Can the Metabolic Theory of Ecology predict respiration in marine bacteria?

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Respiratory oxygen consumption is caused by enzymatic activity of the respiratory electron transfer system (ETS). However, in spite of this understanding, respiration models continue to be based on allometric equations relating respiration to body size, body surface, or biomass. The Metabolic Theory of Ecology (MTE) is a recent example. It is based on Kleiber's Law relating respiration (R) and biomass (M) in the form, $R = K_1 M^{3/4}$, where K_1 is a constant. This law holds because biomass packages the ETS. Consequently, we have been proposing to bypass biomass and model respiration directly from its causal relationship with the ETS activity ($R = f(ETS)$).

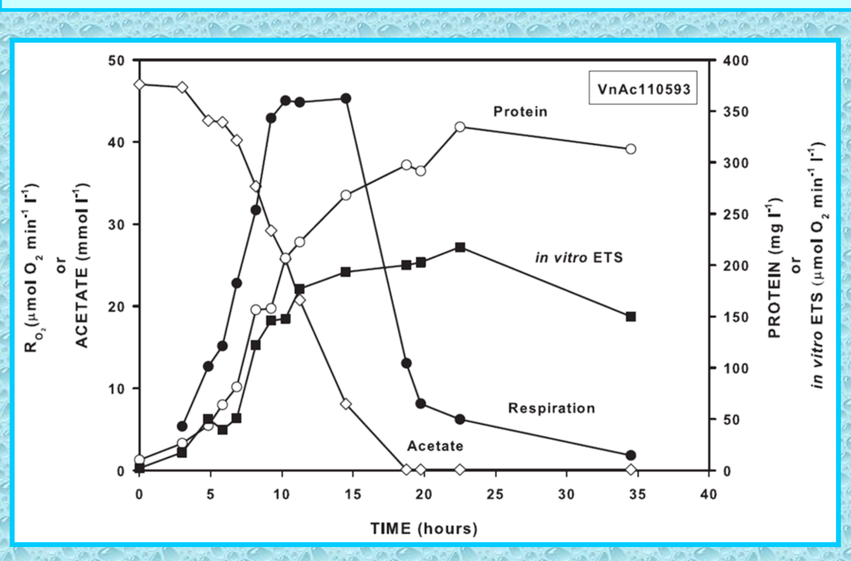

Fig.1 Experiment VnAc110593 showing time-course observations of A_{ETS}, RO_2, protein and acetate.

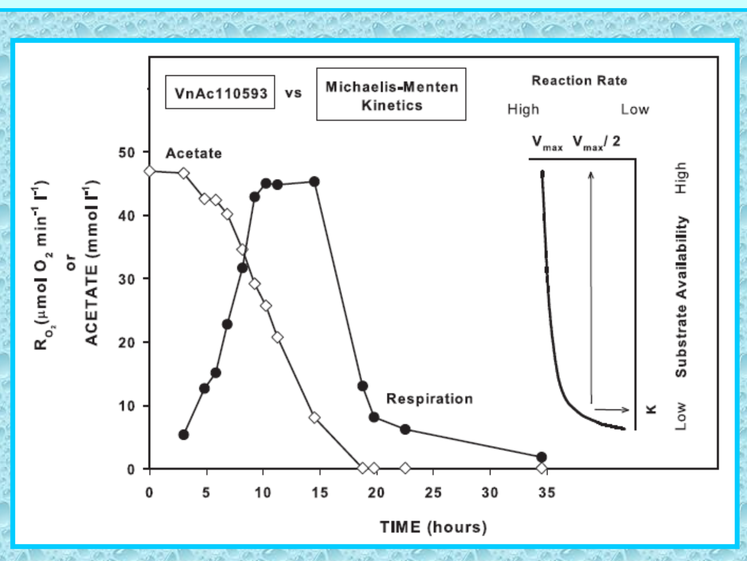

Fig. 2 The inspiration for considering Michaelis-Menten as the throttle mechanism for respiration. Here respiration falls in parallel with the nutrient supply.

