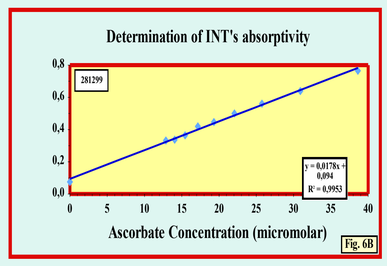
Reagent Blank: Its Better (Seawater example)



R/ETS time course







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