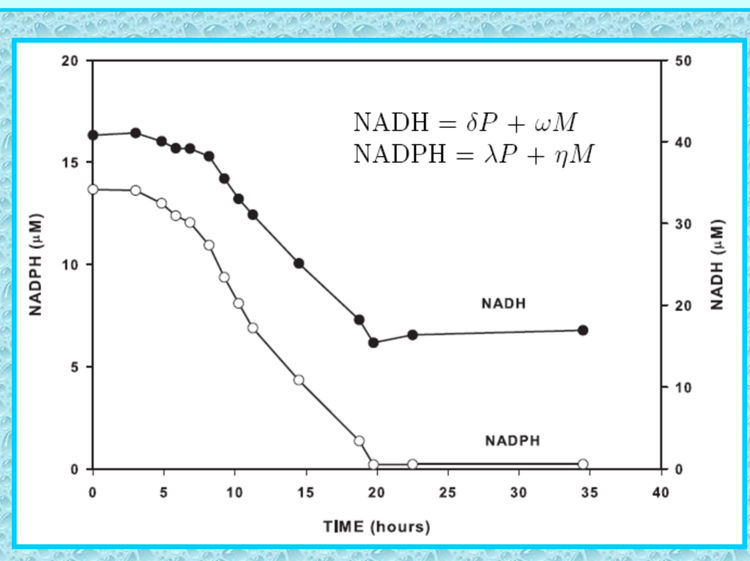

Fig.3 Modelled intracellular NADH and NADPH for experiment VnAc110593. *P* is acetate, *M* is cell protein and δ, ω, λ and η are constants (Table 1).

Enzyme Kinetic Model (EKM)

$$\nu_{ETS} = \frac{A_{ETS}\,[NADH]\,[NADPH]}{K_\beta + [NADH]\,[NADPH]}$$

$$K_\beta = K_{NADH}\,K_{ia} + K_{NADPH}\,[NADH] + K_{NADH}\,[NADPH]$$

Table 1. Kinetic parameters that were used to calculate the in vivo ETS activity (ν_{ETS}) and the intracellular NADH and NADPH concentrations.

Parameter	*Packard et al 1996*	This work
For Eq. (6) and (7)		
K_{ia}	6.7 μM	Same
K_{NADPH}	9.0 μM	Same
K_{NADH}	26.0 μM	Same
For Eq. (8) and (9)		
λ	0.882 μmol NADPH $mmol^{-1}$ pyruvate	0.2905 μmol NADPH $mmol^{-1}$ acetate
η	5.90×10^{-4} μmol NADPH mg^{-1} protein	Same
δ	2.60 μmol NADH $mmol^{-1}$ $mmol^{-1}$ pyruvate	0.8564 μmol NADH $mmol^{-1}$ acetate
ω	5.20×10^{-2} μmol NADPH mg^{-1} protein	Same

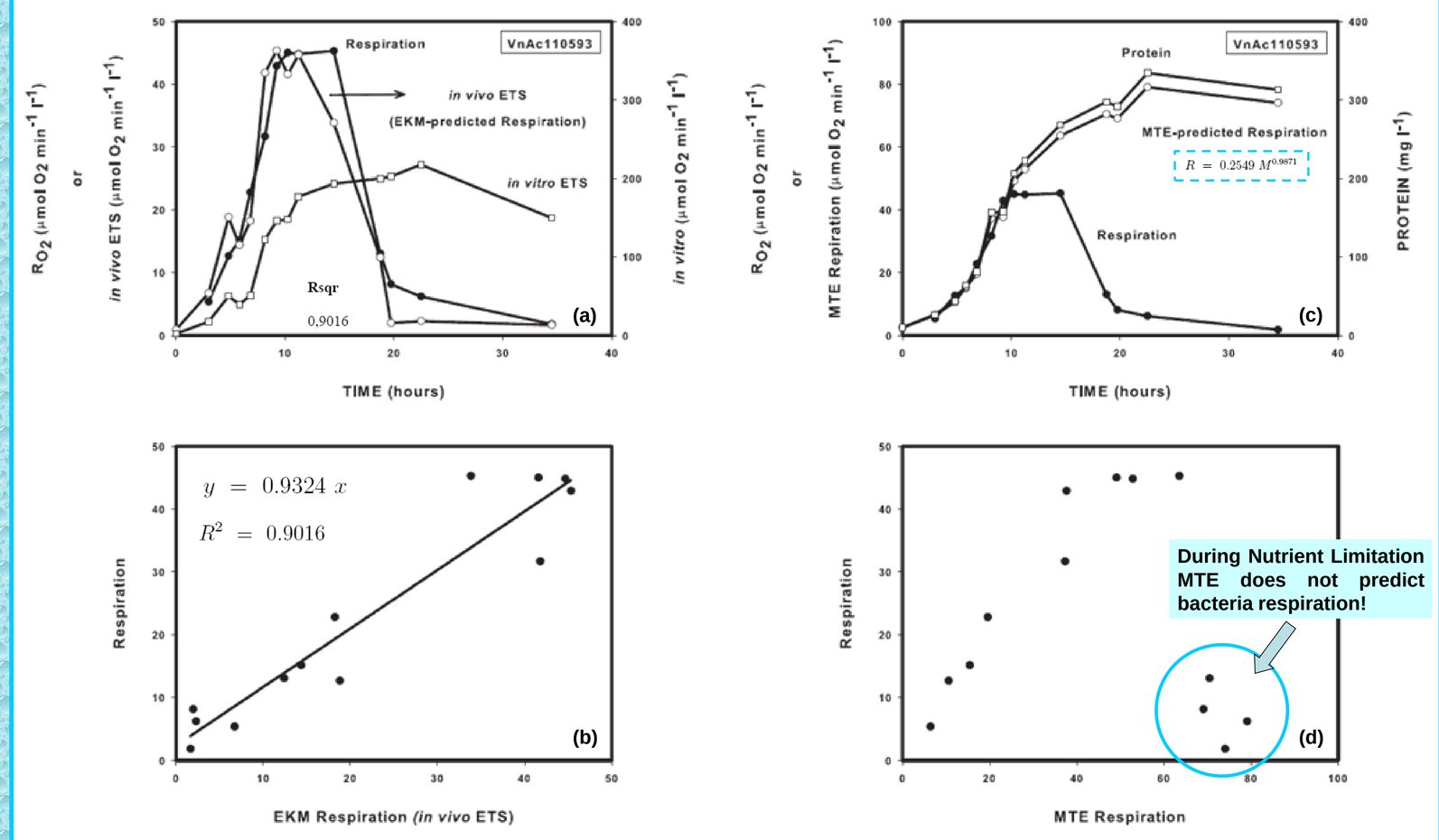

Fig. 4 (a) Modelled time-profile of RO_2 based on *in vitro* ETS from EKM; (b) EKM Predicted-Respiration versus Respiration; (c) Modelled time-profile of RO_2 based on allometric relationship (cell protein) from MTE.; (d) MTE Predicted-Respiration versus Respiration.

Here we present an enzyme kinetic model (EKM) of respiratory oxygen consumption based on the substrate control of the ETS (Fig. 2 and 3). It argues that R is controlled by the maximum velocity, V_{max}, of the enzyme reactions that controls the process (i.e., the ETS), the temperature, and the substrate availability, S (Fig. 3). Kinetics of this thermal-substrate regulation are described by the Arrhenius and Michaelis-Menten equations. The EKM equation takes the form:

$$R_1 = \frac{V_{\max}\; S\; e^{\left(\frac{-E_a}{R_g (T_1 - T_0)}\right)}}{(K_m + S)}$$

where E_a is the molar Arrhenius activation energy, R_g is the molar gas constant, K_m is the Michaelis-Menten constant, and T is temperature.

Here we apply the EKM and the MTE to predict a respiration times-profile throughout the exponential, steady state, and nutrient-limited phases of the marine bacterium *Vibrio natriegens* in an acetate-based culture (Fig. 1). Both models were tested by comparing their output with the measured RO_2 time-profile (Fig. 4 top). They were evaluated quantitatively by least-square regression analysis (Fig.4 bottom). When the predictive capability of both models was compared, serious flaws in the MTE rendered it inaccurate during nutrient limitation. In contrast, the EKM worked well throughout the entire period of the experiment. Results suggest that respiratory control is achieved through changes in the *in vivo,* ν_{ETS} *,* activity of the ETS by substrate modulation of the *in vitro* ETS, A_{ETS}, (V_{max}). We conclude that EKM holds promise for predicting respiration at the different physiological states and time-scales important to microbiological studies.

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